EFFECTS OF SUPPLEMENTAL ENERGY AND PROTEIN ON FORAGE DIGESTION AND UREA KINETICS IN BEEF CATTLE

by

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Abstract

Two experiments quantified effects of supplemental protein and energy on forage digestion and urea kinetics in beef cattle. In experiment 1, energy treatments included: control, 600 g glucose dosed ruminally once daily, and 480 g VFA infused ruminally over 8 h daily. Casein was dosed ruminally once daily (120 or 240 g). Cattle (208 kg) had ad libitum access to low-quality hay (5.8% protein). Infusion of VFA decreased forage intake by 27%. Glucose decreased NDF digestibility. Microbial N flow was greater for 240 than for 120 g/d casein, but was not affected by energy. Retained N increased with casein supply. Urea-N entry rate (UER) and gut entry of urea-N (GER) were not affected by energy, casein, or interactions, but GER/UER was less when 240 rather than 120 g/d casein was provided. Compared to VFA, glucose tended to increase GER/UER. Glucose led to more microbial uptake of recycled urea than VFA. In these young calves, changes in N and energy supply did not greatly impact urea kinetics, likely because increased N was largely retained. In experiment 2, treatments included: 0 or 1.2 kg glucose, and 240 or 480 g casein. Cattle (391 kg) were fed low-quality hay (4.7% protein). Glucose reduced forage intake by 18%, whereas casein did not affect it, and depressed fiber digestion. Microbial N flow to the duodenum and retained N increased as casein increased, but neither was affected by glucose. Increasing casein increased UER 50%. Urinary urea-N increased as casein increased; moreover, GER numerically increased 25% as casein increased. GER/UER decreased as casein increased. Glucose decreased urinary urea, but did not change UER or GER. Microbial uptake of recycled urea was least for steers receiving 480 g/d casein with no glucose, reflecting that this treatment exceeded ruminal requirement for N. In these more mature steers, increases in N intake increased UER, reflecting that only small proportions of the increased N intake were retained. Thus, as steer maturity increased, UER and GER increased, likely because

less N was retained. These studies demonstrate the influence of urea recycling in meeting N needs of cattle fed low-quality forage.

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CHAPTER 1 - A Review of Literature

Producers raising cattle on pasture have many production variables to consider. Manipulating utilization of perennial pastures has proven to be a cost effective nutrition strategy for producers, because of relatively low costs associated with production of perennial forages. Low quality forage (CP<7%) can be detrimental to pasture utilization and animal performance. Cattle consuming a diet consisting only of low quality forages will not be able to achieve optimal performance due to a variety of limitations. Therefore, providing feed supplements is necessary to improve pasture utilization; however, costs associated with supplementation are large.

Due to the low quality of some pastures, producers are often forced to provide supplemental nutrients to their cattle. The question then becomes, "What nutrients must be provided to help maintain performance of the animals in question?" Researchers have evaluated many different answers to this question over the years, with varying levels of success.

Protein and energy have been provided to ruminants as means of improving performance. The benefits of providing supplemental protein to cattle consuming low quality forages are stark and well defined (DelCurto et al., 1990a; Köster et al., 1996). Protein has historically been the most expensive nutrient to use in production settings. This forced the scientific community to take a close look at protein supplements fed to ruminants.

The NRC (1996) classifies protein into two groups: degradable intake protein (DIP) and undegradable intake protein (UIP). DIP is protein degraded in the rumen by the resident microflora. Through DIP supplementation, ruminants may receive protein in the form of microbial cell protein. UIP escapes ruminal degradation, arriving in the small intestine of ruminants unchanged.

DIP Supplementation

Ruminant diets consisting of low-quality forage are most deficient in DIP (Köster et al., 1996). Peptides, amino acids, and ammonia are products of protein degradation in the rumen. Rumen microbes use the aforementioned nitrogenous products to support growth. A decrease in forage digestion occurs in times of inadequate ruminally available nitrogen (RAN) due to a decrease in microbial activity. This has a negative effect on the performance of the animal. With slower digestion, feedstuffs spend more time in the rumen. Concurrently, a reduction in intake occurs, reducing the amount of energy available to the animal.

Satter and Slyter (1974) were among the first researchers to show that increasing ammonia concentration in a fermentation system increased the productivity and growth of the microbial population. They found that 1.4 m*M* ammonia was sufficient to support maximal microbial cell protein production in the rumen, but they recommended 3.6 mM to provide a margin for safety.

Köster et al. (1996) provided definitive research on the efficacy of DIP supplementation in improving utilization of low-quality forage by cattle. They provided cannulated cattle (body weight [BW] = 588 kg) with increasing levels of sodium caseinate as a DIP supplement. This allowed the researchers to study the effect of DIP on forage intake and digestion without any confounding factors in the supplement, as had been a problem with previous research in this area. The provision of up to 540 g/d casein improved forage organic matter intake (OMI). Maximal total tract digestibility of neutral detergent fiber (NDF) was achieved at 180 g/d casein. Koster et al. (1996) recommended providing 11% of TDN as DIP to optimize total digestible OMI.

Looking to build on the work of Koster et al. (1996), Mathis et al. (2000) examined the relationship between forage quality and the response to DIP supplementation. Their research

consisted of three independent studies in which ruminally cannulated steers were fed one of four levels of supplemental DIP daily. The levels of supplementation were 0, 0.041, 0.082 and 0.124% of BW daily. Quality of forage differed among the experiments. The first trial used Bermudagrass, which had a CP content of 8.2%. The second trial used bromegrass with a CP content of 5.9%, and the third trial used forage sorghum with a CP concentration of 4.3%.

Supplementation of DIP had no effect on forage intake or digestion when the cattle were fed Bermudagrass. The only effects were increases in ruminal concentrations of ammonia and minor VFA (isobutyrate, valerate, and isovalerate). For the trial with bromegrass, there was a numeric increase in forage intake and total tract digestion of NDF in response to DIP supplementation. Total digestible OMI was increased by DIP supplementation. Ammonia nitrogen concentrations in the rumen were increased with increasing DIP. Ammonia concentrations were lower at all levels of DIP supplementation in this trial than in the Bermudagrass trial. Supplementation altered the ruminal VFA profile. The acetate:propionate ratio and butyrate concentration decreased with increasing DIP. Once again, the concentrations of the minor VFA were increased by protein supplementation.

The forage sorghum trial showed the largest effects of DIP supplementation. Forage intake and total tract digestibility of both OM and NDF were increased with DIP supplementation, as was passage rate of acid detergent insoluble ash (ADIA). With poor-quality forage, rate of passage slows due to a slow rate of particle size reduction. Ammonia nitrogen and total VFA concentration increased with protein supplementation. The VFA profile showed the same changes as in the bromegrass trial.

UIP Supplementation

Supplementation of protein sources high in UIP concentration is effective in improving forage utilization because of the ability of cattle to recycle nitrogen to the rumen. Bandyk et al. (2001) fed steers low quality forage and infused protein into either the rumen or the abomasum, allowing a comparison of the effects of DIP vs. UIP on forage intake and digestion. Providing a protein supplement improved forage intake, with the improvement being greater when DIP was provided. Organic matter digestibility increased with supplemental protein, with no difference between ruminal and postruminal infusions. Total tract digestibility of NDF was not improved by protein supplementation. Ruminal ammonia N level increased with protein supplementation, the magnitude of increase being larger for ruminal than for postruminal administration.

Wickersham et al. (2009) provided four levels of casein (0, 0.062, 0.124, or 0.186 g/kg BW per head per day) postruminally to steers consuming low quality forage. Urea kinetics were concurrently measured in the steers. They found that even at the highest level of UIP supplementation (0.186 g/kg BW per head per day), steers still recycled 86% of all urea produced in the body back to the rumen. Forage OMI increased quadratically with larger amounts of casein infused into the abomasum. Total tract digestibility of NDF was not impacted by infusing casein postruminally.

Effects of Energy Supplementation to Low-Quality Forage Diets

Low-quality forage can also be deficient in energy; therefore, attempts to improve animal performance have been made using supplemental energy. Supplemental energy has been provided through various sources. Different sources of energy can have major effects on the productivity of cattle fed poor-quality forage, creating a need for research on various sources of supplemental energy.

Research has shown that supplementation of low-quality forage with higher-quality forages causes decreased intake of the low-quality forage. A substitution effect occurs in which low-quality forage consumption decreases with increasing high-quality forage provision. Costs associated with feeding high-quality forages are large; moreover, they take up more storage space than other supplements. There can also be problems in delivering the supplement, especially in production settings with difficult terrain.

Feed ingredients containing significant amounts of non-structural carbohydrates (NSC) have greater energy density than forages. This group includes corn grain, barley, and wheat middlings. Throughout the majority of the 20th century, prices of these grains were low, leading to an opportunity to provide inexpensive supplements. Conversely, research has repeatedly shown that large amounts of NSC supplementation can have negative impacts on forage intake and digestion.

Chase and Hibberd (1987) provided 0, 1, 2, or 3 kg/d of ground corn to mature cows. Total dry matter intake increased with the provision of supplements but the response did not match the magnitude of increase in supplement at each level. Consequently, forage OMI and forage DMI decreased linearly as corn supplementation increased. Digestion of NDF decreased cubically with supplementation. Ammonia nitrogen concentration decreased linearly also with provision of ground corn. This suggested that the starch-utilizing bacteria were capturing RAN to the exclusion of fiber-utilizing bacteria. Horn and McCollum (1987) discussed this effect in subsequent research. According to their theory, fiber digesting bacteria without adequate RAN were less productive, thus explaining the decreases in NDF digestion.

A “carbohydrate effect” also could partially explain the decrease in fiber digestion. Discussed by Arroquy et al. (2005), the carbohydrate effect was classified as a depression in

fiber digestion by NSC supplementation independent of any pH effects. They stated that rumen microbes have an affinity for readily digestible sources of nutrients. When offered a greater vs. a less digestible source of nutrients, the microbes acted upon the more digestible source of nutrients more readily.

Ruminal pH can have a negative impact forage digestion. Rapid digestion of grains occurs in the rumen, leading to swift decreases in ruminal pH. A pH below 6.2 can inhibit the productivity of cellulolytic bacteria (Mould et al., 1983); however, for a number of trials that evaluated energy supplementation in low-quality forage diets (Olson et al., 1999; Krysl et al., 1989; Pordomingo et al., 1991), pH never strayed below the 6.2 threshold.

A number of researchers have tried to ameliorate the depressing effects of low RAN when supplementing NSC. DelCurto et al. (1990) fed ruminally-cannulated steers two levels of supplemental protein and two levels of energy within each level of protein. At the low level of supplemental protein, provision of the high level of energy (18.4 kcal ME/kg BW daily) depressed forage intake and NDF digestion. Overall dry matter (DM) digestion increased when readily digestible carbohydrates were fed. When provided the high level of supplemental protein, the negative effects of energy supplementation were ameliorated. No differences existed between the two levels of energy supplementation within the high protein level, but forage intake improved numerically at the high energy level. Even with a small change in energy level, NDF digestibility still decreased.

Olson et al. (1999) extended this line of research by evaluating two levels of starch supplementation and four levels of protein within each level of starch. Animals received a low quality forage diet (4.9% CP). Starch feeding levels were 0, 0.15, or 0.30% of BW per day. Levels of protein supplementation were 0, 0.03, 0.06, 0.09, or 0.12% of BW per day. Within

each level of starch, forage DM and NDF intakes increased with increasing supplemental protein. With increasing levels of starch, forage DM and NDF intakes were depressed. Increasing starch depressed total tract digestion of NDF. Within each level of starch, increasing protein increased NDF digestion. Not even the highest level of protein (0.12% of BW/d) within each level of starch returned NDF digestion to levels similar to protein supplementation alone. Total tract digestion of DM and OM were most affected at the high level of starch supplementation. At the low level of starch, increasing levels of supplemental protein did not improve digestion to the levels observed for the control animals.

Klevesahl et al. (2003) studied the effects of a wide range of protein levels on forage intake and digestion when supplementing 0.30% of BW/d of starch to a forage-based diet. Fourteen ruminally-cannulated steers were used in a two-period, 14-treatment study. Animals were given one of seven levels of DIP (0, 0.015, 0.051, 0.087, 0.123, 0.159, or 0.195% of BW DIP per day) and received starch in either period one or two. The diet consisted of grass hay (4.9% CP). Intraruminal dosing of DIP occurred once daily for all steers.

Both the DIP and the starch had independent effects on forage OM and NDF intake; there were no significant DIP x starch interactions for intake. Starch depressed intake, whereas DIP yielded a quadratic response. Up to 0.123% of BW/d of supplemental DIP increased intake of forage OM and NDF, but intakes were decreased relative to maximum at levels above this. A significant DIP x starch interaction occurred for total tract NDF digestion. With no supplemental DIP and 0.30% of BW/d starch, NDF digestion was ~30%. As level of DIP increased, so did NDF digestion, up to ~50%, the level observed with DIP supplementation only. Starch without any supplemental DIP resulted in a depression of total tract NDF digestion by 20 percentage

units. Supplemental DIP increased NDF digestion by approximately 5 percentage units in the animals that did not receive starch.

Supplemental starch decreased rumen pH, but the lowest pH was 6.33, greater than the proposed pH of 6.2 for inhibition of cellulolytic bacteria (Mould et al., 1983). Total VFA and ammonia concentrations increased linearly with increasing DIP. The VFA profile exhibited some interesting differences. Acetate concentration decreased with the inclusion of starch in the diet. Concurrently, concentration of propionate and butyrate increased. Supplemental DIP linearly increased the proportions of isobutyrate, valerate, and isovalerate.

In the aforementioned studies, starch was the source of supplemental energy. Other research has evaluated the effects of different types of NSC on forage intake and digestion. Heldt et al. (1999b) looked at this effect through a series of trials. The first trial utilized 13 ruminally cannulated steers with a 2 x 3 x 2 factorial treatment design. This included two levels of DIP (0.031 and 0.122% of BW daily) and three distinct carbohydrate sources (starch, glucose, and fiber) that were fed at two levels (0.15 and 0.30% of BW daily). Another set of steers were fed only hay to provide a baseline measurement of forage intake and digestion. Animals received their supplements once daily intraruminally.

Starch, glucose, and fiber each exhibited unique effects. Starch and DIP did not interact. The level of DIP supplemented did not affect forage OMI. However, NDF digestibility differed by approximately 6% when comparing high and low DIP supplementation. The cattle receiving high DIP treatment digested a greater amount of NDF than those receiving the low DIP treatment.

At the low level of DIP, 0.15% of BW daily as glucose supplementation did not affect forage OMI intake or NDF digestibility, but the 0.30% of BW daily level of glucose depressed

forage OMI and NDF digestion to levels below that of the negative control cattle that only received forage. However, at the high level of DIP, forage OMI and NDF digestion were significantly improved by both levels of glucose supplementation. This once again suggests that glucose-related depressions in ruminal fermentation are related to a deficit in RAN, because an increase in the supplementation of DIP ameliorated the depression associated with glucose supplementation.

The addition of supplemental fiber produced more variable results. At the low level of DIP supplementation, the low level of fiber increased forage OMI above that of the negative control, but the high level of fiber depressed forage OMI to a level below the negative control. The NDF digestibility at both levels of fiber supplementation was greater than when glucose was supplemented. At the high level of DIP, fiber supplementation improved forage OMI intake to a level above that observed for starch supplementation, but below that observed for glucose supplementation. NDF digestibility did not differ between supplemental glucose and supplemental fiber at the high level of DIP supplementation. NDF digestion improved when comparing the high fiber to the low fiber within the high level of DIP. This effect did not occur at the low level of DIP. Perhaps RAN was not adequate to optimize microbial function and thus depressed NDF digestibility. It is somewhat difficult to interpret NDF digestibility in response to fiber supplementation due to the fact that both the forage and supplement contribute to overall dietary NDF. NDF digestibility would be confounded in cases where the quality of the fiber supplemented was much higher than that of the basal forage in the diet. Improvements in basal forage utilization in response to fiber supplementation have been seen in other research (Highfill et al., 1987; Martin and Hibberd, 1990).

The varied sources of supplemental carbohydrates in the work of Heldt et al. (1999b) impacted ruminal fermentation characteristics. Rumen pH generally decreased with the inclusion of supplemental carbohydrates. The higher level of carbohydrate inclusion magnified this effect. Rumen ammonia levels were well below the recommendations of Satter and Slyter (1974) when animals were given the low DIP treatment. Conversely, the high DIP treatment improved ruminal ammonia concentrations. Ruminal ammonia concentrations were less for the glucose supplements than for starch and fiber treatments at the high DIP level. Possibly, the provision of supplemental glucose led to a greater uptake of rumen ammonia than the other treatments. This would make adequate DIP supplementation necessary to prevent depressions in forage intake and digestion with provision of supplemental glucose.

The VFA profile of the cattle showed differences among treatments. Across the board, supplemental DIP and supplemental energy decreased the proportion of acetate. Increasing the amount of DIP or energy magnified this effect. Generally, supplementation increased the proportion of propionate. Due to butyrate and acetate having the same precursor (acetyl CoA), any reductions in acetate concentration were followed by a concurrent increase in butyrate concentration. Glucose supplementation increased butyrate concentration more than the other energy treatments. Lactate concentration was increased by glucose treatments. Supplementation in general and increasing the level of DIP increased the molar proportions of isobutyrate, isovalerate, and valerate. Studies previously mentioned in this review (Köster et al., 1996; Olson et al., 1999) showed that DIP supplementation increased the proportion of the three minor VFA. Heldt et al. (1999b) noted in their discussion that they believed the impact of DIP to be more important than the impact of carbohydrate supplementation on the proportions of minor VFA.

Fermentation of certain amino acids produces branched chain organic acids that are growth factors for fibrolytic bacteria (Baldwin and Allison, 1983).

In a series of trials conducted by Heldt et al. (1999a), the effects of various supplemental sugars and starch fed to steers in combination with DIP were evaluated. The first trial paired an insufficient level of DIP (0.031% of BW/d) with one of four carbohydrate sources (starch, glucose, fructose, or sucrose) fed at 0.30% of BW/d. Level of supplemental DIP was increased to 0.122% of BW/d in the second trial.

In the first trial, no differences were found between starch vs. sugar, monosaccharide vs. disaccharide, or glucose vs. fructose when evaluating intake and digestion parameters (forage OMI, digestible OMI, OM digestibility, and NDF digestibility). Conversely, important differences were found when comparing all supplemented animals to the negative control. The supplemented animals all had depressions in NDF digestibility when compared to the negative control and forage OMI numerically increased with supplementation.

The greater level of DIP supplemented in the second trial led to different effects of carbohydrates on forage intake and digestion. Forage OMI increased when supplements were provided. No differences existed among carbohydrate treatments with regard to forage OMI. Organic matter digestibility also increased with supplementation. Supplemental starch led to lower OM digestibility than did supplemental sugar. Glucose and fructose supplementation led to higher OM digestibility than did sucrose supplementation. In a subsequent comparison, no differences were observed between glucose and fructose. The effects on OM digestibility were similar to those on NDF digestibility, suggesting that differences in OM digestibility among treatments were due to differences in NDF digestion. Supplementation of glucose and fructose led to much higher NDF digestion than did starch when the greater level of DIP supplement was

provided. This provided additional evidence that the shortcomings of simple sugar supplementation can be overcome with additional RAN, whereas the mechanism by which starch inhibits forage digestibility may warrant further investigation.

The effects of supplemental carbohydrates on the VFA profile of the steers were similar for both experiments. There were numeric differences, but the overall trends were the same, suggesting that effects were the result of the energy supplements. It also suggested that increasing DIP did not further alter the VFA profile. Throughout both experiments, supplementation resulted in a decrease in the molar proportion of acetate, with the decrease being larger when sugars were provided. Supplementation increased the proportion of propionate, with no differences among the energy treatments. When acetate concentration decreased, butyrate concentration increased; this effect manifested itself with sugar supplements more than with starch supplements. Results for the minor VFA (isobutyrate, valerate, and isovalerate) were mixed. Starch supplementation increased the proportion of all three minor VFA; however, the increase of isobutyrate and isovalerate were greater for starch than for any of the sugars. These somewhat unexpected results were possibly due to the sugars being supplemented in conjunction with DIP. Valerate increased significantly with provision of supplemental sugar. There were differences between starch vs. sugar and also for monosaccharide vs. disaccharide. Sugar supplementation also increased the concentration of lactate to levels much greater than those of the control or the starch treatment.

Protein to Energy Ratio

A number of researchers have proposed the concept of an ideal energy (total digestible nutrients; TDN) to protein ratio in the rumen. This approach considers the digestibility of the forage, instead of just the crude protein concentration of the forage, with the hope of finding an ideal ratio of protein to energy for maximizing efficiency of ruminal microbes. Moore et al. (1999) evaluated this ratio in a review article. Their database included 444 comparisons between unsupplemented controls and supplemented treatments. The database included various sources of forage and supplements. Forages were grouped into cool season, warm season, native grass, and straw. Supplements included high protein supplements, high energy supplements, liquid feeds, supplements containing non-protein nitrogen, by-product feeds, and plant supplements.

Supplementation had both positive and negative effects on voluntary feed intake. Intake of forages with a TDN:CP ratio of less than 7:1 generally decreased in response to supplementation, whereas forages with a TDN:CP ratio above 7:1 generally had the opposite effect. A TDN:CP ratio of less than 7:1 indicates a relatively high concentration of protein in the forage in relation to the amount of energy available. Hence, the observation that supplementation decreases voluntary intake when the TDN:CP ratio decreases below 7:1 might be expected because the protein concentration should be nearly sufficient to support the rumen ecosystem.

Moore et al. (1999) further investigated the relationship of TDN:CP ratio by exploring the effect of supplemental TDN intake on the change in voluntary forage OMI, as classified by the forage TDN:CP ratio. Generally, when forage TDN:CP ratio was less than 7:1, supplemental TDN intake almost always decreased voluntary OMI. Results for observations with a TDN:CP ratio above 7:1 were mixed. The observations were then sorted by feed type (protein, energy, molasses, and molasses plus feed) and the previously mentioned relationship examined. If

supplemental TDN intake was greater than 0.7% of BW daily, voluntary forage intake always decreased.

In summary of the review by Moore et al. (1999), supplements provided to a forage with a TDN:CP ratio of greater than 7 increased forage intake. Also, providing supplemental TDN at a level above 0.7% of BW daily decreased voluntary forage intake.

Bowman et al. (2004) explored the TDN:CP ratio in grazing cows. The first portion of their research consisted of a digestion trial utilizing cross-bred heifers fitted with ruminal cannulae. The second half of their research involved pregnant crossbred cows in a two-year grazing trial. In both trials, researchers fed increasing levels of NSC and measured effects on forage intake and digestion. Supplements in both trials were fed at three levels (0.32, 0.64, or 0.96 kg/d) with TDN:CP ratios of 9.7, 9.5, and 9.7 for the 0.32, 0.64, and 0.96 kg levels of supplementation, respectively. The diets were not iso-nitrogenous but they were designed to provide 0.34 kg/d of CP and 5.1 Mcal of ME for both trials.

The heifers in the first trial were given ad-libitum access to low-quality orchardgrass (5.5% CP) that had a TDN:CP ratio of 10:1. The cows in the second trial grazed native rangeland with a TDN:CP ratio of 7:1 in the fall after weaning. It is important to note that the heifers used in the first trial received forage considered to be deficient in protein based on the TDN:CP ratio of Moore et al. (1999), whereas the cows grazing native range had access to forage considered adequate in protein (forage TDN:CP < 7). In the second portion of the trial, two ruminally-cannulated cows were included with each treatment to facilitate the collection of forage extrusa samples via ruminal evacuation.

For the first experiment, intake of forage and total dry matter, organic matter, NDF, and CP increased with provision of supplements. There were no differences among the levels of

supplemental NSC. Total tract digestion of forage and total diet DM, OM, and NDF increased with supplementation; however, there were quadratic effects of treatments for forage DM digestion, forage OM digestion, and forage NDF digestion. Forage OMI increased up to the 0.64 kg/d-level of supplementation, along with an improvement in forage NDF digestion. At 0.96 kg/d (the only level above 0.64 kg/d), intake of forage DM and forage OM were decreased, along with a concurrent decrease in forage NDF digestion. Improvement in low-quality forage utilization when high-fiber supplements were provided to beef cattle was previously demonstrated (Heldt et al., 1999a; Highfill et al., 1987; Martin and Hibberd, 1990).

As previously mentioned, the cattle used in the grazing trial of Bowman et al. (2004) had access to forage considered adequate in protein based on the forage TDN:CP ratio. Collection of the ruminal extrusa from cows in each treatment group yielded no significant differences in composition. There was variation in forage quality between the two years (5.1% CP in year one vs. 6.2% CP in year two) but there were no interactions between year and treatments.

For the first year of the grazing trial, there were distinct linear effects of NSC level on forage and dietary intake measurements. Increasing supplemental NSC decreased intake of forage and total diet DM, OM, NDF, and CP. Interestingly, at the highest level of NSC supplementation, the dietary intake of CP was still greater than that of the non-supplemented control, yet intakes of DM, OM, and NDF were decreased, suggesting that NSC supplementation was having a deleterious effect on forage intake.

As previously mentioned, forage quality was greater in year two of the trial than in year one. Increasing the amount of supplemental NSC linearly decreased both forage and total dietary intake of DM, OM, NDF, and CP. Intake of forage OM the cows that received 0.96 kg/d of NSC decreased by 68% relative to the unsupplemented control. The authors attributed the

decrease in forage utilization to the supplements, postulating that the TDN:CP ratio of the supplements was sufficiently high to create a imbalance in the energy to protein ratio in the rumen. This, in turn, would have limited the effectiveness of the ruminal microbial population.

Effect of VFA Infusions on Rumen Function

Ruminal fermentation produces VFA as products. Ruminant nutritionists have known about the role of VFA in providing energy to ruminants for over 50 years. The major VFA of interest in ruminant nutrition are acetate, propionate, and butyrate. VFA are the primary forms of energy ruminant animals receive from their symbiotic partners. Many feedstuffs are degraded to some extent in the rumen, meaning that the fundamental composition of feeds changes between intake and absorption. A large body of research exists on the many facets of VFA production and utilization.

Early work in this area centered on the roles of VFA on the regulation of feed intake in ruminants. Montgomery et al. (1963) infused either acetic (870 g/d), propionic (280 g/d), butyric (260 g/d), or lactic acid (340 g/d) into the rumen of dairy cows. The acids were diluted with 4 liters of water and infused over 4 hours daily. Cows were fed alfalfa-bromegrass hay. Hay intake was decreased by infusions of acetic acid (35% decrease) and butyric acid (17% decrease). Ruminal pH was not affected by treatment; the lowest pH recorded in their measurements was 6.5. Blood metabolites were measured in an attempt to identify a marker for intake inhibition. Increases in blood ketone and decreases in blood urea concentrations occurred in response to acetic acid infusion.

Simkins et al. (1965) also studied the effect of VFA infusions on feed intake by cattle. Their research used cattle fed either pelleted or chopped alfalfa. Isocaloric amounts of acetic acid, propionic acid, butyric acid, or a mixture of the three acids (70% acetate, 15% propionate,

15% butyrate) were infused intraruminally. The infusions were balanced to provide 15% of each animal's daily digestible energy requirement. This led to differences in the actual amounts infused into the cows. Treatments were infused over a 5-hour period for three days. For the cows consuming alfalfa pellets, acetate infusion caused a greater decrease (-30%) in intake relative to other infusions. There were no differences in forage intake when cattle consuming chopped alfalfa were provided the same VFA infusions.

Simkins et al. (1965) also measured blood metabolites in hope of finding an explanation for the decreases in intake. Blood sugar, ketone, VFA, acetate, propionate, and butyrate concentrations were measured from a jugular blood sample taken from each animal. Infusion of butyrate increased ketone and decreased sugar concentrations in the blood. Propionate infusion increased blood sugar concentrations. The VFA mixture decreased ketone concentrations. Based on the inconsistent effects of VFA infusion on the animal intakes and metabolite concentrations, Simkins et al. (1965) were unable to propose a suitable mechanism by which VFA infusions decreased intake of the basal diet.

Infusion of VFA above normal physiological levels may be the explanation for the decreases in intake reported in early research. Papas and Hatfield (1978) conducted a series of experiments to investigate the role of VFA infusions on decreases in feed intake. VFA were administered into the abomasum of sheep in a variety of concentrations. In their first experiment, six treatments were used: 1) water, 2) 60 g sodium acetate, 3) 40 g sodium propionate, 4) 44 g acetic acid, 5) 31 g propionic acid, and 6) 25.8 g butyric acid. VFA in acid form decreased intake, whereas VFA salts had no effect on intake. This led the authors to blame the molar amounts of acid infused for depression of intake. To prove this, the authors conducted another trial where each animal received 0.5 mole (diluted to 750 mL with water) of acetate,

propionate, butyrate, or hydrochloric acid per day. All treatments decreased intake relative to control, but urine pH did not decrease. In a subsequent trial, the animals received twice the molar amount of VFA as in the initial trial. The lambs receiving acid reduced their feed intake to less than 100 grams per day and developed metabolic acidosis. The authors concluded that mass production (or infusion) of VFA into the rumen upsets the acid-base balance of the body, leading to a number of systemic issues.

As observed in previous research, VFA can be provided to animals in one of two forms; in acid form or as sodium salts. Each method has its own challenges. In the acid form, a large dose of VFA will decrease rumen pH and potentially may cause a litany of digestive issues. The VFA salts will increase rumen osmolality. Increased osmolality by the addition of NaCl has been shown to decrease VFA absorption from the rumen (Lopez et al., 1994). Normally, osmotic pressure is lower in the rumen than in the blood, which allows for water to be absorbed from the rumen. If the osmotic pressure in the rumen rises above that of the blood, then water will be moved from the blood into the rumen.

Lopez et al. (2003) studied the effects of VFA supply on VFA absorption and on water kinetics in sheep maintained by intragastric infusions. Sheep were given one of three VFA infusion rates intraruminally. VFA, buffer, and macro minerals were infused ruminally while casein was infused into the abomasum. On data collection days, water and casein were withheld from the animals and VFA were infused at elevated levels (0.5, 1.4 and 1.8 times the basal infusion) within the solution. A marker for the ruminal liquid phase and total ruminal volume were used. Absorption of VFA from the rumen increased as the concentration of VFA in the infusion increased. Absorption from the rumen was less than the rate of infusion, such that VFA

accumulated. This illustrated the risk associated with cattle consuming large meals of carbohydrates that can be rapidly fermented to VFA.

Intraruminal infusions of VFA that are within the physiological range produced by common diets and intake levels can still limit intake. Propionate, infused within the physiological production range, consistently decreases feed intake by cattle. Diets rich in starch increase propionate concentration in the rumen. Ruminal propionate infusion decreased feed intake in dairy cows (Oba and Allen, 2003). In each of two experiments, they infused 8 different mixtures of acetate and propionate into the rumen of lactating dairy cows. The free-acid form of VFA were used in the first experiment, whereas VFA salts were used in the second experiment. Dry matter intake decreased with increasing proportion of infused propionate during both experiments. Total diet metabolizable energy (ME) intake also decreased with increasing infusion of propionate, in spite of the fact that ME content of the infusate increased with as concentrations of propionate in the infusate increased. This suggested that propionate did not play a role in regulating feed intake by cattle. Previous researchers suggested that the hypophagic effects of propionate were due to relatively high energy yielded though oxidation of propionate compared to other VFA.

Causing an aversion to the diet can be another possible mechanism by which VFA decrease voluntary feed intake. Research conducted by Ralphs et al. (1995) showed that gavage of animals with glucose or VFA had different effects on diet preferences. Each morning, sheep were offered straw with one of two unique flavors for 15 minutes. Animals were then ruminally gavaged with 200 mL of either a glucose or propionate solution which provided 13% of the daily energy requirement for the first experiment and 26% in the second experiment. After the

gavage, animals were allowed access to feed for 45 minutes. A reduction in intake during the 45-minute feeding period was judged to indicate satiety.

For both experiments, glucose had no effect on hay intake. Propionate decreased intake in both experiments, with reductions being greater when the sheep received the larger dose of propionate. Propionate also caused aversions to both flavors of straw. For the last two days of each period, animals were offered a choice between both flavors. In the prior days of each period, only one flavor was given in combination with energy treatments. Animals always avoided the flavor that was associated with propionate, leading the authors to conclude that the propionate treatment in their second experiment caused a negative post-ingestive consequence in the animals due.

Villalba and Provenza (1997) investigated the role of VFA in feed preferences. They conducted four experiments to determine if different levels of VFA would cause the animals to develop preferences for or aversions to flavored feeds. In the first experiment, sheep were given one of four doses of sodium propionate (0, 4, 8, or 12% of daily DE requirement). Sheep used in the second experiment received one of four doses of sodium acetate at the same levels of daily DE requirement. Provision of sodium chloride intraruminally tested the effect of increased osmolality on intake in a third experiment. In the fourth trial, various combinations of acetate and propionate were administered to the sheep to discern preferences for either VFA.

In the first trial, intake of straw was depressed with provision of propionate at 8 and 12 percent of daily DE requirement. There were no differences in intake between the control and 4% level. In contrast, acetate did not have an effect on intake at gavage rates of 4 or 8% of DE but the 12% gavage rate of acetate depressed intake. Gavaging the sheep with only NaCl did not

have any effect on intake of straw. Animals given VFA in the fourth trial increased intake compared to those given either water or NaCl.

A reduction in intake is not the only response to ruminal infusion VFA. Increasing VFA concentration in the rumen is associated with increased permeability to urea. Houpt and Houpt (1968) studied the transfer of urea-N across the ruminal wall. Using Pavlov pouches and two-balloon catheters, various concentrations of urea were injected into the jugular vein. The pouches contained fluid of varied ruminal ammonia concentrations. The pouches were treated with anti-microbial agents to inhibit urease activity. With the inhibition of urease, transfer of urea across the rumen wall related directly to the concentration difference between blood urea and rumen fluid. Without urease inhibition, urea entering the Pavlov pouch quickly was hydrolyzed to ammonia. Hydrolysis of urea to ammonia resulted in an increase of transfer of urea from the blood to the rumen. Kennedy and Milligan (1978) corroborated these findings.

Remond et al. (1993) measured the net transfer of urea and ammonia across the ruminal wall of sheep. They were interested in correlating blood flow and transfer of urea into the rumen. Sheep were fed a constant amount of orchardgrass hay daily. Animals received pulse doses of acetohydroxamic acid (a known urease inhibitor), butyric acid, ammonia, or sodium chloride during the end of each period of the trial. Carbon dioxide bubbled into the rumen was an additional treatment to mimic increased gas production. Ammonia absorption and blood flow to the rumen were measured, with correlations made between the two measurements. Ammonia absorption from the rumen increased with butyrate and CO_2 treatments.

Acetohydroxamic acid decreased urease production in the rumen, leading to a decrease in rumen ammonia concentration. Intraruminal ammonia injection increased the net transfer of ammonia but had no effect on blood flow to the rumen. Conversely, NaCl increased ruminal

osmolality and blood flow to the rumen. Concurrently, the net transfer of ammonia was decreased. Carbon dioxide gas decreased concentration of VFA in the rumen. Net absorption of ammonia and blood flow to the rumen increased over time when the CO_2 treatment was applied but the net transfer of urea decreased, leading the authors to postulate that blood flow is not related to net urea of transfer to the rumen, thus corroborating the findings of Dobson et al. (1971).

As rumen microbial activity increases, uptake of nitrogen by microbes in the rumen increases, allowing the digestibility of a diet to have an impact on urea kinetics in cattle. Increased VFA production and increased VFA concentrations in the rumen correspond with increased permeability of the ruminal epithelium to urea.

Kennedy (1980) investigated the effects of sucrose supplementation on the degradation of urea in cattle. Cattle were fed alfalfa hay with sucrose supplemented at either 0.5 or 1.0 kg/d. Urea kinetics were determined by intravenous infusion of ^{14}C-urea. Provision of sucrose in the diet increased urea entry into the rumen by 35%. Production of microbial N improved with provision of sucrose, leading to lesser ruminal ammonia concentrations in those steers.

Norton et al. (1982) measured urea synthesis and degradation in sheep fed grass hay pellets and supplemented with flaked barley. Sheep were fed either 1 kg of pelleted grass cubes or 0.7 kg of pelleted grass cubes and 0.3 kg of flaked barley daily. Urea kinetics were measured with intravenous infusion of ^{14}C-urea. Flaked barley supplementation increased the concentration of butyrate in the rumen but had no effect on overall VFA concentration. The amount of recycled urea utilized in the rumen increased with provision of barley. Flow of urea into the rumen increased also among sheep fed flaked barley.

Conclusion

Maximum performance and efficiency in the rumen cannot be achieved without the supplementation of protein to low-quality diets. The provision of supplemental energy can have detrimental effects on forage digestion without adequate provision of protein. Supplementation with readily digestible carbohydrates or VFA infusions will have a number of effects on ruminal function and nitrogen metabolism in ruminants. The mechanisms by which these effects are mediated require further study.

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CHAPTER 2 - EFFECTS OF SUPPLEMENTAL ENERGY AND PROTEIN ON FORAGE DIGESTION AND UREA KINETICS IN GROWING BEEF CATTLE[1]

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Abstract

We quantified effects of supplemental energy from differing sources on nutrient digestibility and urea kinetics at 2 levels of degradable intake protein. The study was a 6 × 6 Latin square with treatments arranged as a 3 × 2 factorial. Energy treatments included: control, 600 g glucose dosed ruminally once daily, and 480 g VFA (192 g acetic acid, 144 g propionic acid, 144 g butyric acid) infused ruminally over 8 h daily. Casein (120 or 240 g) was dosed ruminally once daily. Six ruminally- and duodenally-cannulated steers (208 kg) had *ad libitum* access to prairie hay (5.8% CP). We infused $^{15}N^{15}N$-urea intravenously to measure urea kinetics. Infusion of VFA decreased ($P < 0.01$) forage intake by 27%; decreases in forage intake due to glucose (7%) and increases due to increasing casein (4.5%) were not significant. Dosing glucose decreased total tract NDF digestibility ($P < 0.01$) and tended to decrease ruminal NDF digestibility; depressions in response to glucose tended to be greater at the lower level of casein. Increasing casein decreased ruminal pH ($P < 0.02$). Infusion of VFA decreased pH during the infusions, but not at other times, whereas glucose decreased pH 2 h after dosing. Ruminal concentrations of NH_3, acetate, and propionate decreased, whereas those of butyrate increased, when glucose was supplemented; glucose may have exacerbated a ruminal NH_3 deficiency. Increasing casein increased ($P < 0.01$) ruminal concentrations of NH_3, acetate, propionate, isobutyrate, and isovalerate. Supplemental energy decreased plasma urea-N concentration ($P = 0.03$), whereas casein level did not affect it ($P = 0.16$). Microbial N flow was greater ($P < 0.04$) for 240 g/d than for 120 g/d casein but it was not affected by supplemental energy ($P = 0.23$). Urea-N entry rate (UER) and gut entry of urea-N (GER) were not affected ($P \geq 0.12$) by supplemental energy or casein, but the proportion of UER that was recycled to the gut was less when 240 g/d rather than 120 g/d casein was provided ($P = 0.01$). When compared to VFA, glucose tended ($P = 0.07$) to increase GER/UER. Supplementation with glucose led to more (*P*

= 0.01) microbial uptake of recycled urea than VFA. The lack of treatment effects on urea production, particularly in response to increased N supplied as casein, may reflect that the complete diets never provided excessive amounts of N and that increased provisions of intestinally-available AA were used efficiently by cattle for protein deposition.

Key words: cattle, supplementation, urea recycling

Introduction

Urea recycling is important to cattle grazing forages deficient in protein (CP < 7%). Wickersham et al. (2008) established that even when cattle consuming low-quality forage received adequate protein, they recycled large proportions (~95%) of urea production to the gastrointestinal tract. Supplementing cattle consuming low-quality forage with non-structural carbohydrates (NSC) is a strategy to increase energy intake by cattle but NSC supplementation without adequate protein content in the diet has detrimental effects on forage utilization (Heldt et al., 1999; Olson et al., 1999; Klevesahl et al., 2003). In contrast, NSC supplementation reportedly increases microbial capture of recycled urea-N (Kennedy, 1980). Therefore, we wanted to investigate how differing sources of supplemental energy (to the animal vs. to the ruminal microbes) affected urea kinetics, forage intake, forage digestion, and the efficiency of N capture by ruminal microbes. Ruminal glucose should stimulate microbial growth, leading to an increase in the microbial cell protein supply to the animal. Conversely, no increases in the protein supply to the animal is expected when VFA are provided ruminally. Infusion of VFA has been associated with increased permeability of the rumen wall to urea (Norton et al., 1982); it has also been shown to increase blood flow to the rumen wall (Sellers et al., 1964).

Our hypothesis was that NSC supplementation would increase microbial growth and exacerbate a ruminal N deficiency, which would increase the amount of urea recycled to the rumen. In addition, we hypothesized that adding VFA to the rumen would increase the amount of urea recycled to the rumen by increasing the permeability of the rumen epithelium to urea. For this work, we supplemented protein at 2 levels, an amount observed to maximize forage intake and digestion (240 g/d; Heldt et al., 1999) and a deficient amount (120 g/d), to measure the effects of adequate and inadequate dietary N on urea kinetics.

Materials and Methods

All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee at Kansas State University.

We studied the effects of providing supplemental protein and energy to growing beef steers consuming low-quality forage, with emphasis on urea kinetics. Six ruminally- and duodenally-cannulated Angus steers (average initial BW = 208 ± 17 kg) were used in a 6 × 6 Latin square with dietary treatments arranged as a 2 × 3 factorial. One of 2 protein treatments (120 or 240 g of sodium caseinate, New Zealand Milk Products Inc., Auckland, New Zealand; Table 1) were pulse dosed into the rumen once daily at 0630 h. One of 3 energy treatments were superimposed on protein treatments: 1) no supplemental energy (control), 2) 600 g glucose (dextrose monohydrate, ADM Corn Processing, Decatur, IL; Table 1) pulse-dosed into the rumen once daily at 0630 h, or 3) 480 g of VFA (40% acetic acid, 30% propionic acid, and 30% butyric acid) infused intraruminally over 8 h daily beginning at 0630 h. All steers had *ad libitum* access to prairie hay (5.8% CP; Table 1) fed at 115% of the average voluntary intake over the previous 4 d.

Each experimental period lasted 14 d. The first 9 d were used for adaption to treatments and the last 5 d for sample collection. For the first 7 d of adaption, steers were housed in individual tie-stalls. For the remainder of each period, steers were placed in metabolism crates that allowed for total collection of urine and feces and facilitated intravenous infusion of labeled urea. At 0630 h on d 10 through d 13 of each period, each metabolism crate had a clean bucket containing 900 mL of 10% (wt/wt) H_2SO_4 placed under the collection funnel to facilitate complete collection of urine from each steer. The acid maintained the pH of the urine below 3 to prevent ammonia volatilization. Feces were collected into a metal bin lined with plastic from d 10 through 13 of each period.

Intake, digestion, and N balance were measured from d 10 through 13. Samples of hay (400 g) were collected on d 9 through 12 and composited within each period to correspond with urine and feces collected from d 10 through 13. Orts were collected just before the daily feeding and orts from d 9 to 12 were composited for each steer. Samples of casein and glucose were collected once each period. Feces and urine collected over a 24-h period were removed each day at 0630 h and sampled. Samples of both feces (5% of total amount collected) and urine (1% of total amount collected) were composited within animal for each period. Two sets of urine samples were collected; one for determination of N balance and another for purine derivative analysis. The urine to be used in purine derivative analysis was diluted 5/1 with 0.05 *M* H_2SO_4.

At 4 h after feeding (1030 h) on d 10, blood (10 mL) was collected by jugular venipuncture into heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed in ice and subsequently centrifuged at 1,200 × *g* for 15 min within 1 h of collection. Plasma was isolated from blood and frozen.

An indwelling ear catheter was placed in each steer on d 10 to allow for infusion of $^{15}N^{15}N$-urea to measure urea kinetics. Sterile saline was infused continuously from the time each catheter was placed until 0630 h on d 11 of each period when infusion of $^{15}N^{15}N$-urea solution began through use of a programmable syringe pump (BS-9000 Multi-Phaser, Braintree Scientific, Inc., Braintree, MA). Label infusion continued through the end of each period. The $^{15}N^{15}N$-urea solution was prepared by combining 3.6 g of $^{15}N^{15}N$-urea (99% $^{15}N^{15}N$-urea, Medical Isotopes, Inc., Pelham, NH) with 1 L of sterile saline solution (0.9% NaCl). The solution was filter sterilized (0.22 µm filter, Sterivex, Millipore Corporation, Billeric, MA), bottled in glass containers, and stored at 4°C. The infusion rate was 4.16 mL/h.

Feces (500 g) and urine (100 mL) collected from d 10 were used for measuring background concentrations of ^{15}N. Feces (500 g) and urine (100 mL) collected on d 13 were used to measure the ^{15}N enrichment at plateau (Wickersham et al., 2009).

On d 14 of each period, samples of rumen and duodenal fluid were collected every 4 h for 24 h beginning 2 h after feeding. Whole rumen contents (1.2 L) were collected from each animal to isolate ruminal bacteria. Contents were first strained through 4 layers of cheesecloth, and the liquid portion was collected and analyzed immediately for pH. An 8-mL sample of ruminal fluid was combined with 2 mL 25% (wt/wt) meta-phosphoric acid and frozen for subsequent analysis of VFA. Another 20-mL sample of rumen fluid was mixed with 2 mL of 6 *M* HCl and frozen for later analysis of ammonia. The remaining fluid and all solids were mixed with 1.0 L of 0.9% (wt/vol) NaCl, blended (NuBlend, Waring Commercial, Torrington, CT) for 1 min and strained through 4 layers of cheesecloth with all liquid collected and frozen for later isolation of bacteria. Bacterial samples collected during each period were composited within animal. Duodenal fluid (300 mL) was collected from each steer concurrent with ruminal samples and was pooled within animal for later analyses.

Laboratory Analyses

The partial DM of hay, ort, and fecal samples were determined by drying in a forced-air oven at 50 °C for 72 h. Duodenal samples were lyophilized. Samples of hay, ort, fecal, and duodenal digesta were ground through a 1-mm screen with a Wiley mill. The DM content of hay, ort, fecal, and duodenal samples as well as casein and glucose was determined by drying for 24 h at 105°C in a forced-air oven and ash content was determined by heating for 8 h in a muffle oven at 450°C.

Hay, ort, fecal, and duodenal samples were analyzed for NDF (without amylase and without ash correction) and non-sequential ADF using an ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY). To determine acid detergent insoluble ash of fecal and duodenal samples, ANKOM bags containing ADF residues were combusted for 8 h at 450°C in a muffle oven. Protein concentration of casein, hay, ort, duodenal, wet feces, and urine samples was determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation, St. Joseph, MI). Crude protein was calculated as N × 6.25.

Ruminal bacteria were isolated from thawed ruminal samples by centrifuging at 500 × *g* for 20 min to remove protozoa and feed particles, centrifuging the supernatant at 20,000 × *g* for 20 min, resuspending the pellet with saline (0.9% NaCl), and centrifuging again at 20,000 × g for 20 min. The bacterial pellet was frozen and lyophilized.

Concentrations of urinary urea (Marsh et al., 1965) and ammonia (Broderick and Kang, 1980) were determined colorimetrically using an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL). Measurement of ^{15}N enrichment in urinary urea was conducted according to Brake (2009). The ^{15}N enrichments of ruminal bacteria, dried feces, and duodenal samples were measured using a stable isotope elemental analyzer (ThermoFinnigan Delta Plus, Thermo Electron Corporation, Waltham, MA).

Concentrations of allantoin, uric acid, and creatinine were determined in composited (d 10 through 13) urine samples by reverse-phase HPLC as described by Brake (2009).

The method of Vanzant and Cochran (1994) was used to measure VFA in ruminal fluid by GLC. Measurements of ruminal ammonia (Broderick and Kang, 1980), plasma urea-N (PUN; Marsh et al., 1965), plasma creatinine (Chasson et al., 1961), and plasma glucose (Gochman and Schmitz, 1972) were accomplished using an AutoAnalyzer (Technicon Analyzer II). Plasma AA

were analyzed by GLC using a GC-FID Free Amino Acid Analysis Kit (EZ:faast, Phenomenex, Torrance, CA).

Calculations

Flows to the duodenum were calculated by dividing the fecal output of acid detergent insoluble ash by the acid detergent insoluble ash concentration in duodenal digesta. Microbial N flow to the duodenum was calculated using 2 methods. The first method ("measured") consisted of multiplying duodenal N flow by the ratio of duodenal ^{15}N enrichment to bacterial ^{15}N enrichment (Wickersham et al., 2009). The second method ("estimated") used the methods of Chen and Gomes (1992) to predict microbial N flow from urinary purine derivative excretion. Flow of undegraded intake protein to the duodenum was the difference between total N flow and measured microbial N flow. Microbial N derived from recycled urea was calculated by multiplying measured bacterial N flow by the ratio of bacterial ^{15}N enrichment to ^{15}N enrichment of urinary urea (Wickersham et al., 2009). Urea kinetics were calculated using the methods of Lobley et al. (2000).

Statistical Analysis

Two steers had very low forage intakes when they were provided the VFA infusions with either level of casein, and the response appeared to be due to the VFA treatment. Treatments were discontinued such that observations were not collected from these 2 steers for either of the VFA-containing treatments; data was collected from those 2 steers for the other 4 periods. The urea kinetics data from 1 steer (control plus 240 g/d casein) were removed as outliers (studentized residual for urea entry rate > 3).

Data were analyzed as a Latin square design with factorial treatments using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included energy, casein,

energy × casein, and period. Steer was a random effect. Orthogonal contrasts were used to delineate differences among energy treatments. These included 1) supplemental energy vs. control, and 2) glucose vs. VFA. Treatment means were calculated using the LSMEANS option. Ruminal measures were analyzed as repeated measures with a model containing terms for energy, casein, energy × casein, hour, hour × casein, hour × energy, and hour × casein × energy. Steer was included as a random term. The repeated term was hour with steer × period as the subject. We utilized a split-plot analysis to make comparisons between the measured microbial N flow to the duodenum and the values predicted by the equation of Chen and Gomes (1992). Significance was declared when $P \leq 0.05$ and tendencies when $0.05 < P \leq 0.10$.

Results

Provision of supplemental energy decreased forage OM intake ($P < 0.01$; Table 2), with a greater decrease for VFA treatment than glucose ($P < 0.01$). There was a tendency for ruminal NDF digestibility to decrease when supplemental energy was provided ($P = 0.09$) but provision of 240 g/d casein ameliorated this effect (energy × casein, $P = 0.05$). Total tract NDF digestibility decreased when energy was supplemented ($P = 0.03$) but the effect of glucose was greater than that of VFA ($P = 0.04$). Provision of additional casein increased total tract digestibility of NDF by 5.4 percentage units when glucose was supplemented but reduced it by 3 percentage units when VFA were provided ([Glucose vs. VFA] × casein, $P = 0.07$). Total digestible OM intake tended ($P = 0.08$) to increase with casein supplementation but it was less when VFA was supplemented than when glucose was supplemented ($P = 0.03$).

Forage N intake was decreased when supplemental energy was provided, reflecting the differences in forage intake among treatments. Fecal N excretion was less when VFA were infused instead of glucose ($P = 0.01$); moreover, it increased with level of casein supplementation ($P = 0.02$). Urinary N excretion increased with level of casein ($P = 0.01$) and was less for glucose than for VFA supplementation ($P < 0.01$). Purine derivative excretion in the urine was not different among energy treatments ($P \geq 0.89$) but increasing casein increased urinary purine derivative excretion. Compared to glucose, N retention was less when VFA were infused ($P = 0.04$) but N retention increased ($P = 0.01$) as casein increased.

Total N flow to the duodenum increased numerically ($P = 0.15$) with casein supplementation but was not affected by the energy supplementation (Table 3). Microbial N flow increased ($P = 0.04$) with additional casein but was not impacted by energy supplementation ($P \geq 0.23$). Infusion of VFA increased ($P = 0.04$) the efficiency of microbial N

synthesis when compared to the glucose treatment. The predictions of microbial N flow based on urinary purine derivative excretions (Chen and Gomes, 1992) underestimated ($P = 0.04$) microbial N flow by approximately 10%, with no interactions between treatments and method of calculating microbial N flow ($P \geq 0.39$).

Capture of recycled urea by rumen microbes was greater ($P = 0.01$) when glucose was provided rather than VFA. Increasing casein supplementation decreased the percent of microbial N flow that was from recycled urea. Providing supplemental energy increased it, with the largest response observed for glucose when 120 g/d casein was provided.

Urea-N entry rate (UER) and gastrointestinal tract entry rate of urea-N (GER) were not affected ($P \geq 0.12$) by supplemental energy, casein, or interactions between them (Table 4). Urinary urea-N excretion increased when casein was increased ($P < 0.01$). Glucose infusion also decreased urinary urea-N excretion when compared to VFA ($P = 0.01$). The amount of urea-N returned to the ornithine cycle was not affected by treatments. Energy supplementation tended (energy × casein interaction, $P = 0.08$) to increase the amount of urea-N utilized for anabolism when 120 g/d of casein was supplemented but not when 240 g/d of casein was provided. Urea-N excreted in feces increased with increasing casein ($P < 0.01$) and was greater for the VFA treatment than for the glucose treatment ($P = 0.01$).

The proportion of UER that was recycled to the gut (GER/UER) decreased as more casein was supplemented ($P < 0.01$) and tended to be less for VFA than glucose ($P = 0.07$). The proportions of GER returning to the ornithine cycle or being used for anabolism were not different ($P \geq 0.21$) among treatments. The proportion of GER excreted in the feces was increased ($P = 0.01$) with increasing casein and tended ($P = 0.06$) to be greater for VFA than for glucose.

Plasma urea-N decreased ($P = 0.03$) when supplemental energy was provided and the decrease was numerically greater ($P = 0.12$) for glucose than for VFA (Table 5). Increasing casein supplementation led only to numeric increases ($P = 0.16$) in PUN. Plasma glucose concentrations were not affected by treatments. Concentration of creatinine in plasma decreased ($P = 0.01$) with additional casein and decreased ($P = 0.05$) with provision of supplemental energy, with a tendency for a greater decrease ($P = 0.07$) in steers given VFA than for steers given glucose.

Creatinine clearance increased ($P \leq 0.03$) with both supplemental energy and casein, with the increase for VFA-infused steers being greater ($P = 0.01$) than for glucose-supplemented steers. Increasing supplemental casein increased urea clearance ($P = 0.01$). In addition, urea clearance was greater ($P = 0.01$) for VFA than for glucose. Urea clearance as a proportion of creatinine clearance was greater ($P = 0.01$) when more casein was supplemented and tended to be less ($P = 0.06$) for steers receiving glucose than for those receiving VFA.

Ruminal fermentation products were measured throughout the day (Table 6). Ruminal pH was lower ($P = 0.01$) for the steers receiving glucose at 2 and 6 h after feeding than for control. In contrast, ruminal pH was lower for control than for glucose-supplemented steers 14 h after feeding (Figure 1). Infusion of VFA caused a decrease in ruminal pH during the time of infusion but rebounded after infusions subsided (Figure 1).

Ruminal ammonia was less ($P = 0.01$) when steers received supplemental energy, a result of lesser concentrations of ruminal ammonia for steers receiving glucose than for those receiving VFA (Figure 2). Infusing VFA did not affect rumen ammonia concentration, as demonstrated by the similarities between the control and VFA treatments (for both 120 and 240 g/d casein) across time (Figure 2). Providing additional casein increased ($P = 0.01$) ruminal ammonia

concentration across all energy treatments; however, this increase was dependent on both energy treatment and time relative to supplementation (energy × casein × hour interaction, $P = 0.05$, Figure 2).

Ruminal acetate concentrations were increased ($P < 0.01$) by increasing casein supplementation (Table 6). Averaged over time, energy supplementation decreased ($P = 0.01$) acetate concentrations; however, the impact of glucose supplementation on acetate concentrations was dependent on time. For steers receiving glucose, acetate concentrations were similar to those of control steers at 2 and 6 h after supplementation but then decreased (energy × hour interaction, $P = 0.01$) steadily throughout the day (Figure 3). Across all time points, steers given VFA infusions had lower ($P = 0.01$) acetate concentrations than control steers.

Ruminal propionate concentrations were increased ($P < 0.01$) by increasing casein supplementation (Table 6). There were marked increases in ruminal propionate concentrations during VFA infusions but after infusions ceased, propionate concentration decreased to a level similar to glucose and control (energy × hour interaction, $P = 0.01$; Figure 4).

For control steers, ruminal butyrate concentration stayed relatively static throughout the sample day (Figure 5). Provision of VFA and glucose led to elevated rumen butyrate concentrations at time points close to infusion but decreased at time points distant from infusion (energy × hour interaction, $P = 0.01$; Figure 5). Casein supplementation level did not affect ($P = 0.18$) ruminal concentrations of butyrate.

Increasing casein supplementation increased ($P = 0.01$) the concentrations of isobutyrate, valerate, and isovalerate in the rumen (Table 6). Providing supplemental energy decreased ($P = 0.01$) concentrations of the branched chain VFA isobutyrate and isovalerate, with the decreases being greater for glucose than for VFA ($P = 0.01$). For isobutyrate, valerate, and isovalerate,

many interactions between treatment and time were significant; however, these interactions reflected the magnitude of change over time rather than a change in temporal pattern. Thus, we concluded that treatment responses could be adequately described by averages over time.

Discussion

Forage Intake and Digestibility

Supplemental energy had deleterious effects on forage OM intake and total tract NDF digestion (Table 2). Ruminal glucose supplementation decreased fiber digestion when the lower level of casein was supplemented but this response largely disappeared when the greater level of casein was supplemented. Thus, the lower level of casein did not meet protein needs to maximize forage digestion for the steers receiving glucose. Although direct comparisons among studies are difficult due to differences in treatment structure, these observations are similar to those of Heldt et al. (1999) and Arroquy et al. (2004) who fed prairie hay diets supplemented with amounts of glucose and casein similar to ours. In our study, forage intake of calves provided with glucose was not affected by protein supplementation, which contrasts with the results of Heldt et al. (1999) and Arroquy et al. (2004). For the steers receiving no energy supplementation, the increases in forage intake in response to increasing casein supply suggested that the low level of casein was inadequate to maximize forage intake. A similar response was noted by (Wickersham et al., 2008),

Infusion of VFA decreased forage OM intake but had only modest effects on NDF digestion when compared to the control treatment (Table 2). Cattle receiving VFA had lesser DMI than cattle receiving glucose; it should be noted that 2 steers, which were excluded from data collection, had even more severely depressed intakes when provided with the VFA infusions. Because forage digestion was not impacted by the VFA infusions, it is possible that the VFA infusion impacted physiological mechanisms that control feed intake. Perhaps steers developed an aversion to feed when infused with VFA because the infusions began concurrent with feeding each morning. The cattle may have associated consumption of forage with

discomfort, in some form, causing them to eat less. Villalba and Provenza (1997) fed artificially flavored forage and infused VFA into sheep at 0, 4, 8, and 12 percent of total daily DE intake. They found that sheep consumed less feed when the flavors paired with the 8 and 12% VFA infusions were fed, even if VFA was not concurrently infused with feeding of the flavor in question. Infusions of VFA alone also have reduced diet intake in cattle (Simkins et al., 1965). Although it is possible that reductions in feed intake associated with VFA infusion could be related to increases in hepatic oxidation (Allen et al., 2009), it seems more likely for our cattle fed poor-quality forage that discomfort associated with the infusions was responsible for depressions in forage intake. It should be noted that the apogee in ruminal pH for steers receiving VFA infusions was 5.8, a level suggesting that ruminal acidity per se would not be responsible for the discomfort.

As more N was provided to steers as casein, there were increases in N retention that amounted 38% of the increased intake of N, as well as increases in fecal N that amounted to 19% of the increased intake of N. Thus, the amount of N provided to the system that was available for urea production was strikingly less than that total amount of N supplemented as casein. Much of the greater fecal N output in response to increased casein supply can be explained by the increased microbial flow to the intestine. This would lead to more indigestible microbial N in feces. The increased N retention in response to casein supplementation was related to increases in microbial N supply to the intestine as well as to increased energy supply. This is further supported by the trend for greater digestible OM intake at the higher level of casein supplementation.

No difference in microbial efficiency was observed between the casein treatments. Similarly, Wickersham et al. (2008) and Neutze et al. (1986) did not observe differences in

microbial efficiency as N supplementation increased. Conversely, Köster et al. (1996) observed linear increases in microbial efficiency as DIP supplementation was increased from 0 to 720 g/d in steers consuming prairie hay.

The efficiency of microbial growth was greater for VFA than for glucose supplementation. It was unexpected that VFA infusion would increase the efficiency of microbial growth because VFA do not serve as substrates for microbial growth. It is possible that increased ruminal passage or changes in microbial populations in response to VFA infusion may have altered microbial efficiency.

Experimental design

We quantified the effects of supplemental energy and protein on urea recycling. Young, growing cattle were used so that we could measure urea kinetics under conditions where animals could use a large proportion of metabolizable protein supply for body protein deposition. Additionally, we provided energy and protein as separate treatments to allow us to measure their individual effects. We designed the study to characterize the effects of providing energy directly to the animal (as VFA) or of providing energy indirectly to the animal via the ruminal microbes (as glucose). The expectation was that fermentation of the glucose would increase production of microbial protein and VFA, whereas the VFA supplement would not impact microbial growth. Although the glucose supplement clearly altered the VFA profile in the rumen (Figures 3, 4, and 5), the increases in digestible OM intake were relatively small in response to glucose. In addition, microbial N flow to the duodenum was not affected by glucose. The lack of a biologically significant increase in digestible OM intake in response to glucose was attributed to reductions in digestion when 120 g/d of casein was provided and to reductions in forage intake when 240 g/d of casein was provided.

We expected that VFA and glucose infusions would both increase urea recycling but potentially through different mechanisms. We assumed that glucose would reduce ruminal ammonia concentrations by stimulating microbial growth and also would lead to production of VFA; the presence of VFA might increase blood flow to the rumen and increase the permeability of the rumen wall to urea, thereby increasing urea transfer to the rumen (Remond et al., 1992). In contrast to our expectations, glucose supplementation strongly reduced ruminal NH_3 concentrations but did not impact microbial N flow to the duodenum. Thus, we were successful in altering the ruminal environment but the impact on microbial N outflow was clearly limited. Across all energy treatments, microbial N flow to the duodenum increased as casein increased (Table 3), which suggested that 120 g/d of casein provided too little available N to maximize microbial activity in the rumen. Wickersham et al. (2008) observed linear increases in microbial N flow to the duodenum of steers when supplemental casein was increased up to 177 mg of N/kg BW daily (similar to our dose of 240 g/d).

Urea Kinetics

For steers not receiving supplemental energy, 81% of UER was returned to the gastrointestinal tract. When glucose was provided, GER/UER increased by 7 percentage units, providing evidence that the glucose-supplemented cattle recycled a greater proportion of urea, likely due to lower ruminal ammonia concentrations. Wickersham et al. (2008) observed that 96% of urea production was recycled to the gut at their highest level of protein supplementation (177 mg N/kg of BW daily), which is slightly greater than what we observed for our calves.

When additional casein was provided, steers excreted more urea in the urine, matching observations of Marini and Van Amburgh (2003). About 34% of the increased N provided by increasing casein supply was excreted in the urine as urea-N. This is more than was observed by

Wickersham et al. (2008) where less than 5% of the increase in N intake was excreted as urinary urea-N. However, Marini and Van Amburgh (2003) observed a greater proportion of increased N intake being excreted as urinary urea-N, reflecting that their heifers received diets containing excessive amounts of N.

Steers supplemented with glucose excreted less urinary urea than steers provided VFA. Other work has demonstrated an increased utilization of ruminally available N when glucose is provided (Kennedy, 1980). An increased demand for N when energy is provided to the rumen likely caused the cattle to salvage more urea-N that otherwise might be excreted in the urine; this was demonstrated by glucose supplementation leading to lower ratios of urea/creatinine clearance by the kidneys. The proportion of UER that was excreted in the urine tended to decrease with glucose supplementation, demonstrating that glucose supplementation may have, at least to a small extent, increased recycling of urea to the rumen. The lower ruminal concentration of ammonia in response to glucose supplementation could account for the increase in recycling (Kennedy, 1980). However, if the relatively small decrease in urinary urea excretion in response to glucose supplementation was matched by a similar increase in recycling, it would be too small to detect statistically as a change in GER.

In our study, UER and GER (daily amounts) were not affected by supplemental energy, casein level, or interactions between them. This differs from other research with supplemental protein and energy. Hennessy and Nolan (1988) observed significant increases in urea production and amount recycled when steers consuming low-quality forage were provided 300 g/d of a protein pellet. Using steers fed low-protein forage, Wickersham et al. (2008) observed linear increases in UER and GER as casein was ruminally supplemented in amounts from 0 to 177 mg of N/kg BW daily. Marini and Van Amburgh (2003) noted that as increasing amounts of

N were fed to Holstein heifers, UER increased linearly, and GER initially increased but then plateaued. Kennedy (1980) observed increases in gut entry of recycled urea when sucrose was provided to steers consuming low-quality forage, and providing readily fermented carbohydrates to forage-fed sheep increased the transfer of urea into the rumen (Kennedy et al., 1981). Our relatively narrow range of protein supplementation may have contributed to our inability to detect differences in urea kinetics when our steers were provided casein; Wickersham et al. (2008) and Marini and Van Amburgh (2003) fed broader ranges of protein to their cattle, allowing them more opportunity to detect differences in urea recycling. The data sets of Marini and Van Amburgh (2003) and Wickersham et al. (2008) demonstrate that when dietary N exceeds the needs of the ruminal microbes and of the host animal, UER will increase along with N intake. However, when the ruminal microbes, the animal, or both are capable of capturing the increases in N intake, then, as in this study, urea production may not be strikingly increased. Our treatments were based on prior research with prairie hay, and the highest level was near the requirement for optimizing forage intake and digestion. Our lack of change in UER in response to treatment may reflect that none of our treatments led either to ruminal ammonia supplies that greatly exceeded the microbes' needs or to metabolizable protein supplies that exceeded the steers' needs.

Microbial Use of Recycled Urea-N

Urea-N that is recycled to the rumen is converted to ammonia and either utilized by microbes or returned to the liver. For urea recycling to allow cattle to conserve N in times of a shortfall, rumen microbes must utilize the recycled urea N. Neutze et al. (1986) measured the microbial capture of recycled urea as urea-N supplementation to sheep fed wheat straw was increased. They reported no differences among treatments. Our steers tended ($P = 0.07$) to have

less microbial capture of recycled urea-N when the greater amount of casein was fed (Table 3), as well as a lesser proportion of microbial N derived from recycled urea. This would be expected because GER did not differ among treatments. In contrast, 240 g/d of casein provided more DIP than 120 g/d of casein; therefore, recycled urea would account for a lesser proportion of ruminally available N when the greater amount of casein was supplemented. In Holstein heifers fed isocaloric diets with increasing N concentration, Marini and Van Amburgh (2003) observed decreasing bacterial N yield from recycled urea with increasing levels of N in the diet. In contrast, Wickersham et al. (2008) observed that microbial capture of recycled urea N increased and that the percentage of microbial N derived from recycled urea was constant with casein supplementation. The different response in the trial of Wickersham et al. (2008) may be explained by the fact that GER increased with casein supplementation in their study.

Supplementation with glucose increased the capture of recycled urea-N by ruminal microbes. Previous work noted differences in uptake when animals were fed concentrate vs. forage diets. Al-Dehneh et al. (1997) fed dairy cows either a high-grain or high-forage diet and measured the incorporation of recycled urea-N into ruminal bacteria. Incorporation of recycled urea-N into bacterial N was proportionally greater when cows were fed grain-based rather than forage-based diets (38% for grain vs. 13% for forage). Kennedy (1980) demonstrated that sucrose supplementation to steers consuming low-quality forage increased microbial N synthesis in the rumen. We provided only small amounts of supplemental energy to forage fed cattle and observed that supplemental energy increased capture of recycled urea-N by ruminal microbes. Infusion of glucose increased microbial capture when compared to VFA. Infusion of glucose likely stimulated microbial uptake of N in the rumen. Conversely, infusion of VFA had no effect

on the rumen microbes, based on responses in ruminal ammonia concentrations and microbial N flow to the duodenum.

Plasma Urea-N and Renal Clearance

Plasma urea-N was increased only numerically as casein supplementation increased from 120 g/d to 240 g/d. In contrast, Wickersham et al. (2008) observed linear increases in PUN concentration as protein supplementation to steers consuming forage was increased. In that study, PUN reached only 2.84 m*M* when casein supplementation levels similar to our largest dose. This was less than what we observed (4.2 and 5.0 m*M* for control steers provided 120 and 240 g/d of casein, respectively). Sunny et al. (2007) found a high correlation between PUN and GER, which supported the conclusions of Kennedy (1980). When our steers were provided supplemental energy, PUN concentration was decreased and GER was not altered. Although lesser PUN might be expected to reduce GER for steers receiving glucose, the concurrent reduction in ruminal ammonia and numerical increases in digestible OM intake would be expected to increase urea recycling (Kennedy, 1980). Kennedy et al. (1981) fed sheep low quality forage and provided 2 levels of sucrose and 2 levels of urea. Concentration of PUN decreased when sucrose was provided, with or without urea. They attributed the decrease in PUN to increased OM fermentation in the rumen, which caused an increased demand for ruminal N. This matched the basic premise in the review of Kennedy and Milligan (1980) that related increased OM digestion to increased recycling of N to the rumen.

Plasma urea-N was numerically less for cattle receiving VFA than for controls. Given that ruminal ammonia was not greatly impacted by VFA infusions and that digestible OM intake was decreased by VFA infusions, reduced urea recycling might be expected. Perhaps the effect of the VFA infusions on ruminal blood flow and urea permeability was such that the net change

in urea recycling was zero or at least too small to detect. Although there were clear increases in ruminal NH_3 as more N was provided to steers, ruminal NH_3 concentrations never exceeded 12 m*M*. This suggested that the amounts of supplemental casein did not greatly exceed the needs of ruminal microbes and, therefore, would not lead to large amounts of ammonia being absorbed from the rumen.

Creatinine and urea clearance in the kidneys were measured to assess renal salvage of urea. Marini and Van Amburgh (2003) reported that 47% of urea filtered by the kidney was reabsorbed when heifers were fed a protein-poor diet (1.45% N) and that reabsorption decreased to 8% as dietary N content increased to 3.40%. By comparison, we observed 81% reabsorption of urea filtered by the kidney for steers receiving 120 g/d casein and 66% reabsorption for those receiving 240 g/d casein. Steers receiving glucose reabsorbed 78% of urea filtered by the kidney, whereas only 70% of urea filtered by the kidney was reabsorbed when VFA was infused. These data provide evidence that glucose increased the ruminal demand for N.

Conclusions

Overall, provision of supplemental energy decreased forage intake or digestion but had no effect on duodenal N flow or urea production in the liver. The proportion of urea that was recycled to the gastrointestinal tract and subsequently captured by ruminal microbes tended to be increased when supplemental energy was provided to the rumen as glucose.

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Table 1. Chemical composition of hay and supplements

Item	Prairie hay	Casein	Glucose	VFA
	----------% of DM----------			
OM	92.5	94.9	100	100
CP	5.8	95.3	---	---
NDF	71.0	---	---	---
ADF	45.4	---	---	---
Acid detergent insoluble ash	4.3	---	---	---

Table 2. Effects of ruminal casein and energy (glucose or VFA) supplementation on intake, digestion, and N balance of steers fed prairie hay

	Control		Glucose		VFA							
	Ruminal casein supply, g/d							*P*-value[1]				
Item	120	240	120	240	120	240	SEM[2]	C	En	GvV	En×C	GvV×C
No. of observations	6	6	6	6	4	4						
OM intake, kg/d												
Forage	3.7	4.2	3.7	3.7	3.0	2.9	0.38	0.42	< 0.01	< 0.01	0.11	0.94
Total	3.8	4.5	4.4	4.4	3.5	3.6	0.38	0.18	0.45	< 0.01	0.11	0.94
Ruminal digestion, %												
OM, true	51.8	50.5	48.9	55.1	53.5	54.4	2.89	0.47	0.58	0.46	0.17	0.32
NDF	53.7	45.3	39.3	46.6	45.1	45.7	4.10	0.96	0.09	0.51	0.05	0.37
Total tract digestibility, %												
OM	56.0	55.7	55.1	60.2	62.6	59.4	2.09	0.74	0.04	0.10	0.68	0.04
NDF	54.0	52.9	44.1	49.5	53.2	50.2	2.45	0.80	0.03	0.04	0.50	0.07
Digestible OM intake, kg/d	2.12	2.51	2.40	2.65	2.18	2.16	0.21	0.08	0.76	0.03	0.25	0.37
Nitrogen, g/d												
Intake	52.9	76.5	53.2	70.1	46.3	62.2	3.50	<0.01	< 0.01	< 0.01	0.06	0.83
Forage	36.4	43.2	36.4	36.8	29.7	28.9	3.50	0.26	< 0.01	< 0.01	0.07	0.79
Supplement	16.7	33.3	16.7	33.3	16.7	33.3	0.06	<0.01	0.70	0.55	0.69	0.53
Fecal	25.0	31.4	29.4	29.9	20.9	24.9	2.55	0.02	0.21	< 0.01	0.17	0.35
Urinary	18.1	25.2	13.4	20.3	18.1	28.4	1.94	<0.01	0.22	< 0.01	0.57	0.28
Ammonia	1.11	1.08	0.50	0.71	1.29	1.63	0.47	0.60	0.84	0.05	0.64	0.88
Total purine derivatives	3.35	3.92	3.46	4.02	3.30	3.84	0.21	<0.01	0.89	0.37	0.94	0.99
Allantoin	3.07	3.60	3.20	3.68	3.02	3.53	0.19	<0.01	0.85	0.33	0.89	0.92
Uric acid	0.28	0.32	0.26	0.34	0.27	0.30	0.03	0.02	0.80	0.69	0.81	0.41
Creatinine	2.13	2.13	2.16	2.14	2.36	2.36	0.12	0.90	0.05	0.01	0.90	0.92
Retained	10.0	19.8	10.3	19.9	7.6	9.7	3.48	0.01	0.20	0.04	0.40	0.20

[1] C = casein level; En = Control vs. energy (glucose plus VFA); GvV = Glucose vs. VFA; En×C = (Control vs. energy) × casein level; GvV×C = (Glucose vs. VFA) × casein level.

[2] Largest value among treatments is reported.

Table 3. Effect of ruminal casein and energy (glucose or VFA) on nutrient flows to the duodenum and microbial efficiency in steers fed prairie hay

	Control		Glucose		VFA							
	Ruminal casein supply, g/d							*P*-value[1]				
Item	120	240	120	240	120	240	SEM[2]	C	En	GvV	En×C	GvV×C
No. of observations	6	5[3]	6	6	4	4						
Duodenal flow, g N/d												
Total N	56.1	72.2	59.3	67.4	50.6	58.7	10.0	0.15	0.49	0.35	0.58	0.99
Microbial N	37.1	53.8	38.0	45.4	31.9	41.0	7.0	0.04	0.23	0.41	0.40	0.89
Predicted microbial N[4]	32.6	41.5	34.5	43.0	32.3	41.3	3.1	<0.01	0.75	0.51	0.98	0.92
Undegraded intake protein	19.0	18.5	21.3	22.0	18.2	16.1	4.1	0.83	0.85	0.24	0.98	0.71
Microbial N from urea	7.7	8.7	15.4	8.2	7.7	6.6	1.9	0.07	0.33	0.01	0.07	0.07
% of microbial N flow	20.5	17.0	41.3	18.0	25.9	17.4	3.8	<0.01	0.03	0.04	0.04	0.04
% of urea entry rate	20.1	16.1	25.3	19.1	17.5	10.7	4.3	0.07	0.98	0.05	0.69	0.93
% of gastrointestinal entry rate	23.7	20.8	27.5	23.4	21.4	15.1	5.3	0.26	0.91	0.15	0.77	0.82
Microbial efficiency,												
g N/kg OM truly fermented [5]	16.7	21.5	17.3	17.3	23.2	23.6	3.1	0.45	0.57	0.04	0.32	0.95

[1] C = casein level; En = Control vs. energy (glucose plus VFA); GvV = Glucose vs. VFA; En×C = (Control vs. energy) × casein level; GvV×C = (Glucose vs. VFA) × casein level.

[2] Largest values among treatments are reported.

[3] For measured microbial N, undegraded intake protein, microbial N from urea, and microbial efficiency, data from 1 steer were excluded because urea entry rate was an outlier.

[4] Predicted from urinary purine derivative excretion based on the equations of Chen and Gomes (1992).

[5] Infused VFA were not included as part of OM truly fermented.

Table 4. Effect of ruminal casein and energy (glucose or VFA) supplementation on urea kinetics in steers fed prairie hay

	Control		Glucose		VFA							
	Ruminal casein supply, g/d							*P*-value[1]				
Item[2]	120	240	120	240	120	240	SEM[3]	C	En	GvV	En×C	GvV×C
No. of observations	6	5	6	6	4	4						
Urea kinetics, g N/d												
UER	38.5	55.3	67.5	44.8	60.5	62.9	11.8	0.89	0.19	0.61	0.14	0.24
UUE	6.7	12.3	2.8	7.8	6.9	15.7	1.8	<0.01	0.36	0.01	0.59	0.21
GER	31.9	42.9	64.8	37.0	54.3	48.0	11.8	0.38	0.14	0.98	0.12	0.32
ROC	13.1	16.4	27.5	15.0	21.9	19.2	6.5	0.40	0.22	0.90	0.27	0.41
UUA	18.2	25.3	37.0	21.4	31.8	27.3	6.2	0.35	0.11	0.94	0.08	0.33
UFE	0.5	1.2	0.3	0.6	0.6	1.6	0.2	<0.01	0.50	0.01	0.94	0.04
Fractional urea kinetics												
UUE/UER (u)	0.17	0.22	0.07	0.18	0.14	0.24	0.037	0.01	0.16	0.07	0.39	0.84
GER/UER	0.83	0.78	0.93	0.82	0.86	0.76	0.037	<0.01	0.16	0.07	0.39	0.84
ROC/UER (p)	0.34	0.31	0.35	0.34	0.32	0.30	0.032	0.33	0.92	0.21	0.71	0.97
ROC/GER (r)	0.42	0.40	0.37	0.41	0.37	0.41	0.032	0.41	0.47	0.92	0.26	0.89
UUA/GER (a)	0.57	0.58	0.62	0.57	0.61	0.56	0.034	0.21	0.49	0.67	0.23	0.97
UFE/GER (f)	0.016	0.029	0.006	0.017	0.012	0.034	0.006	0.01	0.30	0.06	0.68	0.38

[1] C = casein level; En = Control vs. energy (glucose plus VFA); GvV = Glucose vs. VFA; En×C = (Control vs. energy) × casein level; GvV×C = (Glucose vs. VFA) × casein level.

[2]UER = urea-N entry rate; UUE = urinary urea-N elimination; GER = gastrointestinal entry rate; ROC = urea-N returned to ornithine cycle; UUA = urea-N utilized for anabolism; UFE = urea-N excreted in feces

[3] Largest value among treatments is reported.

Table 5. Effect of ruminal casein and energy (glucose or VFA) supplementation on plasma metabolite concentrations and renal clearance in steers fed prairie hay

	Control		Glucose		VFA							
	Ruminal casein supply, g/d							*P*-value[1]				
Item	120	240	120	240	120	240	SEM[2]	C	En	GvV	En×C	GvV×C
No. of observations	6	6	6	6	4	4						
Renal clearance												
Creatinine, kL/d	0.516	0.577	0.550	0.606	0.642	0.720	0.045	0.03	0.01	0.01	0.92	0.75
Urea, kL/d	0.114	0.200	0.079	0.180	0.138	0.276	0.029	0.01	0.57	0.01	0.41	0.47
Urea/creatinine, %	22.6	34.0	14.4	29.4	20.9	38.2	4.1	0.01	0.40	0.06	0.42	0.77
Plasma												
Urea-N, m*M*	4.2	5.0	2.3	3.0	3.4	4.3	0.8	0.16	0.03	0.12	0.96	0.89
Glucose, m*M*	4.3	4.4	4.2	4.3	4.2	4.3	0.2	0.20	0.20	0.89	0.94	0.74
Creatinine, μ*M*	99.3	89.2	96.4	86.5	87.4	80.0	5.1	0.01	0.05	0.07	0.82	0.76
Amino acids, μ*M*												
Ala	269	209	222	183	202	203	29	0.12	0.09	0.98	0.32	0.44
Gly	189	146	204	144	261	204	39	0.05	0.19	0.10	0.77	0.96
Val	307	220	259	243	196	212	33	0.18	0.11	0.11	0.06	0.56
Leu	167	130	149	137	114	123	15	0.17	0.09	0.06	0.09	0.39
Ile	109	91	97	77	77	87	11	0.54	0.30	0.07	0.22	0.41
Thr	79	63	80	69	68	70	8	0.12	0.81	0.39	0.27	0.33
Ser	63	47	58	59	101	87	10	0.15	0.01	0.01	0.50	0.38
Pro	92	75	83	72	77	72	9	0.07	0.22	0.73	0.47	0.72
Asn	27	21	24	21	19	18	2	0.06	0.05	0.07	0.25	0.44
Asp	10	8	9	9	8	7	2	0.54	0.54	0.36	0.63	0.72
Met	27	20	21	20	19	16	2	0.03	0.01	0.12	0.09	0.59
Glu	172	211	210	192	187	195	43	0.76	0.88	0.80	0.49	0.74
Phe	71	59	59	57	53	53	7	0.27	0.05	0.36	0.22	0.89
Gln	86	84	96	83	87	75	17	0.47	0.97	0.59	0.68	0.99
Orn	78	74	79	64	59	55	10	0.31	0.13	0.13	0.70	0.57
Lys	119	97	103	81	75	67	14	0.06	0.01	0.10	0.72	0.54
Tyr	51	48	46	45	37	39	6	0.87	0.08	0.16	0.62	0.81
Trp	31	27	30	26	23	21	4	0.20	0.18	0.11	0.94	0.74

[1] C = casein level; En = Control vs. energy (glucose plus VFA); GvV = Glucose vs. VFA; En×C = (Control vs. energy) × casein level; GvV×C = (Glucose vs. VFA) × casein level.
[2] Largest value among treatments is reported.

Table 6. Effect of ruminal casein and energy (glucose or VFA) supplementation on ruminal fermentation characteristics in steers fed prairie hay

	Control		Glucose		VFA							
	Ruminal casein supply, g/d							*P*-value[1]				
Item	120	240	120	240	120	240	SEM[2]	C	En	GvV	En×C	GvV×C
No. of observations	6	6	6	6	4	4						
Ruminal[3]												
pH	6.62	6.57	6.58	6.48	6.59	6.33	0.10	0.03	0.12	0.37	0.30	0.30
Ammonia, m*M*	2.7	7.0	0.8	2.4	3.6	7.3	0.8	<0.01	0.02	0.01	0.14	0.14
Total VFA, m*M*	98.0	102.9	88.1	97.9	91.8	106.2	5.3	<0.01	0.13	0.11	0.21	0.51
Acetate, m*M*	71.7	72.2	59.3	63.5	58.3	68.1	3.5	<0.01	0.01	0.42	0.06	0.19
Propionate, m*M*	15.8	16.8	13.7	15.7	18.7	21.1	1.2	<0.01	0.15	0.01	0.31	0.82
Butyrate, m*M*	7.5	8.9	12.9	14.6	12.2	12.2	1.2	0.18	0.01	0.14	0.70	0.40
Isobutyrate, m*M*	0.92	1.37	0.68	0.99	0.79	1.28	0.09	<0.01	0.01	0.01	0.70	0.26
Valerate, m*M*	0.97	1.61	0.91	1.67	0.86	1.64	0.14	<0.01	0.85	0.74	0.50	0.96
Isovalerate, m*M*	1.06	1.98	0.61	1.35	0.97	1.76	0.17	<0.01	0.01	0.01	0.49	0.86

[1] C = casein level; En = Control vs. energy (glucose plus VFA); GvV = Glucose vs. VFA; En×C = (Control vs. energy) × casein level; GvV×C = (Glucose vs. VFA) × casein level.
[2] Largest value among treatments is reported.
[3] Average of values collected at 2, 6, 10, 14, 18, and 22 h after feeding.

Figure 1. The effect of ruminal energy (glucose or VFA) supplementation on ruminal pH in steers fed prairie hay. The VFA were infused for 8 h beginning at feeding. Energy × hour interaction; $P = 0.01$; SEM = 0.11.

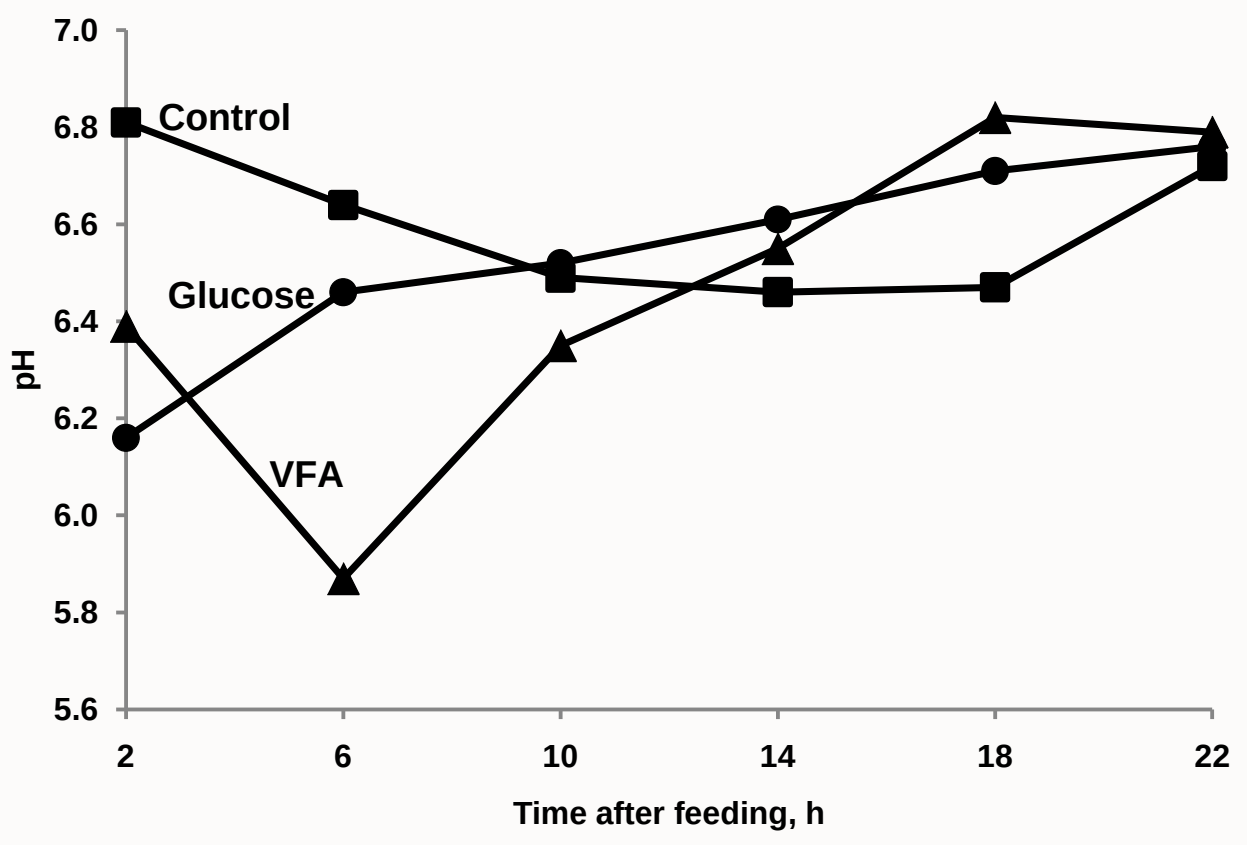


Figure 2. The effect of ruminal casein and energy (glucose or VFA) supplementation on ruminal ammonia concentration in steers fed prairie hay. Casein was dosed ruminally once daily at feeding, and VFA were infused for 8 h beginning at feeding. Energy × casein × hour interaction; $P = 0.05$; SEM = 0.82.

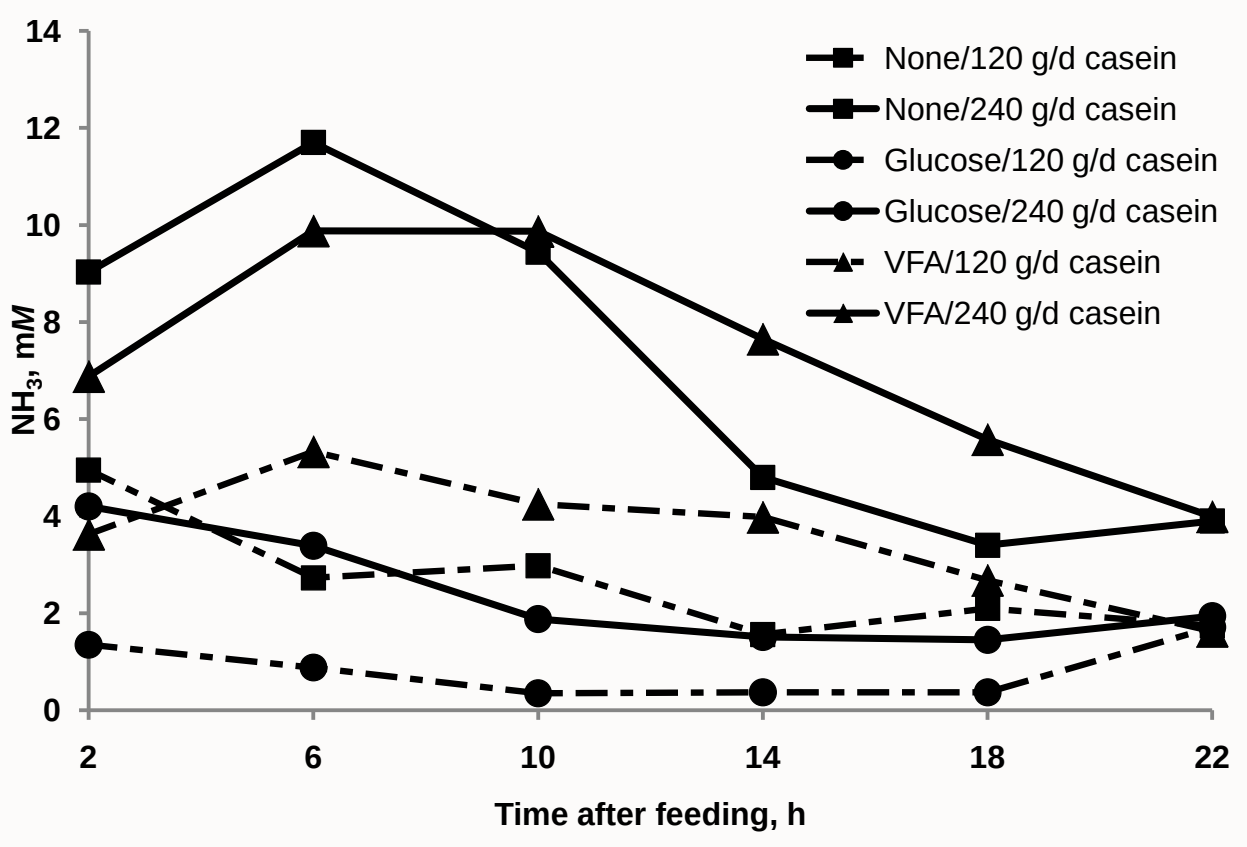


Figure 3. The effect of ruminal energy (glucose or VFA) supplementation on ruminal acetate concentration in steers fed prairie hay. The VFA were infused for 8 h beginning at feeding. Energy × hour interaction; $P = 0.01$; SEM = 3.5.

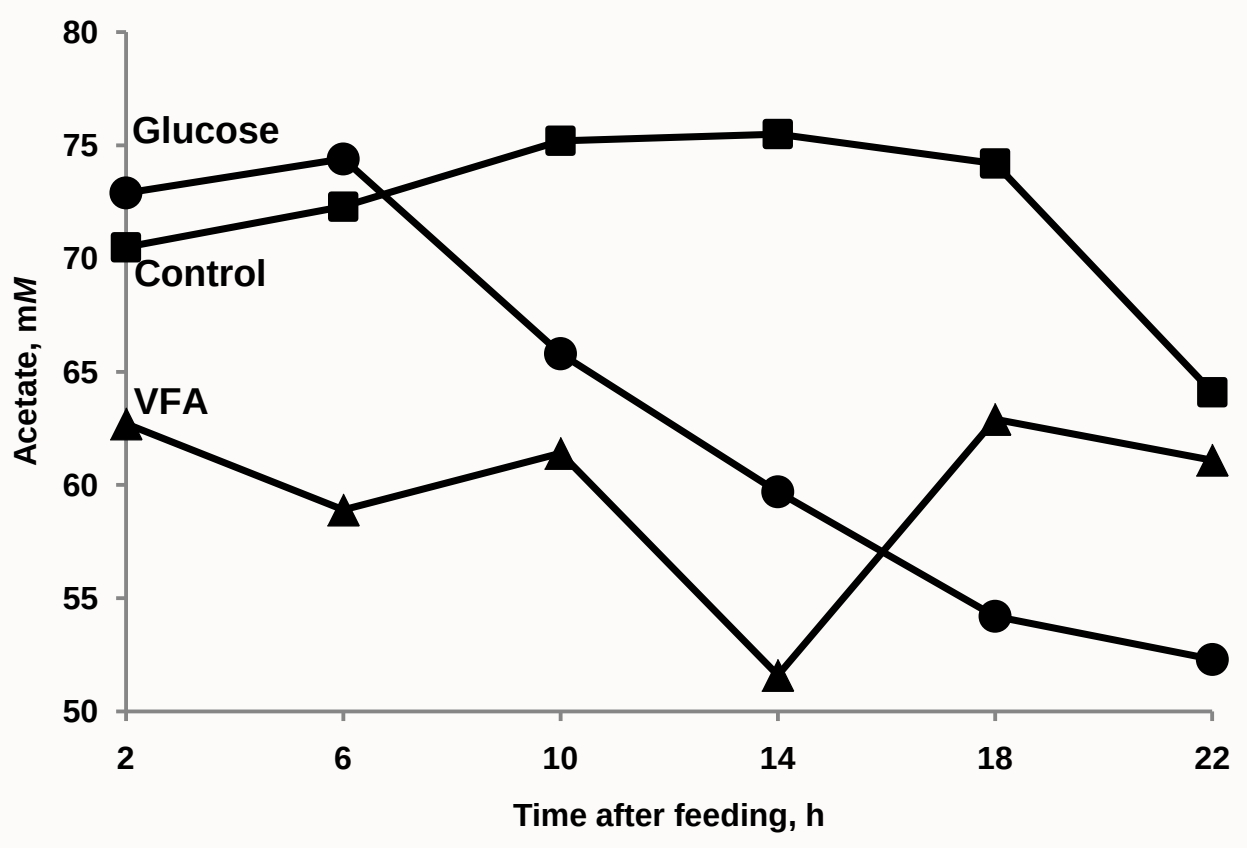


Figure 4. The effect of ruminal energy (glucose or VFA) supplementation on rumen propionate concentration in steers fed prairie hay. The VFA were infused for 8 h beginning at feeding. Energy × hour interaction; $P = 0.01$; SEM = 1.2.

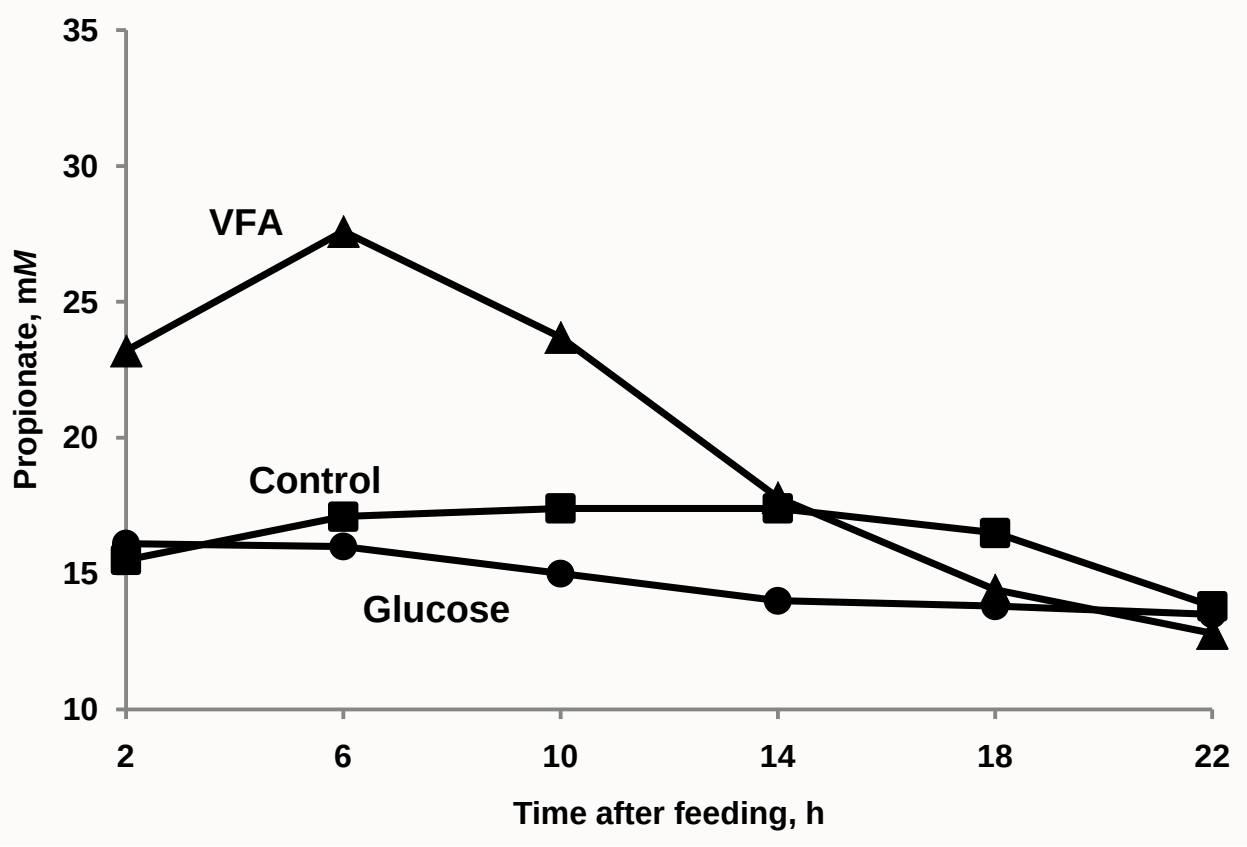
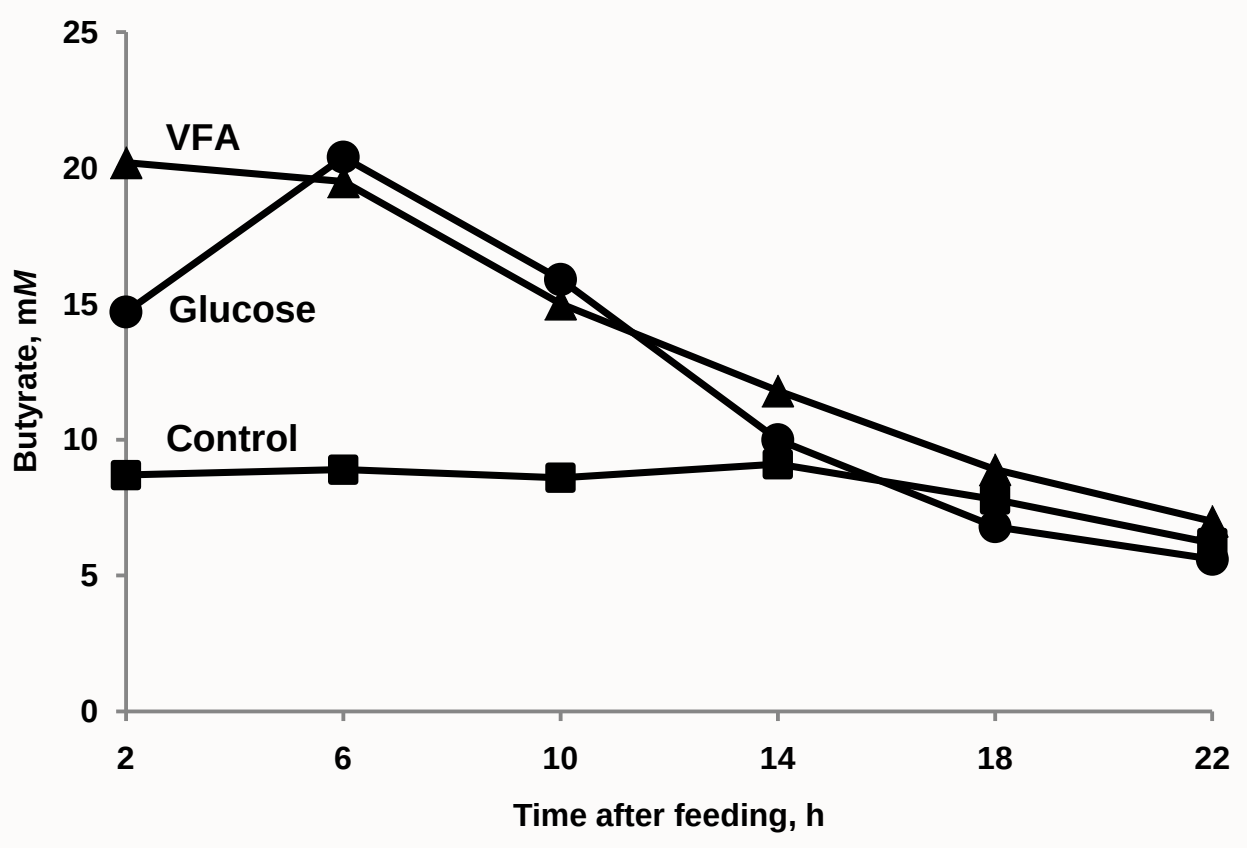


Figure 5. The effect of ruminal energy (glucose or VFA) supplementation on rumen butyrate concentration in steers fed prairie hay. The VFA were infused for 8 h beginning at feeding. Energy × hour interaction; $P = 0.01$; SEM = 1.2.



CHAPTER 3 - EFFECTS OF RUMINAL CASEIN AND GLUCOSE ON FORAGE DIGESTION AND UREA KINETICS IN BEEF CATTLE[1]

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Abstract

We quantified effects of supplemental glucose and degradable intake protein on nutrient digestion and urea kinetics in steers given ad libitum access to prairie hay (4.7% CP). Six ruminally and duodenally cannulated steers (391 kg) were used in a 4 × 4 Latin square with 2 extra steers. Treatments, arranged as a 2 × 2 factorial, were dosed ruminally once daily and included: 0 or 1.2 kg of glucose, and 240 or 480 g of casein. Each period included 9 d for adaptation, 4 d for total fecal and urine collections, and 1 d for ruminal and duodenal sampling. We infused $^{15}N^{15}N$-urea into the jugular vein to measure urea kinetics. Glucose reduced forage intake by 18% ($P < 0.01$), whereas casein level did not affect forage intake ($P = 0.69$). Total tract digestion of NDF was depressed ($P < 0.01$) by glucose. Glucose supplementation decreased ruminal pH 2 h after dosing, but the effect was negligible by 6 h (treatment × time; $P = 0.01$). Providing additional casein increased ruminal concentration of NH_3 ($P < 0.01$), whereas glucose decreased it ($P < 0.01$) with the reduction in response to glucose being greater when 480 rather than 240 g/d of casein was provided. Microbial N flow to the duodenum and retained N increased ($P \leq 0.01$) as casein increased but neither was affected by glucose supplementation. The urea-N entry rate was increased ($P = 0.03$) 50% with increasing casein. Urinary urea-N excretion was increased ($P < 0.01$) as casein increased; moreover, gut entry rate of urea-N was numerically increased ($P = 0.30$) 25% as casein increased. The proportion of urea entry rate that was recycled to the gut decreased ($P < 0.01$) as casein increased. Glucose supplementation decreased ($P < 0.01$) urinary urea excretion but did not change ($P \geq 0.70$) urea entry rate or gut entry rate. The amount of recycled urea-N that was captured by ruminal microbes was less (casein × glucose interaction, $P = 0.05$) for steers receiving 480 g/d casein with no glucose than for the other 3 treatments. This was attributed to an excess of ruminally available N provided

directly to the microbes from the supplement. Overall, the provision of supplemental glucose decreased forage intake and digestibility. Increasing casein altered urea kinetics by increasing urea production, but the proportion of urea-N recycled to the gut was decreased.

Key words: cattle, supplementation, urea recycling

Introduction

Non-structural carbohydrate (NSC) supplementation can decrease forage utilization in cattle, particularly when ruminally available N is limiting (Heldt et al., 1999). Degradable intake protein has been shown to be the first-limiting nutrient in cattle fed low-quality forage (< 7% CP; Köster et al., 1996). One possible mechanism by which NSC supplementation depresses forage utilization is the increased uptake of N by ruminal microbes that do not ferment fiber, thus reducing the amount of N available for the fiber-fermenting microbes.

Wickersham et al. (2008) demonstrated that cattle recycle large proportions (~95%) of urea-N to the rumen when they are fed low-quality forages deficient in protein. Kennedy et al. (1981) found that providing readily fermentable carbohydrates to forage-fed sheep increased the transfer of urea into the rumen. We measured urea kinetics in beef steers when degradable intake protein was supplemented in conjunction with NSC (i.e., glucose). Our hypotheses were that provision of degradable intake protein would ameliorate nutrient imbalances caused by NSC supplementation and that urea recycling would be increased by supplemental glucose.

Another topic of interest was how physiological maturity impacts urea kinetics in cattle. We previously conducted similar research in younger, growing cattle (Chapter II), which allowed us to compare results between physiological states. We hypothesized that urea recycling would be greater in cattle that were more mature because they would use less N for growth and subsequently synthesize more urea.

Materials and Methods

All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee at Kansas State University.

Six ruminally and duodenally cannulated Angus steers (initial BW = 391 ± 49 kg) were used in a 4 × 4 Latin square with 2 additional steers and with dietary treatments arranged as a 2 × 2 factorial. Two protein treatments were 240 or 480 g of casein (sodium caseinate, New Zealand Milk Products Inc., Auckland, New Zealand; Table 1) pulse-dosed into the rumen once daily at 0630 h. Two energy treatments included 0 (control) or 1.2 kg (as fed) glucose (dextrose monohydrate, ADM Corn Processing, Decatur, IL; Table 1) pulse-dosed into the rumen once daily at 0630 h. On a BW basis, treatments were similar to those described in Chapter II. All steers had ad libitum access to prairie hay (4.7% CP; Table 7) fed at 115% of the average voluntary intake over the previous 4 d.

Each experimental period lasted 14 d. The first 9 d were used for adaption to treatments and the last 5 d for sample collection. For the first 7 d of adaption, steers were housed in individual tie-stalls. For the remainder of each period, steers were placed in metabolism crates that allowed for total collection of urine and feces and infusion of labeled urea. At 0630 h on d 10 through d 13 of each period, each metabolism crate had a clean bucket containing 900 mL of 10% (wt/wt) H_2SO_4 placed under the collection funnel to facilitate complete collection of urine from each steer. The acid maintained the pH of the urine below 3, which prevented ammonia volatilization. Feces were collected into a metal bin lined with plastic from d 10 through 13 of each period.

Calculations of intake, digestion, and N balance were made from d 10 through 13. Samples of hay (400 g) were collected from d 9 through 12 and composited within each period to correspond with urine and feces collected from d 10 through 13. Orts were collected before each

daily feeding; orts from d 9 to 12 were composited for each steer. Samples of casein and glucose were collected once each period. Feces and urine collected over a 24-h period were removed each day at 0630 h and sampled. Samples of both feces (5% of total amount collected) and urine (1% of total amount collected) were composited within animal during each period. Two subsamples of urine samples were collected; one for determination of N balance and another for purine derivative analysis. The urine to be used in purine derivative analysis was diluted 5:1 with 0.05 *M* H_2SO_4.

At 4 h after feeding (1030 h) on d 10, blood (10 mL) was collected by jugular venipuncture into heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed in ice and centrifuged at 1,200 × *g* for 15 min within 1 h of collection. Plasma was isolated from blood and frozen.

An indewelling jugular catheter was placed in each steer on d 10 to allow for infusion of $^{15}N^{15}N$-urea. Sterile saline was infused continuously from the time each catheter was placed until 0630 h on d 11 of each period. At that time, infusion of the $^{15}N^{15}N$-urea solution was initiated through use of a syringe infusion pump (Harvard Apparatus, South Natick, MA). Label infusion continued through the end of each period. The $^{15}N^{15}N$-urea solution was prepared by combining 7.2 g of $^{15}N^{15}N$-urea (99% $^{15}N^{15}N$-urea, Medical Isotopes, Inc., Pelham, NH) with 1 L of sterile saline solution (0.9% NaCl). The solution was filter sterilized (0.22 µm filter, Sterivex, Millipore Corporation, Billeric, MA), bottled in glass containers, and stored at 4°C until use. The infusion rate was 4.30 mL/h.

Feces (500 g) and urine (100 mL) collected from d 10 were used for measuring background concentrations of ^{15}N. Feces (500 g) and urine (100 mL) collected on d 13 were used to measure the ^{15}N enrichments at plateau (Wickersham et al., 2009).

On d 14 of each period, samples of rumen and duodenal fluid were collected every 4 h for 24 h beginning 2 h after feeding. Whole rumen contents (1.2 L) were collected from each animal to isolate ruminal bacteria. Contents were first strained through 4 layers of cheesecloth and the liquid portion was collected and immediately analyzed for pH. An 8-mL sample of ruminal fluid was combined with 2 mL 25% (wt/wt) meta-phosphoric acid and frozen for subsequent analysis of VFA. Another 20-mL sample of ruminal fluid was mixed with 2 mL of 6 *M* HCl and frozen for later analysis of ammonia. The remaining fluid and all solids were mixed with 1.0 L of 0.9% NaCl, blended (NuBlend, Waring Commercial, Torrington, CT) for 1 min, and strained through 4 layers of cheesecloth and frozen for later isolation of bacteria. Bacterial samples were composited within animal during each period. Duodenal fluid (300 mL) was collected from each steer at the same times that ruminal samples were collected. Duodenal fluid was frozen and subsequently pooled within animal for later analyses.

Laboratory Analyses

The partial DM of hay, ort, and fecal samples were determined by drying in a forced-air oven for 72 h. Duodenal samples were lyophilized. Samples of hay, ort, fecal, and duodenal digesta were ground through a 1-mm screen with a Wiley mill. The DM content of hay, ort, fecal, duodenal samples, casein, and glucose was determined by drying for 24 h at 105°C in a forced-air oven; the ash content was measured by heating for 8 h in a muffle oven at 450°C. Hay, ort, fecal, and duodenal samples were analyzed for NDF (without amylase and without ash correction) and for ADF non-sequentially using an ANKOM-Fiber Analyzer (ANKOM-Technology, Fairport, NY). To determine acid detergent insoluble ash of fecal and duodenal samples, ANKOM bags containing ADF residues were combusted for 8 h at 450°C in a muffle oven. Nitrogen concentration of casein, hay, ort, duodenal, wet feces, and urine samples was

determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation, St. Joseph, MI). Crude protein was calculated as N × 6.25.

Ruminal bacteria were isolated from thawed rumen samples by centrifuging at 500 × g for 20 min to remove protozoa and feed particles. The supernatant was centrifuged at 20,000 × g for 20 min, the pellet was resuspended with saline (0.9% NaCl), and the resulting solution was centrifuged again at 20,000 × g for 20 min. The bacterial pellet was frozen and lyophilized.

Concentrations of urinary urea (Marsh et al., 1965) and ammonia (Broderick and Kang, 1980) were determined colorimetrically using an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL). Measurement of ^{15}N enrichments in urinary urea was conducted according to Brake (2009). The ^{15}N enrichments of rumen bacteria, dried feces, and duodenal samples were measured using a stable isotope elemental analyzer (ThermoFinnigan Delta Plus, Thermo Electron Corporation, Waltham, MA). Concentrations of allantoin, uric acid, and creatinine were measured in composited urine samples (d 10 through 13) by reverse-phase HPLC as described by Brake (2009). The method of Vanzant and Cochran (1994) was used to measure VFA in rumen fluid by GLC. Measurements of ruminal ammonia (Broderick and Kang, 1980), plasma urea-N (PUN; Marsh et al., 1965), plasma creatinine (Chasson et al., 1961), and plasma glucose (Gochman and Schmitz, 1972) were accomplished using an AutoAnalyzer (Technicon Analyzer II). Plasma AA were analyzed by GLC using a GC-FID Free Amino Acid Analysis Kit (EZ:faast, Phenomenex, Torrance, CA).

Calculations

Flows to the duodenum were calculated by dividing the fecal output of acid detergent insoluble ash by the acid detergent insoluble ash concentration in duodenal digesta. Microbial N flow to the duodenum was calculated using 2 methods. The first method ("measured") consisted

of multiplying duodenal N flow by the ratio of duodenal ^{15}N enrichment to bacterial ^{15}N enrichment. The second method ("estimated") utilized the methods of Chen and Gomes (1992) to predict microbial N flow from urinary purine derivative excretion. Flow of undegraded intake protein to the duodenum was calculated as the difference between total N flow and microbial N flow. Microbial N derived from recycled urea was calculated by multiplying measured bacterial N flow by the ratio of bacterial ^{15}N enrichment to ^{15}N enrichment of urinary urea (Wickersham et al., 2009). Urea kinetics were calculated using the methods of Lobley et al. (2000).

Statistical Analysis

Data from 2 observations were not obtained from 1 steer (for treatments control/480 g/d casein and glucose/480 g/d casein) due to temporary problems related to the duodenal cannula. Data were analyzed as a Latin square with a factorial arrangement of treatments using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model included glucose, casein, glucose × casein, and period. Steer was a random effect. Treatment means were calculated using the LSMEANS option. Ruminal fermentation parameters were analyzed as repeated measures with the model containing glucose, casein, glucose × casein, hour, hour × glucose, hour × casein, hour × glucose × casein, and period. Steer was included as a random term. The repeated term was hour with steer × period serving as the subject. Compound symmetry was the covariance structure. We utilized a split-plot analysis to compare the measured microbial N flow to the duodenum with values predicted by the equations of Chen and Gomes (1992). The model included glucose, casein, glucose × casein, method, glucose × method, casein × method, glucose × casein × method, and period. Steer and steer × period × glucose × casein were included as random effects. Significance was declared when $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Results

Forage Intake and Digestibility

Providing additional casein had no impact ($P = 0.69$) on forage intake but tended ($P = 0.09$) to decrease total tract NDF digestion (Table 8). Supplemental glucose decreased ($P < 0.01$) forage OM intake but tended ($P = 0.08$) to increase total OM intake (Table 8). Supplemental glucose increased ($P = 0.03$) true ruminal OM digestion 6 percentage units. Total tract digestibility of NDF decreased ($P < 0.01$) 8 percentage units when glucose was provided but total tract digestibility of OM increased ($P < 0.01$) with glucose. Glucose supplementation increased total digestible OM intake ($P < 0.01$).

Nitrogen Intake, Retention, and Flow at the Duodenum

Increasing the amount of supplemental casein increased ($P < 0.01$) N intake (Table 8). When glucose was provided, N intake decreased ($P < 0.01$) relative to casein alone. There were no differences ($P \geq 0.18$) in fecal N excretion between casein or glucose treatments. Urinary N excretion increased ($P < 0.01$) as more casein was provided and decreased ($P < 0.01$) when glucose was provided. Total purine derivative excretion increased ($P < 0.01$) when more casein was provided and also when glucose was added ($P < 0.01$). There also was a casein × glucose interaction ($P = 0.02$); increasing the amount of supplemental casein led to greater purine derivative excretion when glucose was supplemented than when it was not. Increasing the N supply to the animal improved N retention ($P < 0.01$).

A casein × glucose interaction ($P = 0.02$) was observed for total N flow to the duodenum (Table 9). Glucose supplementation to steers receiving the lower level of casein decreased N flow to the duodenum but it increased duodenal N flow when the higher level of casein was provided. Microbial N flow to the duodenum increased when casein supply was increased ($P =$

0.01) but was not affected by supplemental glucose. In addition, microbial N flow numerically ($P = 0.11$) interacted with glucose and casein with a pattern similar to that for total N flow. Supplementing glucose decreased microbial efficiency ($P = 0.04$) but casein supply did not affect it. As would be expected, the predicted microbial N flows interacted with casein and glucose in a manner similar to that for urinary excretion of purine derivatives. Increasing the amount of supplemental casein led to greater predicted microbial N flow when glucose was supplemented than when it was not. The predictions of microbial N flow based on urinary purine derivative excretions (Chen and Gomes, 1992) underestimated ($P < 0.01$) microbial N flow by 24%. In addition, the purine derivative method underestimated microbial N flow by a smaller margin (~9% underestimation) when glucose was provided than when glucose was not provided (~38% underestimation; glucose × method interaction, $P < 0.01$). This effect of glucose supplementation on the accuracy of prediction may relate to changes that carbohydrate source might impart on the N:purine ratio of ruminal microbes.

Urea Kinetics

Urea-N entry rate increased ($P = 0.03$) as we provided more casein to the steers (Table 10), whereas glucose did not have an effect ($P = 0.70$). A significant casein × glucose interaction was observed for urinary urea-N excretion (UUE). Providing more casein markedly increased UUE and glucose decreased it but the magnitude of decrease in response to glucose was less for 240 than for 480 g/d casein. The amounts of urea-N returned to the ornithine cycle and the amount of urea-N utilized for anabolism were not affected by supplemental glucose or casein. Increasing casein supply increased the amount of urea-N excreted in feces ($P < 0.01$).

The proportion of UER that was recycled to the gut (GER/UER) decreased ($P < 0.01$) as more casein was supplemented ($P < 0.01$). However, these decreases in the proportion of UER

that was recycled to the gut in response to greater amounts of casein were not large enough in magnitude to completely offset the greater UER, such that GER was numerically increased (P = 0.30) in response to increased casein supply as well (94 vs. 75 g/d; Table 4). The proportions of GER returning to the ornithine cycle or being used for anabolism were not different ($P \geq 0.21$) among treatments. The proportion of GER excreted in the feces was increased (P = 0.03) with increasing casein.

Microbial Use of Recycled Urea-N

We observed a casein × glucose interaction for microbial capture of recycled urea (Table 9; P = 0.05). When no glucose was provided, increasing casein from 240 to 480 g/d decreased microbial capture of recycled urea. In contrast, microbial capture of recycled urea was similar for both levels of casein when glucose was supplemented. Increasing casein supplementation decreased (P = 0.02) the percentage of microbial N flow that was derived from recycled urea. Increasing casein also decreased (P = 0.01) the percentages of urea-N entry rate (UER) and of gastrointestinal entry rate of urea-N (GER) that were captured by ruminal microbes.

Plasma Urea-N and Renal Clearance

Renal creatinine clearance (Table 11) was greater (P = 0.03) when 480 rather than 240 g/d of casein was provided. Urea clearance increased ($P \leq 0.01$) with both increasing casein and with provision of glucose. Increasing casein supply increased ($P < 0.01$) PUN and glucose supplementation decreased it ($P < 0.01$). A casein × glucose interaction was manifested also for PUN (P = 0.05); the magnitude of increase in PUN in response to increasing casein was less when glucose was provided than when it was not provided. Neither plasma glucose nor plasma creatinine concentrations were affected by treatments.

Ruminal Fermentation

Ruminal fermentation products were measured throughout the day and average values over time are presented in Table 12. Average ruminal pH was not different among treatments but it was decreased (glucose × hour interaction, $P = 0.01$) by glucose supplementation 2 h after feeding (Figure 6).

Glucose decreased ($P < 0.01$) ruminal ammonia concentrations (Table 12, Figure 7) and increasing casein increased ($P < 0.01$) ruminal ammonia concentrations. We also observed a casein × glucose interaction ($P < 0.01$) for rumen ammonia concentration (Table 12). The magnitude of increase in ammonia concentration in response to increasing casein supply was less when glucose was supplemented. The pattern for ruminal ammonia concentrations over time differed also among treatments. When no supplemental glucose was provided, ruminal ammonia concentration peaked sometime before 6 h post-feeding but it peaked later and at a lower concentration when glucose was provided, (glucose × casein × hour, $P = 0.01$; Figure 7).

Supplemental glucose decreased ruminal acetate concentrations across all time points (Figure 8). Concentrations of acetate stayed relatively steady throughout the day when no glucose was provided but decreased between 2 and 10 h after feeding when glucose was added. Supplemental glucose caused propionate concentration to increase immediately after feeding; it then declined to pre-feeding concentrations by 10 h post-feeding (Figure 9). Ruminal butyrate was greater 2 and 6 h after feeding when glucose was supplemented but it was not impacted by glucose supplementation at other sampling times (Figure 10). Cattle not provided glucose maintained a steady ruminal butyrate concentration throughout the day.

Increasing casein supply did not affect ruminal concentrations of acetate, propionate, or butyrate but it increased ($P = 0.01$) concentrations of isobutyrate, valerate, and isovalerate (Table 12). Increases in isobutyrate in response to increasing casein were less (casein × glucose

interaction, $P = 0.03$) when glucose was supplemented. Supplemental glucose decreased ($P = 0.01$) ruminal isobutyrate, valerate, and isovalerate. For the minor VFA, a number of interactions between treatment and time were significant, but these interactions reflected the magnitude of change over time rather than a change in temporal pattern and, thus, we concluded that treatment responses could be adequately described by averages over time.

Discussion

Forage Intake and Digestibility

A shortage of ruminally-available N is a common cause of poor utilization of low-quality forages, which are often deficient in protein. We provided a N source (casein) that was completely available in the rumen. The complete ruminal availability of casein-N was demonstrated in our study by the fact that the largest amount of supplemental casein did not increase flow of undegraded intake protein to the duodenum (Table 9). We provided a level of supplemental casein that was designed to maximize forage utilization (480 g/d casein; Köster et al., 1996) and a level designed to be inadequate (240 g/d casein) in order to study urea recycling when N was deficient and adequate. Our glucose treatments were designed to study the impact of NSC when cattle were adequate or deficient in ruminally-available N.

In our study, the lesser amount of casein did not seem to limit forage utilization based on forage intake and fiber digestion. In contrast, microbial N flow to the duodenum was depressed when glucose was provided to steers receiving only 240 g/d casein. This demonstrated a deficiency in ruminally-available N that limited microbial growth in the rumen for that treatment combination. Across the other 3 treatment combinations, no differences in microbial N flow to the duodenum were observed. It is likely that both casein treatments provided adequate N to the control steers; however, glucose increased the ruminal demand for N and caused a N deficit for the glucose × 240 g/d casein treatment. Based on ruminal ammonia concentrations (Figure 7), it appeared no glucose × 240 g/d casein and glucose × 480 g/d casein did not provide excessive N to the rumen. In contrast, control × 480 g/d casein seemed to provide ruminally-available N beyond the microbial requirement. Wickersham et al. (2008) observed linear increases in forage intake and total tract digestion of NDF when increasing the supply of casein to steers fed prairie

hay; however, but they also observed much lower PUN and ruminal ammonia concentrations then we did. This might explain why they observed improved forage intake and digestion with additional casein, whereas we did not.

Forage OM intake and total tract NDF digestibility (Table 8) were not improved by increasing the amount of supplemental protein, which suggested that factors beyond a N deficiency contributed to the glucose-induced depression in forage intake. Mould et al. (1983) stated that when ruminal pH decreases below 6.2, activity of fiber-digesting bacteria is inhibited. At 2 h after feeding, ruminal pH was 5.3 for steers receiving supplemental glucose, but pH was similar between glucose treatments 6 h after feeding (Figure 6). In addition, ruminal pH was well above the threshold for optimal fiber digestion from 6 to 24 h after feeding (Mould et al., 1983). Heldt et al. (1999) observed a similar, transient decrease in rumen pH when supplemental glucose was provided and concluded that temporarily depressed ruminal pH had little impact on fiber digestion. It is possible that much of the glucose-induced depression in forage intake and NDF digestibility was mediated through a carbohydrate-specific effect. Fiber-digesting bacteria can utilize glucose as an energy source; however, Arroquy et al. (2005) demonstrated that preferential use of glucose and oligosaccharides can delay the hydrolysis of cellulose until the non-structural carbohydrate source is depleted.

Ruminal OM digestibility, total-tract OM digestibility, and total digestible OM intake were increased when glucose was provided. This was likely caused by the fact that glucose was readily digested and it represented about 24% of total OM intake. Similarly, total digestible OM intake was increased when glucose was provided, in spite of the fact that glucose supplementation reduced forage intake, because the increased supply of readily-digested glucose more than compensated for the depression in forage intake.

The cattle's nutrient requirements for growth in this study were apparently limited more by protein supply than by energy supply. Increasing the amount of supplemental casein increased duodenal N flow and increased N retention, whereas supplemental glucose increased total digestible OM intake but did not change N retention. The pattern in N retention across our treatments was similar to that for duodenal N flow.

Urea Kinetics

Urea production in the body and the amount of urea recycled to the gut increased as we provided additional casein (Table 4), although the effect for GER was not significant. Wickersham et al. (2008) reported that UER and GER linearly increased in mature beef steers as supplemental casein supply was increased. In contrast, Marini and Van Amburgh (2003) reported that UER increased as additional N was provided in the diet but that GER plateaued as N content of the diet increased above 1.89%.

The proportion of UER recycled to the gut decreased as we supplemented additional casein, showing less efficient use of urea-N as N supply increased. Any urea-N not recycled to the gut is excreted in the urine, a basic premise of the model of Lobley et al. (2000), and we observed an increase in UUE as additional casein was provided. Both Marini and Van Amburgh (2003) and Wickersham et al. (2008) noted similar increases in UUE as they provided more N in the diet.

Supplemental glucose had no impact on UER or GER in our study, in contrast to some previous research. Kennedy (1980) observed increased transfer of urea to the rumen of cattle when sucrose was supplemented to a forage-based diet. We expected that glucose would change urea kinetics due to the fact that it provides an easily-digested energy source for ruminal microbes and, thereby, creates a demand for additional N (Kennedy and Milligan, 1980).

Supplemental glucose decreased ruminal ammonia concentrations (Figure 7) and PUN (Table 10), but it did not increase total or microbial N flow at the duodenum (Table 9). Although glucose supplementation reduced UUE, the magnitude of the decrease in UUE (3.8 or 10.6 g N/d for 240 and 480 g/d casein, respectively) was not large enough to significantly impact either GER or the proportion of UER that was recycled (GER/UER).

Microbial Use of Recycled Urea-N

For urea recycling to conserve N in time of shortfall, the ruminal microbes must utilize the urea-N recycled to the gut. In this trial, we observed a casein × glucose interaction for MNU. Increasing casein supply from 240 to 480 g/d decreased MNU when no glucose was provided. In contrast, MNU was unaffected by level of casein when glucose was provided. This likely reflected an excess of ruminally available N for the glucose/480 g/d casein treatment; our other treatments did not greatly exceed the requirement for ruminally-available N. Although 480 g/d of casein alone exceeded the requirement for ruminally-available N of control steers, this was probably not the case for the glucose/480 g/d of casein treatment. Additional casein decreased the proportion of microbial N flow that was derived from recycled urea N (MNU as, % of microbial N flow), matching observations of Marini and Van Amburgh (2003). This likely occurred because microbes had access to a greater supply of N provided directly from the supplemental casein.

The effect of supplemental N on MNU was likely related to the effects of supplements on urea recycling to the rumen and on DIP. Neutze et al. (1986) found no differences in MNU when increasing amounts of urea were provided to sheep consuming low-quality forage. In contrast, Wickersham et al. (2008) observed that the microbial capture of recycled urea N increased with casein supplementation and that the percentage of microbial N derived from

recycled urea was constant. The different response observed by Wickersham et al. (2008) may be explained by the fact that GER increased with casein supplementation at a rate very similar to that for UER (GER/UER ≥ 95%), whereas the proportion of UER that was utilized for GER decreased in our study when additional casein was supplemented. Only 43% of the increase in UER in response to increasing casein supplementation was recycled to the gut. Additionally, the cattle of Wickersham et al. (2008) demonstrated greater N deficits than our steers based on ruminal ammonia concentrations, making any additional ruminally-available N more subject to capture by rumen microbes.

Plasma Urea-N and Renal Clearance

In agreement with Wickersham et al. (2008), we observed an increase in PUN as supplemental casein increased. The increase in PUN in response to casein for control steers was greater than that for those receiving glucose. This likely reflected that the greater level of casein exceeded the ruminal requirement for available N in the absence of glucose but not in the presence of glucose. Kennedy et al. (1981) fed sheep low quality forage and provided 2 levels of sucrose and 2 levels of urea. Concentration of PUN in their study decreased when sucrose was provided, with or without urea. They attributed the decrease in PUN to increased OM fermentation in the rumen, which caused an increased demand for ruminal N.

Creatinine and urea clearances by the kidneys were measured to assess renal salvage of urea. Marini and Van Amburgh (2003) observed that 47% of urea filtered by the kidney was reabsorbed when heifers were fed a low protein diet (1.45% N) and that the reabsorption decreased to 8% as dietary N content increased to 3.40%. By comparison, we observed 58% reabsorption of urea for steers receiving 240 g/d casein and 35% reabsorption for those receiving 480 g/d casein. Sunny et al. (2007) found that renal salvage of urea was highly dependent on

PUN concentration in sheep. Our responses to casein in terms of renal urea salvage were expected because PUN concentration increased as we provided more casein. Surprisingly, glucose did not have a significant effect on fractional renal salvage of urea, even though it depressed PUN concentration. Perhaps this was due to less urea being filtered when glucose was supplemented (based on similar creatinine clearance volumes but lower PUN when glucose was supplemented), thereby eliminating the physiological need for a reduction in fractional salvage.

Effects of Cattle Maturity

We were interested in comparing this work to a generally similar study (Chapter II) conducted with less mature steers. Two major differences existed between trials; 1) animal size (208 kg in Chapter II vs. 391 kg herein), and 2) amount of supplementation. Supplement size was twice as large in this study when compared to Chapter II but forage intake (kg/d) did not differ greatly between trials. Therefore, the proportion of total intake comprised by supplements was greater in this study.

The efficiency with which supplemental N was utilized appeared to be greater for the younger cattle of Chapter II than for the more mature cattle in this study. The increase in N retention in response to increasing supplemental casein was only 5.9 g/d when casein supply increased by 34.5 g N/d in this study, compared to a 9.7 g/d increase in N retention when casein supply increased by 16.7 g N/d in the younger cattle receiving the control and glucose energy treatments. The greater use of supplemental N for protein deposition by the younger steers than by the older steers may explain, in part, why UER was responsive to increasing casein in the older but not the younger steers. The greatest level of casein supply to the younger steers was 240 g/d. It is possible that UER would have been responsive to increasing N intake at levels of casein supplementation greater than 240 g/d, as was observed with the older steers.

With both the younger and older steers, one identical treatment was applied: control/240 g/d casein. For this treatment, we observed similar levels of forage OM intake (4.4 vs. 4.2 kg/d), N intake (68.5 vs. 76.5 g/d), and duodenal N flow (73.4 vs. 72.2 g/d) between the 2 experiments, which allowed for a reasonable comparison of the results. The mature cattle in this trial produced more urea-N (88.0 vs. 55.3 g N/d) and recycled more of it to the gut (73.7 vs. 42.9 g N/d) than the less mature cattle. A portion of the greater production (and subsequent recycling) of urea can be attributed to less retention of N in the tissues by the more mature steers (7.6 vs. 19.8 g/d). In addition, ruminal ammonia concentrations of steers receiving control × 240 g/d casein were less for the older steers than the younger steers (3.7 vs. 7.0 m*M*), likely reflecting slight differences in the protein contents of the forage and in the proportion of total intake accounted for by the supplemental casein. As might be expected from the lower ruminal ammonia concentrations and greater GER, ruminal microbes of the older steers in this study captured more recycled urea-N than did the younger steers in Chapter II (18.7 vs. 8.7 g N/d). Overall, steers were better able to recycle urea to meet their N needs as maturity increased.

Conclusions

Providing glucose to steers consuming low-quality forage decreased forage intake and digestion via mechanisms that could not be corrected by increasing the provision of degradable intake protein. Providing increasing amounts of protein (as readily degradable casein) increased urea production in the body and recycling to the gut but decreased the proportion of urea production that was recycled to the gut. Providing N in excess of ruminal requirements decreased MNU but when additional ruminally-available NSC was provided in the face of adequate ruminally available N, MNU was not changed.

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Table 7. Chemical composition of hay and supplements

Item	Hay	Casein	Glucose
	----------% of DM----------		
OM	91.4	96.4	100
CP	4.7	96.0	---
NDF	72.1	---	---
ADF	46.9	---	---
Acid detergent insoluble ash	5.7	---	---

Table 8. Effects of ruminal casein and glucose supplementation on intake, digestion and N balance of steers fed prairie hay

	Control		Glucose					
	Ruminal casein supply, g/d					*P*-value		
Item	240	480	240	480	SEM[1]	Casein	Glucose	Casein × glucose
No. of observations	6	5	6	5				
OM intake, kg/d								
Forage	4.43	4.13	3.43	3.59	0.25	0.69	< 0.01	0.19
Total	4.64	4.56	4.74	5.12	0.25	0.39	0.08	0.19
Ruminal digestion, %								
OM, true	46.1	48.9	52.7	54.1	2.2	0.34	0.03	0.74
NDF	44.6	45.6	37.4	40.9	3.8	0.55	0.16	0.74
Total tract digestibility, %								
OM	55.7	55.6	60.0	60.9	1.6	0.78	< 0.01	0.70
NDF	53.9	48.8	43.4	43.1	2.1	0.09	< 0.01	0.12
Digestible OM intake, kg/d	2.58	2.54	2.84	3.12	0.13	0.21	0.01	0.10
Nitrogen, g/d								
Intake	68.5	100.1	60.1	96.8	2.0	< 0.01	< 0.01	0.11
Forage	33.9	31.6	25.6	27.7	1.9	0.93	< 0.01	0.12
Supplement	34.6	69.1	34.5	69.1	0.02	< 0.01	0.59	0.53
Fecal	32.6	33.1	31.6	35.3	2.2	0.18	0.69	0.29
Urinary	28.3	57.0	23.5	47.6	2.7	< 0.01	< 0.01	0.07
Ammonia	1.51	2.41	1.10	2.63	0.47	< 0.01	0.79	0.39
Total purine derivatives	3.95	4.40	4.14	5.46	0.30	< 0.01	< 0.01	0.02
Allantoin	3.59	3.97	3.77	4.96	0.26	< 0.01	< 0.01	0.02
Uric acid	0.36	0.42	0.36	0.50	0.04	< 0.01	0.15	0.14
Creatinine	3.96	4.12	3.91	4.56	0.27	0.03	0.27	0.15
Retained	7.6	10.5	5.0	13.9	3.6	< 0.01	0.86	0.14

[1] Largest value among treatments is reported.

Table 9. Effect of ruminal casein and glucose supplementation on nutrient flows to the duodenum and microbial efficiency in steers fed prairie hay

	Control		Glucose					
	Ruminal casein supply, g/d					*P*-value		
Item	240	480	240	480	SEM[1]	Casein	Glucose	Casein × glucose
No. of observations	6	5	5	5				
Duodenal flow, g N/d								
Total N	73.4	70.3	56.2	79.2	6.1	0.06	0.43	0.02
Microbial N	48.9	55.0	39.5	59.2	4.6	0.01	0.55	0.11
Predicted microbial N[2,3]	30.5	37.2	33.4	53.5	4.5	< 0.01	< 0.01	0.02
Undegraded intake protein	24.5	15.3	16.7	20.0	2.5	0.07	0.37	< 0.01
Microbial N from urea	18.7	7.3	18.0	18.3	3.1	0.06	0.10	0.05
% of microbial N flow	39.3	13.6	46.2	29.4	7.7	0.02	0.17	0.54
% of urea entry rate	21.5	5.3	21.2	14.5	2.7	0.01	0.13	0.08
% of gastrointestinal entry rate	27.2	8.6	24.9	19.6	3.5	0.01	0.25	0.07
Microbial efficiency								
g N/kg OM truly fermented	20.8	23.2	15.1	19.3	2.0	0.11	0.04	0.64

[1] Largest values among treatments are reported.

[2] Predicted from urinary purine derivative excretion based on the equations of Chen and Gomes (1992).

[3] Predicted microbial N was less than measured microbial N ($P < 0.01$), and the difference between measured and predicted values was greater for control than for glucose (method × glucose treatment, $P < 0.01$).

Table 10. Effect of ruminal casein and glucose supplementation on urea kinetics in steers fed prairie hay

	Control		Glucose					
	Ruminal casein supply, g/d					*P*-value		
Item[1]	240	480	240	480	SEM[2]	Casein	Glucose	Casein × glucose
No. of observations	6	5	6	5				
Urea kinetics, g N/d								
UER	88.0	137.2	86.0	124.6	21.1	0.03	0.70	0.77
UUE	14.1	41.6	10.3	31.0	3.1	< 0.01	< 0.01	0.04
GER	73.7	94.4	75.8	92.8	20.6	0.30	0.91	0.99
ROC	28.3	31.6	26.9	41.0	7.0	0.17	0.53	0.36
UUA	43.9	60.3	47.8	49.1	15.0	0.52	0.80	0.58
UFE	1.5	3.0	1.1	3.1	0.5	< 0.01	0.68	0.54
Fractional urea kinetics								
UUE/UER (u)	0.19	0.33	0.15	0.27	0.046	0.01	0.25	0.84
GER/UER	0.81	0.67	0.85	0.73	0.045	0.01	0.25	0.84
ROC/UER (p)	0.31	0.26	0.31	0.33	0.031	0.35	0.14	0.15
ROC/GER (r)	0.39	0.35	0.37	0.46	0.041	0.46	0.27	0.14
UUA/GER (a)	0.59	0.61	0.61	0.50	0.043	0.29	0.32	0.13
UFE/GER (f)	0.02	0.03	0.02	0.04	0.006	0.03	0.63	0.47

[1] UER = urea-N entry rate; UUE = urinary urea-N elimination; GER = gastrointestinal entry rate; ROC = urea-N returned to ornithine cycle; UUA = urea-N utilized for anabolism; UFE = urea-N excreted in feces.
[2] Largest values among treatments are reported.

Table 11. Effect of ruminal casein and glucose supplementation on plasma metabolite concentrations in steers fed prairie hay

	Control		Glucose					
	Ruminal casein supply, g/d					*P*-value		
Item	240	480	240	480	SEM[1]	Casein	Glucose	Casein × glucose
No. of observations	6	5	6	5				
Renal clearance								
Creatinine, L/d	772	816	756	933	66	0.03	0.28	0.14
Urea, L/d	285	491	352	624	62	< 0.01	0.01	0.33
Urea/creatinine, %	36.5	62.2	47.6	67.7	7.1	< 0.01	0.15	0.59
Plasma								
Urea-N, m*M*	3.5	6.4	2.0	3.4	0.47	< 0.01	< 0.01	0.05
Glucose, m*M*	3.9	4.0	3.9	3.6	0.16	0.67	0.18	0.29
Creatinine, µ*M*	122.2	125.4	123.8	118.6	7.3	0.88	0.71	0.52
Amino acids, µ*M*								
Ala	208	179	166	148	24	0.30	0.13	0.80
Gly	215	178	237	208	26	0.20	0.31	0.87
Val	262	276	190	200	34	0.73	0.05	0.94
Leu	152	137	92	102	17	0.86	0.01	0.38
Ile	88	83	56	60	10	0.98	0.01	0.61
Thr	80	67	56	52	11	0.31	0.05	0.60
Ser	45	40	36	34	7	0.63	0.29	0.85
Pro	76	69	60	61	8	0.65	0.14	0.57
Asn	27	26	18	17	4	0.75	0.02	0.91
Asp	7	6	7	10	1	0.50	0.12	0.15
Met	26	24	17	17	3	0.61	0.01	0.63
Glu	115	106	121	110	17	0.53	0.77	0.98
Phe	57	56	41	46	6	0.75	0.05	0.62
Gln	229	230	178	181	26	0.93	0.04	0.99
Orn	80	81	58	68	11	0.97	0.28	0.38
Lys	115	99	68	70	15	0.63	0.03	0.54
Tyr	48	46	33	39	6	0.71	0.07	0.41
Trp	32	27	25	29	4	0.94	0.45	0.23

[1] Largest value among treatments is reported.

Table 12. Effect of ruminal casein and glucose supplementation on ruminal fermentation characteristics in steers fed prairie hay

	Control		Glucose					
	Ruminal casein supply, g/d					*P*-value		
Item	240	480	240	480	SEM[1]	Casein	Glucose	Casein × glucose
No. of observations	6	5	6	5				
Ruminal[2]								
pH	6.7	6.7	6.6	6.6	0.1	0.65	0.15	0.20
Ammonia, m*M*	3.7	11.5	2.2	5.4	0.7	< 0.01	< 0.01	< 0.01
Total VFA, m*M*	93.7	97.6	75.1	83.0	5.9	0.07	< 0.01	0.51
Acetate, m*M*	72.2	70.9	47.1	51.6	4.3	0.49	< 0.01	0.19
Propionate, m*M*	13.0	14.2	16.0	16.3	0.9	0.32	< 0.01	0.58
Butyrate, m*M*	5.0	6.0	8.2	9.2	1.1	0.14	< 0.01	0.96
Isobutyrate, m*M*	1.0	1.9	0.7	1.1	0.1	< 0.01	< 0.01	0.03
Valerate, m*M*	1.1	2.2	2.4	3.0	0.3	< 0.01	< 0.01	0.36
Isovalerate, m*M*	1.4	2.5	0.8	1.6	0.2	< 0.01	< 0.01	0.47

[1] Largest value among treatments is reported.
[2] Average of values collected at 2, 6, 10, 14, 18, and 22 h after feeding.

Figure 6. The effect of glucose supplementation on ruminal pH in steers fed prairie hay. Glucose was dosed ruminally once daily at feeding. Energy × hour interaction, $P = 0.01$; SEM = 0.06.

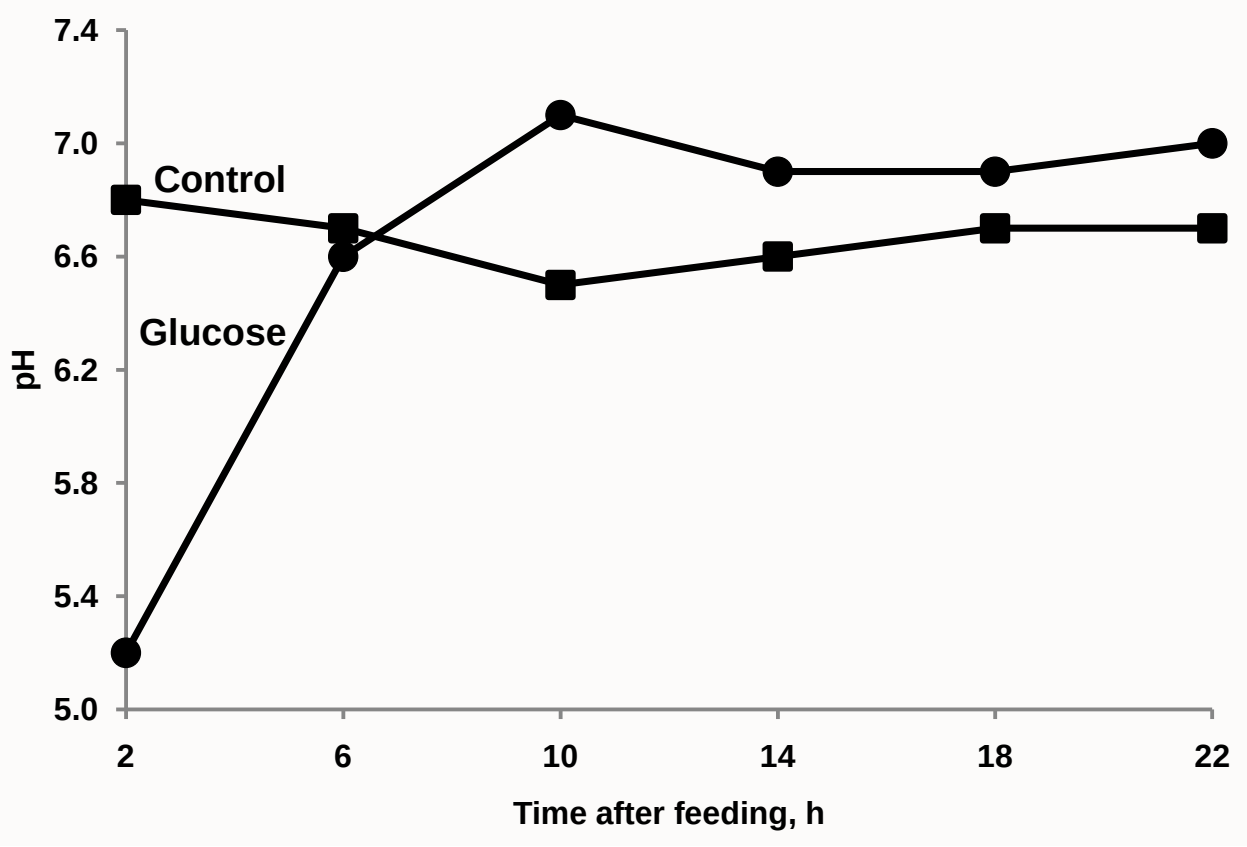

Figure 7. The effect of casein and glucose supplementation on ruminal ammonia concentration in steers fed prairie hay. Casein and glucose were dosed ruminally once daily at feeding. Energy × casein × hour interaction, $P = 0.01$; SEM = 0.66.

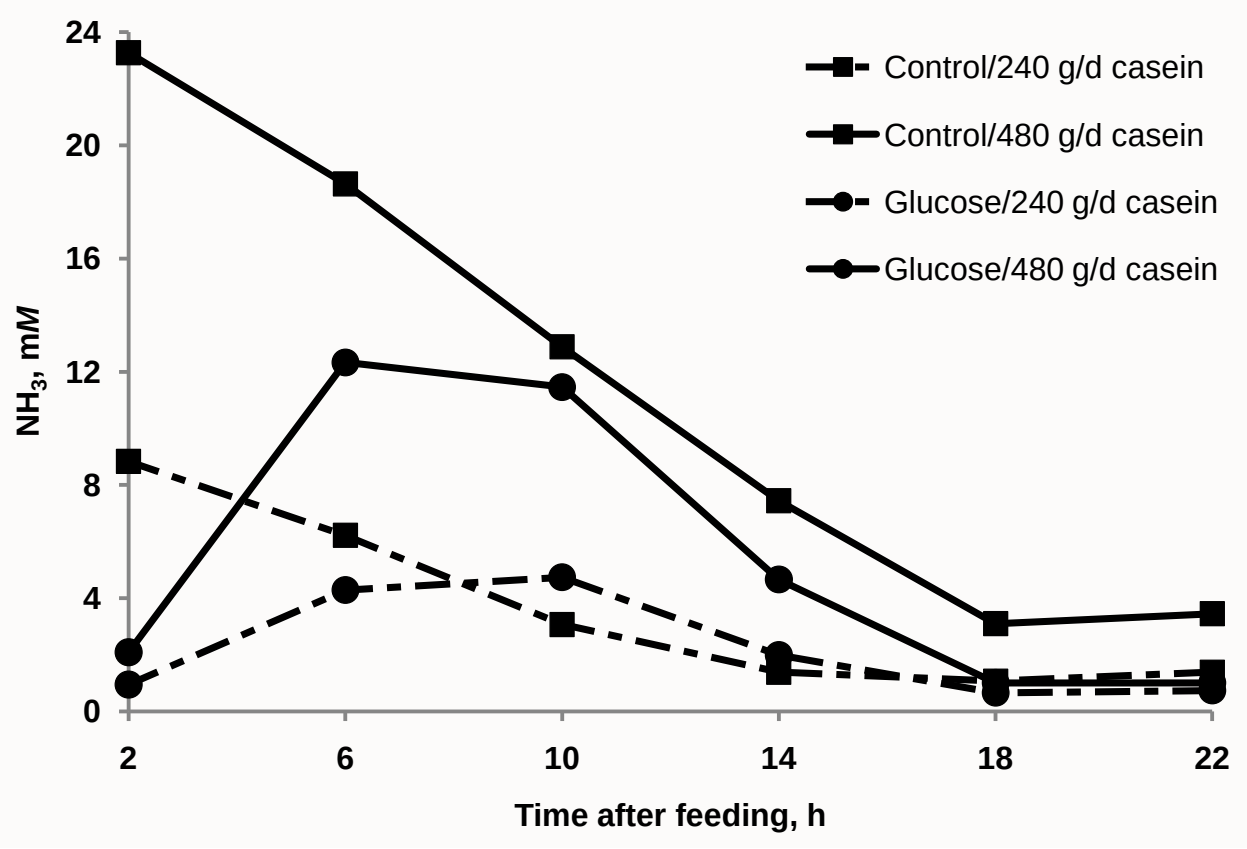

Figure 8. The effect of glucose supplementation on ruminal acetate concentration in steers fed prairie hay. Glucose was dosed ruminally once daily at feeding. Energy × hour interaction, P = 0.01; SEM = 4.3.

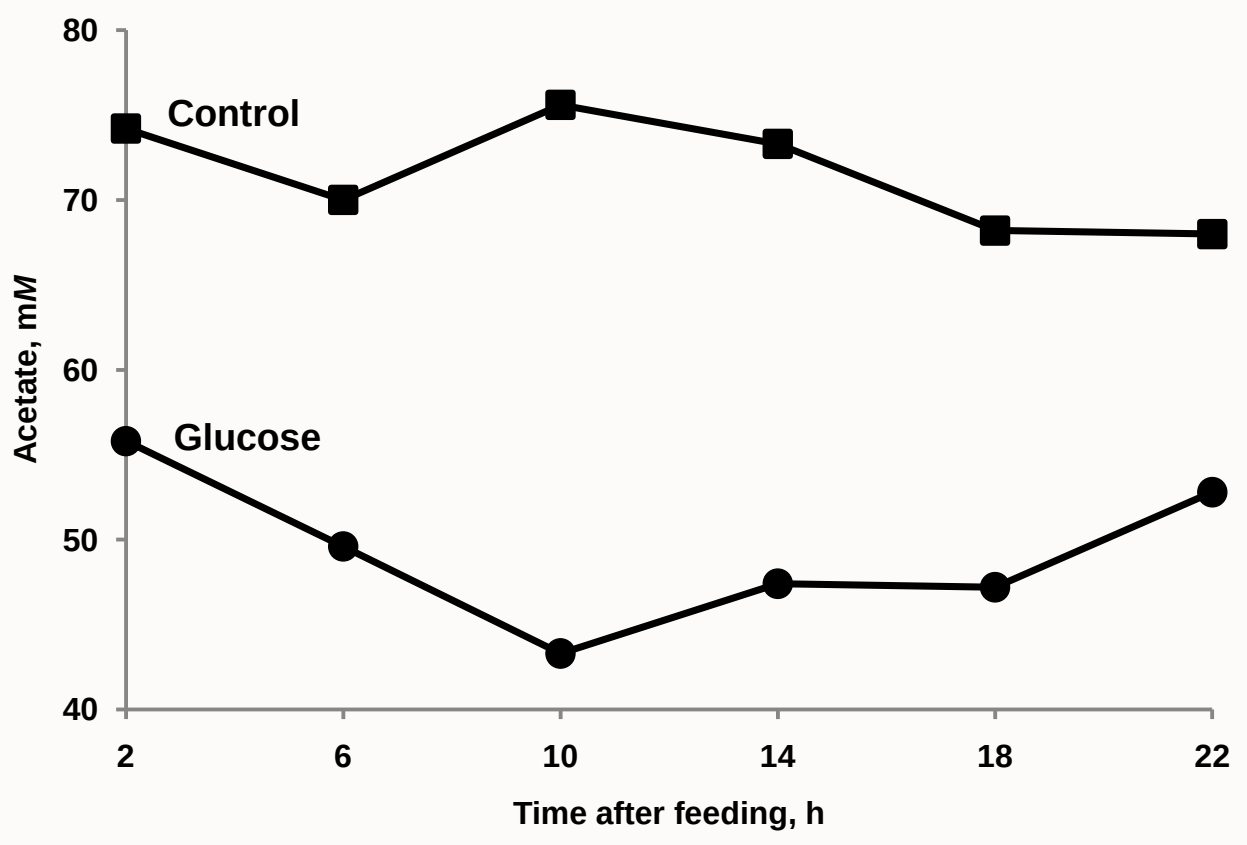

Figure 9. The effect of glucose supplementation on ruminal propionate concentration in steers fed prairie hay. Glucose was dosed ruminally once daily at feeding. Energy × hour interaction, $P = 0.01$; SEM = 0.94.

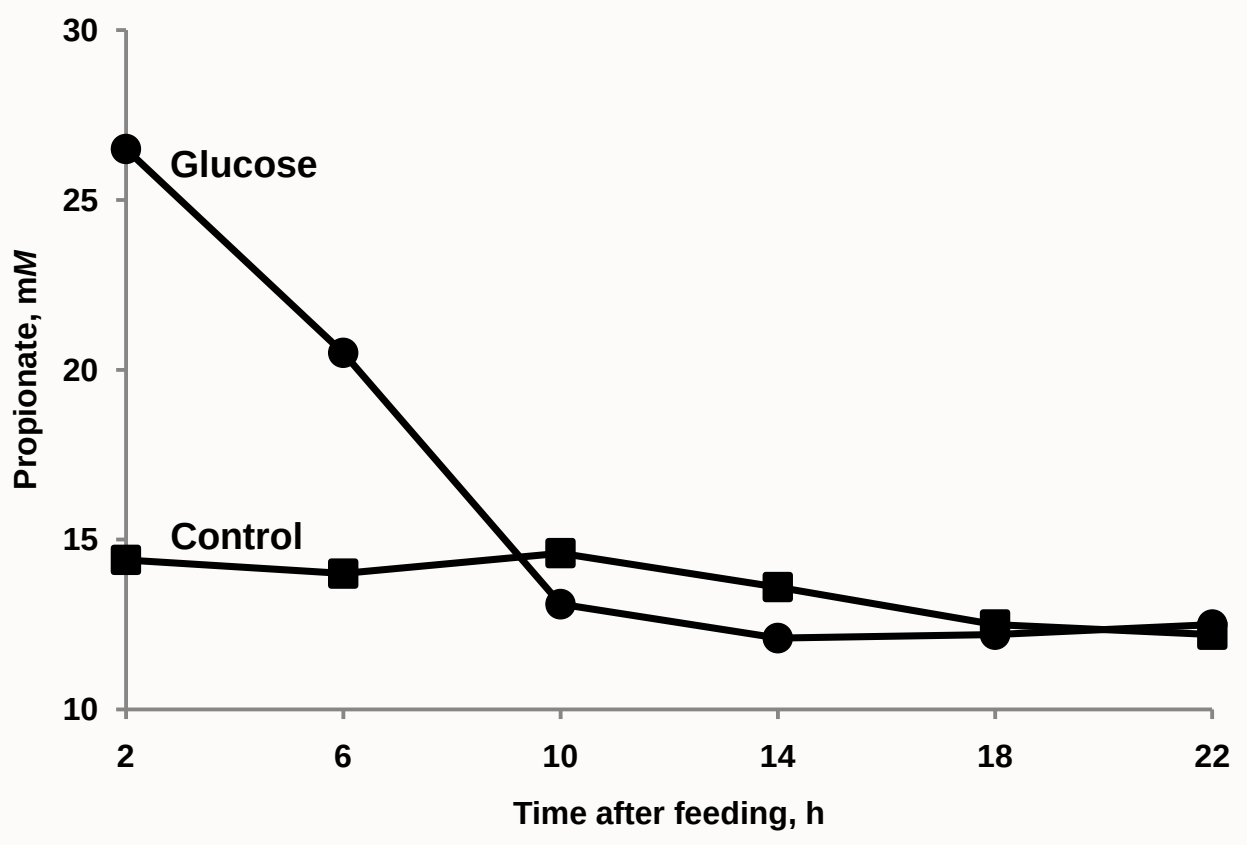

Figure 10. The effect of glucose supplementation on ruminal butyrate concentration in steers prairie hay. Glucose was dosed ruminally once daily at feeding. Energy × hour interaction, *P* = 0.01; SEM = 1.1.