AN ABSTRACT OF THE THESIS OF

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Data is limited evaluating infrequent supplementation of urea or biuret to ruminants consuming low-quality forage (< 6% crude protein). Therefore, a series of experiments were designed to compare the effects of daily (D) and alternate day (2D) supplementation of two non-protein nitrogen (NPN) sources (urea or biuret) to ruminants consuming low-quality forage. Experiment 1 was a N balance study using five wethers in an incomplete 5 x 4 Latin square design (five treatments; four 24-d periods) with a 2 x 2 factorial arrangement of treatments (two sources of NPN and two supplementation frequencies) and an unsupplemented control. Supplements, consisting of urea or biuret mixed with ground soy hulls and dried molasses, were isonitrogenous (approximately 26% crude protein; dry matter basis) and offered D or 2D. The 2D supplemented lambs received double the quantity of supplemental N on their supplementation day compared with D lambs; therefore, all D and 2D treatments received the same amount of supplemental N over a 2-d period. Experiment 2 was a 70-d cow performance study using 80 spring-calving

cows during the last third of gestation. Cows were stratified by age, body condition score, and expected calving date, and assigned randomly within stratification to one of the five treatments described in Experiment 1 above. They were then sorted by treatment and randomly assigned to 1 of 20 pens (4 cows/pen, 4 pens/treatment). Experiment 3 was a site of digestion study using five ruminally and duodenally fistulated steers to compare D and 2D supplementation of urea or biuret on forage intake, ruminal fermentation, site and extent of nutrient digestion, and rumen microbial efficiency. Five ruminally and duodenally fistulated steers were used in an incomplete 5 x 4 Latin square design with the same treatments described in Experiment 1. The results for Experiment 1 reported that DM, OM, and N intake, DM, OM, and N digestibility, N balance, and digested N retained were greater ($P < 0.03$) for supplemented wethers compared with CON with no difference ($P > 0.05$) because of NPN source or SF. Supplemented lambs had increased plasma urea N (PUN) compared with CON ($P < 0.01$) and urea treatments had greater PUN compared with biuret ($P < 0.01$). Also, PUN was increased ($P = 0.02$) for D compared with 2D treatments. In addition, data suggest that PUN exhibited less fluctuation on the day of a supplementation event for biuret compared with urea. Experiment 2 demonstrated that pre- and post- calving (within 14 d and 24 h of calving, respectively) cow weight and body condition score changes were more positive ($P<0.05$) for supplemented groups compared with the CON. In Experiment 3, forage OM intake and OM digestibility were not affected ($P > 0.05$) by NPN supplementation, NPN source, or SF. However, total OM and N intake

were increased ($P < 0.01$) with supplementation. Duodenal flow of OM tended ($P = 0.08$) to increase with NPN supplementation while N flow was greater ($P = 0.04$) with NPN supplementation compared with the control. In addition, duodenal bacterial N flow was increased with NPN supplementation ($P = 0.04$) and for biuret compared with urea ($P < 0.01$). Bacterial efficiency (g bacterial N/kg OM truly digested in the rumen) was greater for the control compared with NPN treatments ($P < 0.01$) while biuret had greater true N disappearance compared with urea ($P = 0.01$). Intestinal disappearance (% of duodenal flow) of OM and N was not affected by NPN supplementation, NPN source, or SF. However, apparent total tract N digestibility was increased with NPN supplementation ($P < 0.01$) and not affected by NPN source or SF. In addition, ruminal NH_3-N increased ($P < 0.04$) on the day all supplements were provided and the day only daily supplement were provided with supplemental NPN. However, an NPN source × SF interaction ($P = 0.03$) on the day all supplements were provided indicated NH_3-N increased at a greater rate for urea as SF decreased compared with biuret. Ruminal NH_3-N on the day only daily supplements were provided was greater for D compared with 2D ($P = 0.02$). This data suggests that ruminal degradation of biuret to NH_3-N was more moderate and prolonged compared with urea, possibly improving use by ruminal microflora. On the day all supplements were provided, D treatments had increased ($P = 0.05$) ruminal indigestible acid detergent fiber passage rate and ruminal liquid volume compared with 2D treatments. Overall, NPN supplementation when feeding low-quality forage (<6% CP) was more beneficial than compared to a

negative control, for increasing efficiency of forage digestion, N use, and animal performance. While at the same time indicating that the infrequent supplementation of urea or biuret was not detrimental to forage nutrient utilization, N efficiency or cow performance. This research will provide researchers and ruminant livestock producers with original information that can be used in designing winter supplementation strategies that decrease supplementation costs.

KEY WORDS: Urea, Biuret, Forage, Non-Protein Nitrogen, Supplementation, Frequency

Daily and Alternate Day Supplementation of Urea or Biuret to Ruminants Consuming Low-Quality Forage

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CONTRIBUTION OF AUTHORS

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INTRODUCTION AND LITERATURE REVIEW

Cattle and other ruminants have been foraging on low quality (<6% CP) rangelands since before the domestication of cattle by humans. It has been demonstrated that cattle are able to harvest forage and produce meat, milk and/or fiber. This allows for the productive use of rangeland that is unsuitable for farming. The importance of the ruminant animal can be better defined in retrospect to total landmass. Approximately one-third of the Earth's surface is land and approximately 40% of this is made up of rangeland. Therefore, grazing ruminants provide land managers with a management tool that can be used to manipulate plant density and plant vigor while producing consumer products. However, the difficulty arises from the fact that the nutritional value of these forages in the late summer through winter is not sufficient to meet the nutritional requirements for most ruminants (Turner and DelCurto, 1991). This low-quality forage can be deficient in energy and (or) protein. Therefore, alternative methods need to be developed to efficiently utilize these forage resources in late summer and winter.

Supplementation of low-quality (<6% CP) forage with protein has been shown to increase cow weight gain and body condition score (Clanton and Zimmerman, 1970; Beaty et al., 1994) and forage intake and digestibility (Köster et

al., 1996). In addition, infrequent supplementation of natural protein to ruminants consuming low-quality forage has been shown to result in acceptable performance (Huston et al., 1999; Bohnert et al., 2002b) and nutrient utilization (Coleman and Wyatt, 1982; Krehbiel et al., 1998; Bohnert et al., 2002b) compared with daily supplementation. However, information is lacking concerning infrequent supplementation of non-protein N (**NPN**) to ruminants consuming low-quality forage. Non-protein N is an attractive source of supplemental protein because of its low cost compared with most sources of natural protein (N basis). However, a major concern of infrequent supplementation of NPN is the potential for NH_3 toxicity. Ammonia toxicity can result from the rapid production of ruminal NH_3-N often observed with NPN supplementation. We are aware of limited data comparing urea and biuret as CP supplements to ruminants consuming low-quality forage. Therefore, the objectives of this research was to compare daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage and measure the effects on forage intake, efficiency of N use, weight and body condition score change, site of nutrient digestion, microbial CP production, and ruminal fermentation.

RUMINANT N METABOLISM

When ruminants are consuming low-quality (<6% CP) forage, the microbial flora found in the rumen are converting what structural carbohydrates and protein (N) are available in the forage into microbial crude protein and/or volatile fatty

acids. Optimal use of ruminally available protein (including non-protein N) would logically occur if protein and carbohydrate degradation in the rumen were occurring simultaneously; however, this does not occur with most diets. Typically, forage protein degradation is rapid while degradation of energy-yielding components of neutral detergent fiber (carbohydrate) are much slower (NRC, 1996; Tamminga, 1979). Degradable intake protein (**DIP**) is the portion of ingested or recycled N that is degraded in the rumen to NH_3-N and free amino acids or peptides. These can then be used by ruminal bacteria to reproduce and, consequently, produce additional microbial CP (**MCP**; NRC, 1996). Microbial CP is only a portion of the protein available to meet the animal's protein requirements. The protein flowing to the small intestines of ruminants is composed primarily of MCP and undegraded intake protein (**UIP**; NRC, 1996). Undegraded intake protein is the portion of feed protein that passes through the rumen undegraded and is potentially absorbable in the small intestine (NRC, 1996).

Ammonia (NH_3)

Hungate (1966) supported earlier research suggesting dietary protein is fermented in the rumen to simpler N compounds and reincorporated into MCP. Ammonia makes up the largest portion of the ruminal N pool (Hungate, 1966). Three sources introduce NH_3-N into the rumen, one is the degradation of dietary protein or hydrolysis of dietary non-protein N, second is hydrolysis of urea recycled to the rumen, and third is the degradation of microbes within the rumen

(Hungate, 1966). Bloomfield et al. (1960) indicated that urea hydrolysis occurred four times faster than uptake of liberated NH_3-N, thus resulting in eventual loss of N available for microbial protein synthesis. The fate of NH_3-N in the rumen is controlled by three factors. These are: 1) microbial uptake and incorporation into MCP, 2) absorption from the rumen into the portal blood for transport to the liver where it is detoxified to urea, and 3) passage through the reticulo-rumen and absorbtion from the omasum and/or small intestine for transport to the liver and detoxification to urea (Egan, 1980; Owens and Bergen, 1983).

Many cellulolytic ruminal bacteria prefer, or require, N in the form of NH_3-N (Russell et al., 1992). Assimilation of NH_3-N into MCP by rumen bacteria implies they have the ability to construct the necessary carbon skeletons from carbohydrate sources or fermentation end-products (Hoover et al., 1963). Ruminal bacteria have a high affinity for NH_3-N and can efficiently maintain growth and reproduction with NH_3-N concentrations $\geq$2 mg/100 ml rumen fluid (Funk et al., 1987).

Amino Acids, Peptides, Branched Chain Volatile Fatty Acids

Many rumen bacteria have a requirement for branched chain volatile fatty acids (**VFA**; Yokoyama and Johnson, 1988). These bacteria are predominantly cellulolytic and, therefore, are important to the ruminal digestion of low-quality forage. In addition, cellulolytic bacteria make up a significant portion of the rumen bacterial population of forage fed ruminants. Branch chain VFA's result from the

ruminal degradation of the amino acids valine, leucine, isoleucine and proline to isobutyrate, isovalerate, 2-methyl butyrate and valerate, respectively (Leng, 1973). As a result, fiber digestion is at least partially dependant on the supply of branched chain VFA's from the diet or from other bacteria in the rumen (Owens and Zinn, 1988). Consequently, DIP from sources of natural protein is a common supplement to ruminants consuming low-quality forage.

Microbial Crude Protein

Ruminal production of microbial crude protein (MCP) is dependent on the quantity of organic matter fermented in the rumen, which is a measure of the energy available to the ruminal microbes (Zinn and Owens, 1983). Several factors have been found to influence the efficiency of microbial growth. They include the extent and rate of N and carbohydrate degradability in the rumen and rumen dilution rate (Stern and Hoover, 1979). It has been estimated that 50 to 80% of the microbial N in the rumen comes from ruminal NH_3-N (Mercer and Annison, 1976). The remaining microbial N comes from preformed amino acids.

Sheep fed purified diets containing urea as a sole source of N showed that the microbial biomass contained all of the essential amino acids required for ruminants (Loosli et al., 1949). However, ruminal MCP may not meet the essential amino-acids needs of a high producing ruminant (Huber and Kung, 1981). Bergen et al. (1968a,b) determined the biological value of MCP to be 80 using N balance studies to determine net protein utilization. Approximately 40 to 80% of the total

protein reaching the small intestine of ruminants is MCP, with the quantity dependent on the UIP content of the diet, the energy sources in the rumen for microbial growth, and the ruminal digesta and liquid passage rate (Owens and Bergen, 1983).

Stern and Hoover (1979) compared the results of numerous studies and reported that approximately 27 g of microbial N is synthesized/kg of organic matter (OM) apparently digested in the rumen. Also, Zinn and Owens (1983) noted that microbial efficiencies with and without N supplementation averaged 24 g microbial N/kg OM fermented, ranging from 19 to 30 g microbial N/kg OM fermented for unsupplemented and supplemented treatments, respectively. Köster et al. (1996) supplemented cows consuming dormant tallgrass prairie forage with increasing levels of DIP (casein) and reported that microbial efficiency (g microbial N/kg OM truly digested in the rumen) increased from 12 for unsupplemented cows to 20 for cows receiving 720 g/d of casein.

A reduction in N utilization by ruminal microbes is possible with a ruminal sulfur deficiency. This is because ruminal microorganisms require sulfur for the synthesis of methionine and cysteine. This is especially important when NPN is used as a CP supplement. Hume and Bird (1970) compared production of MCP in sheep fed a semi-purified diet in which urea was used as the source of dietary N and the N:sulfur ratio was varied from approximately 6:1 to 34:1. They noted that production of MCP increased when the N:sulfur ratio was approximately 10:1

compared with 34:1. Also, they reported that lowering the N:sulfur ratio to 6:1 did not increase MCP production compared with a ratio of 10:1.

N Recycling

The ability of ruminants to recycle N is useful because the animal is able to incorporate a portion of the recycled N into MCP. Estimates of N recycled to the rumen have ranged from 6-15 g/d for sheep and 24-60 g/d for cattle (Houpt, 1970; Owens and Zinn, 1988). Huntington and Archibeque (1999) suggested that the amount N recycled was related to N intake and the productive priorities of the animal. Also, they noted 19 to 96% of endogenous urea could be recycled to the gut, with 15 to 94% of that being transferred via saliva. Additionally, they estimated 10 to 42% of N intake could be transferred directly across the gut wall as urea. The entire process begins with passive absorption of NH_3-N from the rumen or small intestine into the portal blood (Owens and Zinn, 1988). In addition to simple diffusion, there are mechanisms, such as association with bicarbonate or VFA anions, that provide for transport of small quantities of ammonium ions into the blood (Parker et al., 1995). The NH_3-N is then transported via the hepatic portal vein to the liver to be detoxified into urea (Cocimano and Leng, 1967). Once in the liver, the enzymes of the ornithine cycle and catalyzing transamination reactions form urea (Katz, 1992). Plasma urea then exits the liver via the hepatic veins to the inferior vena cava and into the general circulation. Plasma urea can

then be recycled back to the rumen through saliva or by simple diffusion across the ruminal epithelium (Chalmers et al., 1976; Cocimano and Leng, 1967).

Studies indicate that 15 to 50% of N transfer is by the salivary route for cattle and sheep fed forage-based diets (Kennedy and Milligan, 1980). The amount of urea that is involved with salivary secretion appears to be determined mainly by factors such as intake and the proportion of forage and concentrate in the diet (Kennedy and Milligan, 1980). The diffusion of urea into the rumen is associated with urease in the ruminal epithelium, probably by the bacteria adhering to the rumen wall, which hydrolyzes into free NH_3-N (Owens and Bergen, 1983; Bunting et al., 1989). The free NH_3-N then diffuses through the ruminal epithelium into the rumen where it is trapped by conversion to the ammonium ion (NH^+_4; Kennedy and Milligan, 1980). The quantity of N recycled to the rumen appears to be negatively related to ruminal NH_3-N concentration and positively correlated to plasma urea concentration and OM digestion in the rumen (Chalmers et al., 1976; Kennedy and Milligan, 1980). A high ruminal NH_3-N concentration reduces recycling by inhibiting urease associated with the rumen wall and decreasing the NH_3 diffusion gradient across the ruminal epithelium (Huntington and Archibeque, 1999). It has been determined that plasma urea concentration is positively related to N intake, with approximately 70% of N intake passing into the plasma urea pool (Harmeyer and Martens, 1980). It has been estimated that 0 to 80% of NH_3-N resulting from urea degradation is incorporated into bacterial N (Slyter et al., 1979), with energy

availability being the major determinant of the percentage (increased incorporation with high energy and decreased incorporation with low energy).

N recycling serves to complement low N diets consumed by ruminants. N is conserved by decreasing urinary excretion of plasma urea via slowing of renal clearance (Schmidt-Nielsen, 1957). Therefore, a greater quantity of plasma urea is available for recycling to various portions of the digestive tract. Urea is an important source of N entering the gut, with 10 - 42% of N intake and 23-92% of the plasma urea being recycled to the digestive tract. The higher values are associated with lower N intakes and vise versa (Huntington, 1986; Kennedy and Milligan, 1980). Therefore, low N intake results in a greater proportion of N returning to the rumen and less excreted in the urine (Harmeyer and Martens, 1980). This can result in negative apparent ruminal N digestibility and duodenal N flow that exceeds N intake. This is common with low protein diets (Köster et al., 1996; Bohnert et al., 2002a).

Urinary N Excretion

Cocimano and Leng (1967), Thornton (1970), and Harmeyer and Martens (1980) reported that plasma urea concentration was highly correlated with urinary urea excretion in both cattle and sheep. Renal excretion of urea is increased when N intake is adequate (based on requirements) and is reduced when N intake is below requirements (Harmeyer and Martens, 1980). Bunting et al. (1989) and Huntington (1989) found factors such as ruminal digestiblity of dietary

carbohydrate and N intake affect the quantity of urea, expressed as a percentage of N intake, excreted in urine. Also, the quantity of urea that is excreted by the kidneys is probably in response to changes in plasma urea concentration and corresponding changes in filtered urea loads, changes in glomular filtration rate, and/or changes in tubular resorption of urea (Harmeyer and Martens, 1980). In addition, Harmeyer and Martens (1980) and Kennedy and Milligan (1980) suggest that daily changes, such as restricted feeding and (or) consumption of low-protein diets, can alter the permeability of the gastrointestinal tract to urea and change regulation of renal urea excretion. Schmidt-Nielson et al. (1957) reported that 40% of urea N filtered by the glomeruli in camels consuming a maintenance diet was excreted in the urine compared with 1 to 2% when a N-deficient diet was fed. A similar phenomenon was observed with sheep (Schmidt-Nielson et al., 1957; Schmidt-Nielson and Osaki, 1958). Schmidt-Nielson and Osaki (1958) noted that the proportion of urea excreted by the kidney decreased from 42% of that filtered by the glomeruli in ewes consuming a 7.5% digestible CP diet to 14% for those ewes consuming a 3% digestible CP diet. This adaptation began within 24 h and seemed to stabilize within 4 d.

CRUDE PROTEIN SUPPLEMENTATION OF LOW QUALITY FORAGE

What is Low-Quality Forage

In terms of defining low-quality forage, a baseline needs to be set, and for this area of research an obvious baseline would be the nutrient requirements of a beef cow during the last third of gestation. Therefore, the nutrient requirements of a 550 kg mature beef cow during the last third of gestation are 53% total digestible nutrients (**TDN**) and 8% CP (DM basis; NRC, 1984). Applying this base line in context to forage quality, forage TDN and CP should be equal to or greater than 53 and 8%, respectively. Therefore, forage with TDN or CP less than the aforementioned values are considered low-quality for this discussion. Another indicator of low-quality forage is neutral detergent fiber (NDF). Forages that are greater than 70% NDF are likely suspects for a deficiency in either TDN or CP. In addition, research has suggested that forage NDF is inversely related to forage DMI by ruminants (Mertens, 1994). Briefly, as NDF concentration increases forage intake can be expected to decrease and vice versa.

Why Supplement Low-Quality Forage

Minimize N Deficiency

Protein tends to be the most beneficial supplemental nutrient when feeding low-quality roughages ad libitum (Campling et al., 1962). Therefore, when

supplementing low-quality forage, the total ration should be balanced to meet, but not exceed, the CP requirement of that animal (DelCurto et al., 1999). Funk et al. (1987) noted that the efficiency of MCP synthesis in steers grazing blue-gamma rangeland throughout the growing season was depressed in the later stages of summer dormancy and could be attributed to the ruminal NH_3-N concentration dropping below 2 mg/100 ml. Furthermore, they go on to state that CP supplementation during this period should correct the ruminal N deficiency and thereby improve the N status of the animal.

Stimulate Forage Intake

Crude protein supplementation may be effective in increasing voluntary forage intake when the CP content of the forage is low (Clanton and Zimmerman, 1970). This has been demonstrated in numerous studies with beef cattle consuming low-quality forage (DelCurto et al., 1990b; Köster et al., 1996; Bandyk et al., 2001). DelCurto et al. (1990b) provided supplements varying in CP concentration from 12 to 41% to steers consuming dormant tallgrass prairie forage (isonitrogenous basis) and increased forage intake compared with an unsupplemented control. Also, Köster et al. (1996) provided increasing amounts of supplemental protein (casein) to beef cows fed dormant tallgrass prairie forage and linearly increased forage intake compared with an unsupplemented control. Simularly, Bandyk et al. (2001) infused casein ruminally or post ruminally in steers consuming low-quality, tallgrass prairie hay and increased forage intake by

approximately 62 and 28%, respectively, compared with an unsupplemented control.

Improve Nutrient Utilization

Nutrient utilization by ruminants consuming low-quality forage can be increased with CP supplementation. This can occur by improving diet digestibility, increasing intake while maintaining DM digestibility, or by a combination of the two. Crude protein supplementation of low-quality forage has been shown to increase DM digestibility (DelCurto et al., 1990b; Beaty et al., 1994; Bohnert et al., 2002a). This is most likely the result of improved N availability for the ruminal microflora (Petersen, 1987) and (or) provision of a CP supplement that is more digestible than the basal forage. In addition, research has suggested that supplemental CP can increase the rate of digestion of fibrous constituents (Caton et al., 1988). This increased rate of digestion with protein supplementation of low-quality forage may also be related to observed increases in digesta passage (McCollum and Galyean, 1985; Caton et al., 1988).

Increased Performance

Clanton and Zimmerman (1970) reported that CP supplementated cow gained more weight and were in a more positive N balance compared with unsupplemented cows fed 8% CP bromegrass hay. This has been supported by recent research (DelCurto et al., 1990a; Mathis et al., 1999; Bohnert et al., 2002b).

DelCurto et al. (1990a) supplemented beef cows grazing dormant tallgrass prairie forage with low, moderate, and high protein supplements (14, 25, and 39% CP respectively) and linearly improved cow weight and BCS change during the last third of gestation as CP concentration increased. Also, Mathis et al. (1999) provided soybean meal at increasing levels (0.08, 0.12, 0.16, 0.20, 0.24, 0.32, 0.40, and 0.48% of BW/day) to beef cows grazing dormant, tallgrass prairie forage and improved cow weight and BCS change in the 69 days before calving. Bohnert et al. (2002b) supplemented beef cows consuming 5% CP meadow hay with a DIP or UIP supplement every day, once every 3 d, or once every 6 d during the last third of gestation. They noted that cow weight and BCS were improved with CP supplementation compared with an unsupplemented control.

Improved Reproduction

Sasser et al. (1988) reported that a protein intake below requirements could increase the postpartum interval to first estrus, first service to conception, and decrease the number of cows that demonstrate estrus and conceive. They fed beef cows a diet that was either adequate in CP (21%) or deficient in CP (7%) for normal performance beginning at approximately 150 d prepartum and continuing until 110 d postpartum. They noted the number of cows exhibiting estrus by 110 d postpartum increased from 63% with the inadequate diet to 89% with the adequate diet. Similarly, overall pregnancy rate at 110 d postpartum was 74% compared with 32% for the cows receiving the adequate and inadequate diets, respectively.

Additionally, Clanton (1980) reported results from a five-year study that evaluated the effects of supplemental CP during the last third of gestation on cow reproductive performance, noting that the number of days from calving to first estrus was 70 and 64 days for cows receiving a diet that was approximately 6 or 8% CP, respectively. Clanton (1980) also stated that conception rate of the cows that calved was increased by 11% for those receiving the 8% diet compared with those receiving the 6% diet.

Urea

Nutritionists have known for over a century that urea can be used by ruminants and converted to protein (Helmer and Bartley, 1971). The chemical formula of urea is $(NH_2)_2CO$. Therefore, urea contains 46.7% N or 291% CP (N% * 6.25). Urea is rapidly degraded by the urease enzyme to two molecules of NH_3 and one molecule of CO_2. Research has demonstrated that ruminal bacteria possess inherent urease activity and can rapidly hydrolyze urea at a rate that exceeds their ability to assimilate the NH_3-N to bacterial protein (Bloomfield et al., 1960).

The high CP content of urea makes it an attractive CP supplement because it can be provided in small amounts (compared with sources of natural protein) while greatly increasing the CP content of the diet or ration. Early work by Virtanen (1966) and Oltjen (1969) noted that ruminants could survive and be productive on diets in which urea provided the sole source of N (CP). However, the generally accepted guidelines for use of urea in ruminant diets are that urea

should not constitute more than 3% of a concentrate mix (supplement), it should be less than 1% of the total diet, and it should provide not more than one-third of the total CP in the diet (Chalupa, 1968).

Disadvantages Associated with Urea

Toxicity

The rapid hydrolysis of urea to NH_3-N in the rumen often exceeds the capacity of assimilation by rumen bacteria (Helmer and Bartley, 1971). This can lead to toxicity. Toxicity occurs when there is an inability of the liver to convert all absorbed NH_3-N to urea, thereby creating increased levels of NH_3-N in peripherial blood (Helmer and Bartley, 1971). Lewis (1960) noted a direct relationship between rumen NH_3-N concentration, blood NH_3-N concentration, rumen pH, and NH_3-N toxicity. Lewis (1960) found that toxicity occurred when rumen NH_3-N exceeded 176 mg/100 ml or when blood NH_3-N exceeded 0.4 mg/100 ml. Also, as ruminal pH approached the pKa of NH_3-N ($\approx$9), blood NH_3-N greatly increased because of the greater absorption of ruminal NH_3-N. The liver's capacity to convert NH_3-N to urea is overwhelmed at rumen fluid concentrations greater than 84 mg NH_3-N /100 ml. According to Fonnesbeck et al. (1975) and Owens and Zinn (1988) ruminal NH_3-N concentrations approach toxic levels at 85 to 100 mg NH_3-N /100 ml. However, there is no consensus on the concentration of ruminal NH_3-N that results in toxicity. Urea intake, expressed as g/kg BW, has been used

to minimize the potential for urea toxicity. Helmer and Bartley (1971), in a thorough review of urea as a protein replacement for ruminants, suggest that urea toxicity is probable if urea intake is greater than 0.30 to 0.51 g/kg BW. As a result, it is not recommended to formulate diets that will result in urea intakes that exceed 0.30 g/kg BW.

Palatibility

Chalupa (1968) stated in his review of the problems associated with feeding urea to ruminants that substituting urea for sources of true protein could result in decreased supplement intake. This has been shown in numerous studies (Forero et al., 1980; Owens et al., 1980; Köster et al., 2002). Forero et al. (1980) supplemented cows grazing dormant native range in Oklahoma with natural protein, urea, or slow-release urea. They reported that the natural protein and slow-release urea supplements were consumed readily while only approximately 60% of the urea supplements were consumed. Similarly, Owens et al. (1980) supplemented wethers consuming rolled corn/alfalfa hay diets with urea or slow release urea. The slow release urea is a prilled urea coated with a tung oil-linseed oil-talc-catalyst mixture that was intended to slow the rate of urea degredation to NH_3-N in the rumen. Wethers supplemented with the slow release urea had approximately 12% greater supplement intake compared with the urea-supplemented wethers. Also, Köster et al. (2002) conducted two experiments with beef cows grazing tallgrass prairie pasture. They provided four isonitrogenous

supplements with increasing amounts of urea substituting for soybean meal. They noted in one of the experiments that the cows receiving the greatest quantity of urea (4.8% of supplement) totally refused to consume the supplement. However, there was no palatability problem in the second experiment. This was attributed to the possibility that forage quality and management conditions may have affected supplement acceptability.

Advantages Associated with Urea

Non-Protein N Economics

Non-protein N is an attractive CP substitute for sources of natural protein. This is because NPN is often much less expensive than natural proteins. For example, when pricing alfalfa, soybean meal, cottonseed meal, and urea on a cost per pound of CP, an economical advantage appears with urea (Table 1.1). This economical advantage should be viewed with caution; however, because of the negative factors often associated with feeding urea. These were discussed previously in the section entitled negatives associated with feeding urea. Nevertheless, if utilized properly, urea and other NPN sources can be used effectively to decrease the cost of supplemental CP.

Table 1.1: Cost of various crude protein supplements per pound of feed

Supplement	CP % DM	lbs. CP	Cost / Ton	Cost / lb CP[a]
Alfalfa	18 %	360	$90	$0.25
Soybean Meal	49 %	980	$250	$0.26
Cottonseed Meal	44 %	880	$225	$0.26
Urea (100% Utilization)	281 %	5620	$180	$0.03
Urea (50% Utilization)	281 %	2810	$180	$0.06

[a] Cost / lb CP calculated from equation in Cattle Producer's Library, (Cow-Calf Section, CL313), Pricing Protein and Energy Supplements.

Crude Protein Supplement

Urea has been effectively used as a CP supplement to ruminants consuming low-quality forage (Coombe and Tribe, 1963; Egan, 1965; Köster et al., 1997). Coombe and Tribe (1963) conducted three experiments with sheep investigating the effect of urea added to a diet of straw and molasses on roughage intake, digestion, and N status of the animal. When urea was added to straw and molasses at 3% of the amount of straw, DMI, rate of cellulose digestion, and digesta passage rate increased. Also, they noted that once sufficient urea had been supplemented to bring sheep into a positive N balance, additional N supplied by urea was almost quantitatively excreted in the urine. In another experiment with sheep, Egan (1965) infused 4.5 g of urea into the duodenum and increased DMI by 23% compared with an unsupplemented control. In addition, Egan (1965) noted increased cellulose digestion with supplemental urea. Köster et al. (1997) supplemented beef steers consuming dormant, tallgrass prairie hay with five supplements in which casein was substituted for urea. Each supplement was 40% CP; however, the proportion of urea in each supplement was increased so that the five supplements contained 0, 3.5, 7.0, 10.5, and 14.0% urea (DM basis), respectively. They reported that forage DMI, microbial efficiency (g bacterial N/kg OM truly digested in the rumen), and total tract CP digestibility were not affected by the proportion of supplemented N which came from urea. Therefore, research suggests that ruminants consuming low-quality forage can effectively use urea as a source of supplemental CP.

Biuret

Urea, with its rapid hydrolysis in the rumen and potential toxicity concerns, has not been used routinely by nutritionists and ruminant livestock producers with ruminants consuming low-quality forages. Biuret is another form of NPN that has been used as a CP replacement. It is a condensation by-product of urea production that is formed when two molecules of urea are joined under high pressure and heat (Merchen, 1988). The chemical formula of biuret is $(NH_2)_2(CO)_2NH$; therefore, biuret contains 40.8% N or 254% CP (N * 6.25). Feed grade biuret is a commonly utilized mixture of compounds and is defined as having a minimum of 55% biuret, a maximum of 15% urea, a maximum of 30% cyanuric acid and triuret, and a minimum of 35% N (Fonnesbeck et al., 1975). Fonnesbeck et al. (1975) reviewed the potential for biuret as a CP replacement. They noted that the primary advantages of biuret include its slow hydrolysis to NH_3-N (low potential for toxicity) and its lack of palatability concerns compared with urea. They go on to conclude that biuret is a desirable NPN product for making self-fed supplements for grazing ruminants or supplements for ruminants consuming low-quality forages.

Disadvantages Associated with Biuret

Researchers have confirmed that the utilization of biuret by ruminants becomes more efficient with time; therefore, a required adaptation period is needed (Clemens and Johnson, 1973a). This time-period is variable depending on the

basal diet and DMI. Schröder and Gilchrist (1969) stated that the rate of adaptation of rumen microflora to biuret was inversely proportional to the level of digestible protein in the basal diet. Consequently, Clemens and Johnson (1973a) concluded that providing ruminants low-quality forage (0 to 2% digestible protein) prior to the initiation of an experiment may enhance the rate of their adaptation to biuret. Schröder and Gilchrist (1969) determined that the period of time it took for ruminal adaptation to biuret supplementation was 15, 30, and 70 d for lambs consuming low (3.5% CP), medium (6.0% CP), and high (10.3% CP) protein diets, respectively. Also, they noted that biuretolytic activity was increased when starch was included in the supplement. This agrees with the work of Clemens and Johnson (1973b) which showed sheep consuming diets with low to moderate levels of starch developed significant biuretolytic activity within 2 to 4 d of being provided biuret, with maximum activity achieved within the first 10 to 14 d of adaptation.

While adaptation of ruminal microflora to biuret can be slow, deadaptation can be rapid. Clemens and Johnson (1973b) found that when biuret was removed form the diet of adapted sheep, biuretolytic activity was not affected the first day; however, biuretolytic activity was severely depressed 2 d after removal and completely lost after 4 d. Therefore, it has been recommended to provide biuret as a daily supplement to maintain biuretolytic activity and efficient use of biuret N.

Advantages Associated with Biuret

Biuret is much less toxic than (Fonnesbeck et al., 1975). This is mostly because of its insolubility. Fonnesbeck et al. (1975) indicated that greater than 200g of urea will solubilize in 100 ml of water at 37°C compared with only 2 g of biuret. This suggests that urea will be much more available to action by microbial enzymes and, consequently, rapidly degraded to NH_3-N. In contrast, biuret is essentially insoluble and microbial access is greatly reduced. Fonnesbeck et al. (1975) provided a review of toxicity studies concerning the use of urea and biuret in ruminants. They noted that urea, provided in amounts ranging from 0.22 to 0.88 g/kg BW could result in the death of cattle and sheep. However, biuret could be provided in amounts up to 5.5 g/kg BW without signs of distress or toxicity. In addition, the low solubility of biuret provides an advantage over urea when the feed or supplement is offered to ruminants in a humid climate or when rain and (or) snow could solubilize urea. This could result in a loss of dietary N and lower than anticipated N intakes. Furthermore, biuret is stable when stored at temperatures up to 60°C. In addition, biuret stability is maintained when exposed to the temperatures, steam, and pressures that are commonly present when pelleting, cubing, or blocking feeds.

Biuret has exhibited no undesireable palatability characteristics compared with urea which has been shown to decrease intake (Clanton, 1978). Clanton (1978) conducted a series of studies comparing urea and biuret as CP supplements to ruminants consuming low-quality forage. The urea supplements contained from

0 to 6% urea while the biuret supplements contained from 0 to 12% biuret. He noted that the supplement containing 6% urea was not as palatable as the other supplements. Specifically, he stated that most calves would consume the 6% urea supplement but some would eat little to none. In contrast, he reported that no calves refused the biuret supplements, even when incorporated at 12% of the supplement DM.

Urea versus Biuret

Urea and biuret, the two most common forms of NPN used in ruminant diets, have been beneficial as CP supplements to ruminants consuming low-quality forage. Also, the majority of research suggests that urea and biuret are used with a similar efficiency by ruminants consuming low-quality forage. Chicco et al. (1971) compared biuret and urea as CP supplements for young growing bulls grazing 4% CP forage. They noted that forage intake was not significantly affected by NPN source or by NPN supplementation. However, they did report that biuret supplemented bulls retained 26% more N compared with those receiving the urea supplement. In addition, Oltjen et al. (1969) evaluated urea and biuret as CP supplements to steers consuming timothy hay. They noted that it took at least 21 d for steers to adapt to biuret and effectively use it as a source of supplemental N. Nitrogen balance, expressed as a percentage of N intake, was 28 and 7% for urea and biuret supplemented steers, respectively, during the first 7 d of the experiment. In contrast, N retention was not different from d 21 to d 126 for urea and biuret

treatments. Oltjen et al. (1969) also conducted a growth study with the same treatments discussed above and noted that urea and biuret supplementation resulted in similar ADG and feed/gain over an 84 d period. Thomas and Armitage (1972) compared daily and alternate day supplementation of urea, biuret, or soybean meal to pen fed steers consuming grass hay during a 126 d study. They reported gains for biuret and urea supplemented steers were 5 and 13% less, respectively, compared with those receiving soybean meal. Consequently, biuret supplementation resulted in approximately 9% greater gains compared with urea supplementation. Similarly, Turner and Raleigh (1969) supplemented steers consuming an 8% CP meadow hay with urea, biuret, or cottonseed meal for 113 d and noted that cottonseed meal increased ADG compared with urea and biuret. However, they did not report a difference between urea and biuret. For more performance data pertaining to urea and biuret supplemented diets please refer to the Appendix (Table 5.1).

Effects of Supplementation Frequency

Infrequent supplementation (as infrequently as once every 7 d) of CP to ruminants consuming low-quality forage has been shown to result in acceptable levels of performance and nutrient utilization compared with daily supplemented animals (Coleman and Wyatt, 1982; Huston et al., 1999b; Bohnert et al., 2002b). Coleman and Wyatt (1982) supplemented steers consuming range hay (8% CP) with cottonseed meal every day, every other day, or once every fourth day. They

reported that infrequent supplementation did not affect DMI, N balance, or digested N retained compared with daily supplementation. Also, Huston et al. (1999b) supplemented beef cows grazing mature pasture in west Texas with cottonseed meal daily, three times per week, or once per week. They reported that supplementation resulted in less weight and body condition score loss than no supplementation and infrequent supplementation resulted in performance similar to daily supplementation. Relatedly, Huston et al. (1999b) noted that cows supplemented three times and one time per week had a more consistent supplement intake compared with daily supplemented individuals. This was attributed to less competition for supplement during a supplementation event, which allowed more cows to consume supplement when it was provided infrequently. Briefly, they stated daily supplementation resulted in cows running back and forth between feed bunks because dominant cows attempted to keep others away from the feed bunks. However, with the cows receiving supplement once every week or three times per week there was a greater quantity of supplement available. This allowed more cows to consume supplement because many dominant cows would consume supplement and leave while supplement remained in the bunks. Consequently, the more timid cows were able to consume supplement, thereby increasing the number of cows consuming supplement compared with the daily supplemented group. Bohnert et al. (2002b) supplemented cows (during the last third of gestation) and lambs consuming low-quality meadow hay (5% CP) with a high-DIP supplement or a low-DIP supplement daily, once every 3 d, or once every 6 d. They reported that

infrequent supplementation linearly decreased forage and total intake by lambs. In addition, they noted that N balance decreased linearly as supplementation frequency decreased while digested N retained was not influenced by supplementation frequency. Also, cow weight and body condition score change at calving were more positive with CP supplementation and not affected by supplementation frequency. Similarly, Farmer et al., (2001) supplemented cows grazing dormant tallgrass prairie forage with a 43% CP supplement 2d/wk, 3d/wk, 5d/wk, or 7d/wk during the last third of gestation. Cow body condition score change at calving was not affected by supplementation frequency. However, they did report that cow weight loss was increased as supplementation frequency decreased.

There is only limited research concerning infrequent supplementation of NPN to ruminants consuming low-quality forage. This is primarily because of the potential for toxicity when feeding large quantities of urea. In addition, the research of which we are aware concerns the affects of daily and alternate day supplementation of NPN on ruminant performance (Thomas and Armitage, 1972; Farmer et al., 2002) with no research available concerning the effects of infrequent NPN supplementation on site of digestion, ruminal fermentation, and microbial efficiency (g microbial N/kg OM digested in the rumen). There are a couple of studies that evaluate N balance and N efficiency with daily and alternate day supplementation of urea to ruminants consuming low-quality forage-based diets (<8% CP; Tudor and Morris, 1971; Romero et al., 1976). However, these studies

dosed urea as an oral drench and/or sprayed solubilized urea on the basal diet. Consequently, the results may not be indicative of a practical application of NPN supplementation.

Thomas and Armitage (1972) supplemented steers consuming grass hay with soybean meal, urea, or biuret daily or every other day. They reported no significant difference in weight gain because of N source or supplementation frequency. Farmer et al. (2002) supplemented beef cows grazing winter pasture (4% CP) during the last third of gestation with CP supplements that contained increasing portions of supplemental DIP from urea (0, 15, 30, or 45% of the DIP) either daily or three times per week. Soybean meal made up the remainder of supplemental DIP. Their data suggest urea can provide up to 15% of the supplemental DIP and be fed daily or three times per week with no difference in cow weight, body condition score change, or pregnancy rate. They did note that cows would not consume the supplement in which 45% of the supplemental DIP came from urea, regardless of supplementation frequency.

Tudor and Morris (1971) conducted two experiments in which they orally dosed urea to lambs consuming forage-based diets once a day, twice a day, three times a day, or twice on alternate days. No differences in forage intake were observed because of altering supplementation frequency. In addition, when lambs were offered a 7.5% CP basal diet they found no difference in N balance because of urea supplementation or supplementation interval compared with an unsupplemented control. In contrast, when lambs consumed a 2.8% CP basal diet,

N balance was increased with urea supplementation and not affected by supplementation interval. Similarly, Romero et al. (1976) supplemented steers consuming low-quality forage (2% CP) with urea administered at different frequencies (50g once daily as an oral drench, 50g once daily sprayed on the feed, 25g twice daily as an oral drench, or 100g as an oral drench once every other day). They noted that urea supplementation increased forage intake compared with an unsupplemented control. In addition, N balance was increased with urea supplementation compared with the control but not affected by supplementation frequency.

Research suggests that ruminants consuming low-quality forage can be supplemented infrequently with CP and maintain acceptable levels of performance. In addition, NPN can be effectively used as a source of supplemental CP by these animals. However, there is a lack of information that comprehensively compares urea and biuret as CP supplements to ruminants consuming low-quality forage, specifically when supplemented infrequently. Therefore, three experiments are proposed to comprehensively evaluate daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage. The experiments will include a N balance study to evaluate efficiency of N use by lambs, a site of digestion study to evaluate nutrient digestion and ruminal microbial efficiency in steers, and a cow performance study during the last third of gestation. The research proposed will provide ruminant nutritionists and ruminant livestock producers with

information that can be used in designing winter supplementation strategies that reduce winter feed costs while maintaining acceptable levels of production.

DAILY AND ALTERNATE DAY SUPPLEMENTATION OF UREA OR BIURET TO RUMINANTS CONSUMING LOW-QUALITY FORAGE: I. EFFECTS ON COW PERFORMANCE AND EFFICIENCY OF NITROGEN USE IN WETHERS

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ABSTRACT

Two experiments were conducted to determine the influence of supplemental non-protein N (NPN) provided daily (D) or every other day (2D) on ruminant performance and N efficiency. Treatments included an unsupplemented control (CON) and a urea (28.7% CP) or biuret (28.6% CP) supplement provided D or 2D at 0700. In experiment 1, five wethers (39 ± 1 kg BW) were used in an incomplete 5 × 4 Latin square design with four 24-d periods to determine the influence of supplemental NPN source and supplementation frequency (SF) on efficiency of N use in lambs consuming low-quality forage (4% CP). In experiment 2, 80 Angus x Hereford cows (540 ± 4 kg BW) in the last third of gestation were used to determine the effect of NPN source and SF on cow performance. The NPN treatments were calculated to provide 90% of the degradable intake protein requirement assuming a microbial efficiency of 11%. All supplemented treatments were provided on an isonitrogenous basis. In other words, the urea or biuret treatments received the same amount of total supplemental N over a 2-day period; therefore, the 2D treatments received double the quantity of

supplemental N on their respective supplementation day compared with D treatments. In experiment 1, total DM, OM, and N intake, and DM, OM, and N digestibility, N balance, and digested N retained were greater ($P < 0.03$) for supplemented wethers compared with CON with no difference ($P > 0.05$) because of NPN source or SF. Supplemented lambs had increased plasma urea N (PUN) compared with CON ($P < 0.01$) and urea treatments had greater PUN compared with biuret ($P < 0.01$). Also, PUN was increased ($P = 0.02$) for D compared with 2D treatments. In addition, data suggest that PUN exhibited less fluctuation on the day of a supplementation event for biuret compared with urea. In experiment 2, pre- and post- calving (within 14 d and 24 h of calving, respectively) cow weight and body condition score change were more positive ($P < 0.05$) for supplemented groups compared with the CON. These results suggest that supplements containing urea or biuret as the supplemental N source can be effectively used by lambs and cows consuming low-quality forage without adversely affecting N efficiency or performance, even when provided every other day.

Key Words: Urea, Biuret, Forage, Non-protein N, Supplementation, Frequency

INTRODUCTION

It has been 36 yr since Virtanen (1966) demonstrated ruminants could convert non-protein N (**NPN**) to milk protein. Sources of NPN are an attractive protein replacement because of their low cost compared with natural proteins (N basis). Consequently, numerous studies have been conducted evaluating NPN as a source of supplemental N for ruminants. Urea, the most commonly used NPN source, is extremely soluble in water and rapidly hydrolyzed to NH_3-N within the rumen. This can lead to NH_3-N toxicity if urea is consumed in large quantities within a short period of time (Raleigh and Wallace, 1963; Helmer and Bartley, 1971; Bartley et al., 1976). In contrast, biuret is not very soluble in water and is degraded to NH_3-N at a slower rate than urea (Fonnesbeck et al., 1975). As a result, biuret is comparatively non-toxic compared with urea and, therefore, can be incorporated into supplements at higher concentrations than urea (Hatfield et al., 1959). Also, biuret does not elicit the negative effects on supplement palatability and intake often observed with urea (Fonnesbeck et al., 1975; Clanton, 1978).

Decreasing the frequency of supplementation is a management practice that decreases labor costs. Cocimano and Leng (1967) suggested that recycling of absorbed N to the rumen may support fermentation between supplementation events. In addition, research has shown that protein supplements can be fed at infrequent intervals while maintaining acceptable levels of performance (Hunt et al., 1989; Huston et al., 1999b; Bohnert et al., 2002b); however, data is limited comparing urea and biuret supplemented at infrequent intervals on forage intake, N

efficiency, and cow performance. Consequently, this study was designed to determine if daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage (<6% CP) would allow for acceptable levels of performance and nutrient utilization. This information will assist in developing management strategies that help reduce winter feed costs.

MATERIALS AND METHODS

Experiment 1: N Balance Study

Five wethers (39 ± 1 kg) were used in an incomplete 5 × 4 Latin square design (Cochran and Cox, 1957) to evaluate the efficiency of N use in lambs supplemented with a urea or biuret supplement (Table 2.1) every day or every other day. Estimates of degradable intake protein (**DIP**) were determined based on in situ degradability using techniques similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for hard fescue straw and supplements, respectively. Wethers were randomly allotted to treatments and housed in individual metabolism crates within an enclosed barn with continuous lighting. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Oregon State University.

Wethers had continuous access to fresh water and chopped (4 – 8 cm length) hard fescue (*Festuca trachyphylla*) straw (Table 2.1). Treatments were arranged as a 2 × 2 factorial, two sources of supplemental NPN and two

Table 2.1: Ingredient and nutrient content of hard fescue straw and supplements

Item	Hard Fescue Straw	Urea Supplement[a]	Biuret Supplement[a]	Hard Fescue Straw	Urea Supplement[a]	Biuret Supplement[a]
	Lamb Study			Cow Study		
Supplement Composition (%)						
Urea	-	5.3	-	-	5.3	-
Biuret	-	-	6.1	-	-	6.1
Soybean Hulls	-	91.0	90.2	-	91.0	90.2
Dried Molasses	-	3.7	3.7	-	3.7	3.7
Nutrient Composition						
CP, % DM	4.3	28.7	28.6	4.0	29.1	28.6
DIP[b], % CP	76.0	83.0	84.2	76.0	83.0	84.2
OM, % DM	93.6	90.2	92.4	93.8	93.1	94.0
NDF, % DM	73.8	57.9	55.4	75.9	58.6	55.6
ADF, % DM	32.0	38.1	38.2	42.0	40.8	40.0

[a] Pelleted supplements were provided by ADM Alliance Nutrition, Inc., Quincy, IL.

[b] Degradable intake protein. Estimates are based on dacron bag degradabilities. Techniques were similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for straw and supplements, respectively.

supplementation frequencies (**SF**), with a negative control (**CON**; no supplementation). Crude protein supplements were offered every day (**D**) or every other day (**2D**) at 0700. The urea and biuret treatments received the same amount of total supplemental N over a 2 d period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day compared with D treatments. Urea and biuret intake was approximately .175, .350, .207, and .416 g/kg BW on each supplementation day for urea D, urea 2D, biuret D, and biuret 2D, respectively. The amount of CP supplied by each supplement was approximately 0.10% of BW/d (averaged over a 2-day period). To avoid bias because of different BW changes as a result of treatment during each period, the quantity of supplement provided in each period was based on initial BW. Forage was provided daily at 120% of the average intake for the previous 5 d in two equal portions (0715 and 1900), with feed refusals from the previous day determined before the 0700 feeding. Also, 35 g of a trace mineral salt mix (2.4% Ca, 2.3% P, 20.4% Na, 31.65 Cl, 0.2% K, 0.4% mg, 0.1% S, 1309 ppm Mn, 2046 ppm Fe, 7 ppm Cu, 1930 ppm Zn, 42 ppm Co, 120 ppm I, 16 ppm Se, 1325 IU/kg Vitamin E, and 552 and 50 kIU/kg Vitamins A and D, respectively) was provided daily to each lamb at 0700. In addition, an intramuscular injection of vitamins A, D, and E (200,000, 20,000, and 600 IU of Vitamins A, D, and E, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each lamb at the onset of the trial to safeguard against deficiency.

Experimental periods were 24 d with at least 3 d between periods (to remove wethers from metabolism crates). Dry matter intake was determined on d 17 to 22. In addition, samples of hard fescue straw and CP supplements were collected on d 17 to 22 while orts were collected on d 18 to 23. Samples of feed and orts were dried at 55°C for 48 h. On d 19 to 24, total fecal and urine output was collected. Sufficient 6 *N* HCl (100 mL) was added daily to urinals to maintain urine pH < 3. This was verified with pH paper at the start of the urine collection period and monitored randomly over the 6 d collection period. The pH was maintained below 3 in order to prevent bacterial growth and N loss. Urine was composited daily by wether (50% of total; weight basis) and stored at 4°C. A sub-sample of each daily fecal sample (7.5%; wet weight basis) was dried at 55°C for 96 h for calculation of fecal DM. On d 19 to 24, 12 mL of blood was collected via jugular venipuncture 2, 4, and 6 h after the 0715 straw feeding using a heparinized syringe. Blood samples were immediately transferred to vacutainers (Fisher Scientific, catalog no. 0268360), placed on ice for transport to the lab, centrifuged (5000 × *g* for 15 min, 4°C), and plasma harvested and stored (-20°C).

Dried samples were ground through a Wiley mill (1-mm screen). Samples of ground hard fescue straw and CP supplements were composited by period and daily orts composited by lamb (within period) on an equal weight basis (20% as-fed). Ground fecal samples were composited by lamb within period. Feed, orts, and fecal samples were analyzed for DM, OM (AOAC, 1990), NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures

modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Feed, orts, fecal, and urine samples were analyzed for N (Leco CN-2000, Leco Corporation, St. Joseph, MI). Plasma samples were assayed for urea-N using the Sigma Diagnostics Procedure 535 (Sigma Chemical Co., St. Louis, MO) and a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

Experiment 2: Performance Study

Eighty pregnant (approximately 210 d) Angus x Hereford beef cows (540 ± 4 kg BW) were stratified by age, body condition score (**BCS**; 1 = emaciated, 9 = obese; Herd and Sprott, 1996), and weight, and assigned randomly within stratification to one of five treatments (as described in Experiment 1) in a 2 x 2 factorial arrangement (two types of NPN and two SF) with a negative CON (no supplementation). They were then sorted by treatment and allotted randomly to 1 of 20 pens (4 cows/pen; 4 pens/treatment). A trace mineralized salt mix was available free choice (7.3% Ca, 7.2% P, 27.8% Na, 23.1% Cl, 1.5% K, 1.7 % Mg, 0.5% S, 2307 ppm Mn, 3034 ppm Fe, 1340 ppm Cu, 3202 ppm Zn, 32 ppm Co, 78 ppm I, 90 ppm Se, 79 IU/kg vitamin E, and 397 kIU/kg vitamin A). Cows were provided ad libitum access to hard fescue straw (Table 2.1).

The NPN treatments were formulated to provide 90% of the estimated DIP requirement assuming a microbial efficiency of 11% (NRC, 1996). The urea and biuret treatments received the same amount of total supplemental N over a 2-day

period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day compared with D treatments. Crude protein supplements were offered D or 2D at 0700 to provide approximately 0.04% of BW/d of CP until calving. The experiment began on January 11, 2002, with experimental diets fed from start date to calving (70 ± 1 d).

Cow BW and BCS were measured every 14 d up to calving and within 24 h after calving. All weights were obtained following an overnight shrink (16 h). Cow BCS was judged independently by four observers. The same technicians measured BCS throughout the experiment. In addition, calf weights were obtained within 24 h of birth. Hard fescue straw and supplement samples (approximately 200 g) were collected weekly, dried at 55°C for a minimum of 48 h, ground through a Wiley mill (1-mm screen), and composited by period for analysis of ADF and NDF, N, and OM as described in Experiment 1.

STATISTICAL ANALYSIS

Experiment 1: N Balance Study

Data were analyzed as an incomplete 5 × 4 Latin square (Cochran and Cox, 1957) using the GLM procedure of SAS (1996). The model included period, wether, and treatment. Because the treatment structure consisted of a 2 × 2 factorial plus a negative CON, orthogonal contrasts were used to partition specific treatment effects. Contrast statements included: 1) CON vs CP supplementation; 2)

urea vs biuret; 3) D vs 2D supplementation; 4) NPN source × SF. Response variables included: 1) DM and OM intake; 2) total tract digestibility of DM, OM, and N; 3) N balance; and 4) digested N retained. Plasma urea-N was analyzed using the REPEATED statement with the MIXED procedure of SAS (1996). The model included lamb, period, treatment, hour, frequency, treatment × frequency, treatment × hour, and treatment × hour × frequency. In addition, lamb × period × treatment was used to specify variation between animals (using the RANDOM statement). Autoregression was used as the covariance structure. The same contrasts noted above were used to partition treatment sums of squares.

Experiment 2: Performance Study

Cow performance data were analyzed as a randomized complete block design using the GLM procedure of SAS (1996). The model included block and treatment. The same orthogonal contrasts described in the lamb N balance study were used to partition specific treatment effects. Response variables included: 1) cow weight change; 2) cow BCS change; and 3) calf birth weight.

RESULTS

Experiment 1: N Balance Study

Intake of straw DM and OM by lambs was not affected by CP supplementation while there was a tendency ($P = 0.08$) for straw DM and OM

intake to decrease as SF decreased (Table 2.2). Total DM, OM, NDF, and N intake increased ($P < 0.03$) with supplementation. Also, total DM and OM intake tended to decrease ($P = 0.08$) as SF decreased, while NDF intake decreased ($P = 0.04$) as SF decreased.

Total tract digestibility of DM, OM, NDF, ADF, and N were increased ($P < 0.03$) with CP supplementation (Table 2.2). Daily fecal and urinary N excretion (g/kg BW) were increased ($P < 0.02$) with CP supplementation; however, no differences were noted because of NPN source or SF. Daily N balance and digested N retained were greater ($P < 0.03$) with CP supplementation with no difference because of NPN source or SF.

Treatment × hour, treatment × SF, and treatment x hour x SF interactions ($P < 0.01$) were observed for plasma urea-N. However, after considering the nature of the interactions, we concluded that discussing treatment means while providing the treatment × hour x SF figure would aid in interpretation and discussion of the data (Figure 2.1). Lamb plasma urea-N was greater ($P < 0.01$) for CP supplemented lambs and urea had greater ($P < 0.01$) plasma urea-N than biuret (Table 2.2). In addition, plasma urea-N decreased ($P = 0.02$) as SF decreased. Figure 2.1 provides an illustration of average plasma urea-N means for 2, 4, and 6 h post-feeding on the day all supplements were offered and the day only daily supplements were offered over the 6 d collection period. Interestingly, plasma urea-N was similar at 2, 4, and 6 h post-feeding on the day all supplements were offered for UD but increased from 2 to 6 h post-feeding for U2D. In contrast, plasma urea-N was similar over

Table 2.2: Effects of non-protein nitrogen (NPN) source and supplementation frequency on intake, diet digestibility, and nitrogen balance in lambs consuming low-quality forage

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source × SF
Daily DM Intake, g/kg BW										
Straw	26.4	28.2	26.1	28.2	26.1	1.1	0.56	0.99	0.08	0.99
Supplement[d]	0.0	3.3	3.3	3.4	3.4					
Total	26.4	31.5	29.4	31.6	29.4	1.1	0.009	0.97	0.08	0.99
Daily OM Intake, g/kg BW										
Straw	24.8	26.5	24.5	26.4	24.4	1.0	0.56	0.97	0.08	0.97
Supplement[e]	0.0	3.0	3.0	3.1	3.1					
Total	24.8	29.5	27.5	29.5	27.5	1.0	0.01	0.96	0.08	0.97
Daily NDF Intake, g/kg BW	19.6	22.9	21.3	22.8	20.8	0.7	0.02	0.68	0.04	0.75
Daily N Intake, g/kg BW	0.183	0.347	0.331	0.343	0.347	0.012	<0.001	0.58	0.63	0.42
Total Tract Digestibility, %										
DM	39.2	48.0	47.9	47.8	45.3	1.9	0.006	0.50	0.51	0.55
OM	42.8	51.4	51.2	51.0	48.7	2	0.009	0.49	0.56	0.63
NDF	42.2	50.8	51.1	49.5	46.1	2.3	0.02	0.20	0.51	0.44
ADF	42.9	52.5	52.0	51.8	46.9	2.4	0.02	0.27	0.31	0.40
N	24.3	53.0	48.5	51.9	52.3	2.9	<0.001	0.66	0.51	0.43
Daily N excretion, g/kg BW										
Fecal	0.136	0.160	0.170	0.167	0.164	0.008	0.01	0.92	0.71	0.46
Urinary	0.059	0.144	0.148	0.135	0.142	0.007	<0.001	0.27	0.45	0.79
Daily N balance, g/kg BW	-0.012	0.042	0.013	0.041	0.041	0.014	0.02	0.36	0.34	0.35
Daily digested N retained[f], %	-54.4	16.3	6.5	28.6	19.4	15.6	0.003	0.45	0.56	0.98
Plasma urea-N, mM	2.40	5.86	5.36	4.46	4.05	0.16	<0.001	<0.001	0.02	0.78

Continued Table 2.2: Effects of non-protein nitrogen (NPN) source and supplementation frequency on intake, diet digestibility, and nitrogen balance in lambs consuming low-quality forage

[a] CON = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source × SF = interaction of NPN source vs supplementation frequency.
[d] UD received 3.3 g/kg BW daily; U2D received 6.6 g/kg BW every other day; BD received 3.4 g/kg BW daily; B2D received 6.8 g/kg BW every other day.
[e] UD received 3.0 g/kg BW daily; U2D received 6.0 g/kg BW every other d; BD received 3.1 g/kg BW daily; B2D received 6.2 g/kg BW every other day.
[f] Calculated as (Daily N retention, g/kg BW / Daily N digested, g/kg BW) × 100.

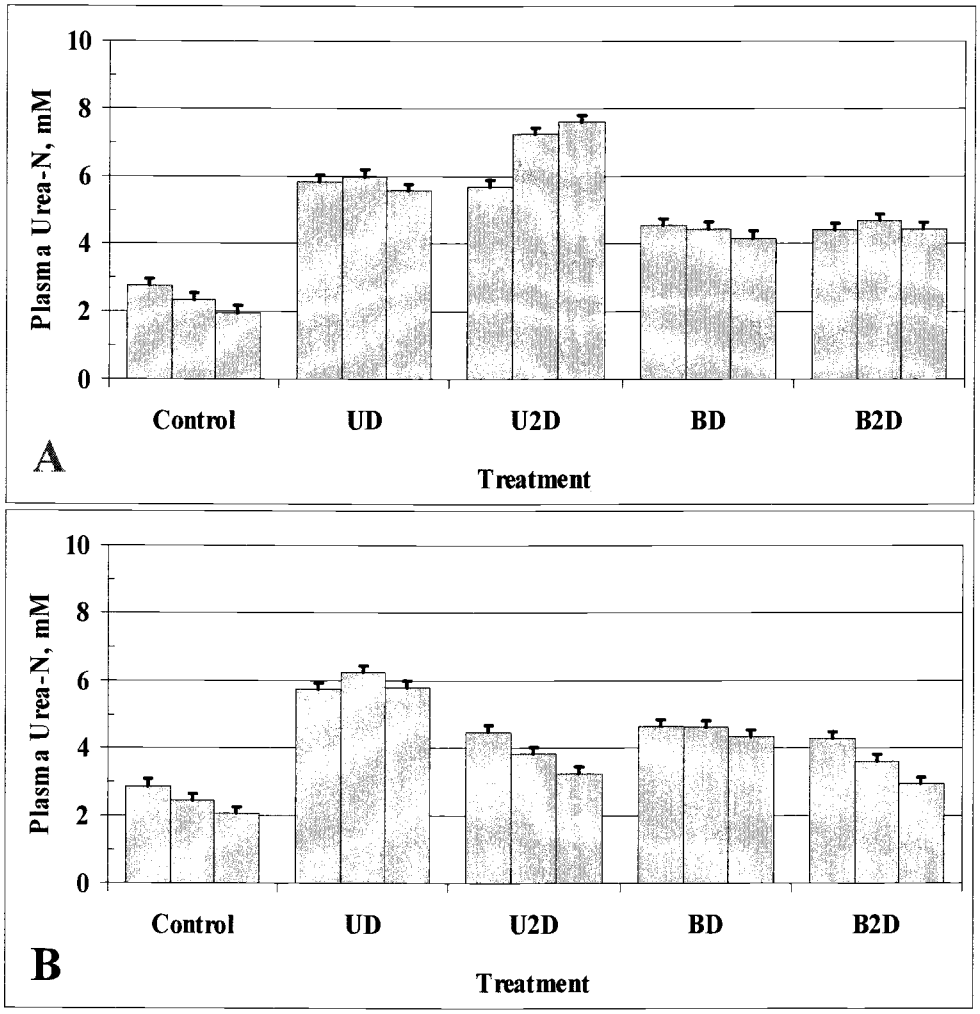

Figure 2.1: Effects of non-protein nitrogen source and supplementation frequency on lamb plasma urea-N (mM) on the day all supplements were offered (A) and the day only daily supplements were offered (B). Columns from left to right for each treatment represent lamb plasma urea-N at 2, 4 and 6 h post-feeding. Treatments were: Control; UD = Urea supplement every day; U2D = Urea supplement every other day; BD = Biuret supplement every day; B2D = Biuret supplement every other day. Treatment x hour x SF interactions for A and B are ($P < 0.0001$). SEM for treatment x hour x SF is 0.20.

the collection period on the day all supplements were offered for BD and B2D. On the day only daily supplements were offered, plasma urea-N responded in a like manner for the U2D and B2D treatments (decreasing over the collection period). However, the average difference between D and 2D treatments was less for BD and B2D (0.93 mM) compared with UD and U2D (2.05 mM).

Experiment 2: Performance Study

Precalving (within 14 d of calving) and postcalving (within 24 h of calving) weight and BCS change were more positive ($P < 0.01$) with CP supplementation (Table 2.3). In addition, calf birth weight was not affected by NPN supplementation, NPN source, or SF ($P > 0.10$).

DISCUSSION

Experiment 1: N Balance Study

There is little data available concerning the effects of infrequent supplementation of NPN on efficiency of N use and performance by ruminants consuming low-quality forage. Also, certain self-fed supplements, such as liquid-molasses mixes, dry molasses blocks (tubs), and dry or pressed blocks, are becoming increasingly popular with beef producers because of the decreased labor associated with there use. However, these types of supplements are generally consumed at infrequent intervals and often contain a significant portion of their

Table 2.3: Effects of non-protein nitrogen (NPN) source and supplementation frequency on cow performance and calf birth weight

	Treatment[a]						*P*-Value[c]			
	Con	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source x SF
Supplement DMI, g/d[d]	0.0	689	689	738	738					
Initial Wt., Kg	538	556	541	536	531					
Initial BCS	4.85	4.85	4.86	4.87	4.89					
Weight change, kg										
Precalving[e]	10	31	33	35	33	5	0.002	0.70	0.92	0.65
Postcalving[f]	-40	-13	-19	-6	-14	5	<0.001	0.27	0.19	0.88
Body condition score change										
Precalving[e]	-0.08	0.29	0.20	0.18	0.18	0.08	0.006	0.44	0.56	0.56
Postcalving[f]	-0.55	0.20	0.21	0.18	0.02	0.11	<0.001	0.40	0.51	0.48
Calf birth date, Gregorian d	75	69	66	67	73	3	0.08	0.41	0.58	0.10
Calf birth weight, kg[f]	37	38	39	37	38	1	0.59	0.19	0.33	0.86

[a] Con = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.

[b] n = 4

[c] Con vs Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source × SF = interaction of NPN source vs supplementation frequency.

[d] UD received 689 g daily; U2D received 1378 g every other day; BD received 738 g daily; B2D received 1476 g every other day.

[e] Within 14 d of calving.

[f] Within 24 h after calving.

supplemental CP in the form of NPN. Therefore, this study was conducted to comprehensively compare daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage. This data should add to our understanding of N metabolism in ruminants consuming N deficient forage-based diets. In addition, this work provides ruminant nutritionists with information that can be used in designing CP supplementation strategies that lower supplementation costs and improve the economic sustainability of livestock operations that rely on low-quality forages as a primary feed source.

The lack of a CP supplementation effect on straw DM and OM intake contrasts with other studies in which protein supplementation increased forage intake (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001). However, Bohnert et al. (2002a,b) reported that CP supplementation did not increase forage intake of steers and lambs consuming low-quality forage. This coincides with the results observed in the current study. Bohnert et al. (2002a,b) suggested that the lack of an increase in forage intake could be related to NDF intake. They based this on the concept proposed my Mertens (1985,1994) that DMI is maximized when NDF intake is approximately 12.5 $g \cdot kg\ BW^{-1} \cdot d^{-1}$. Bohnert et al. (2002a,b) noted that NDF intake by steers and lambs ranged from 13.9 to 16.0 $g \cdot kg\ BW^{-1} \cdot d^{-1}$ and 12.7 to 15.6 $g \cdot kg\ BW^{-1} \cdot d^{-1}$, respectively. Therefore, based on NDF intake, Bohnert et al. (2002a,b) did not expect to increase forage intake with CP supplementation. Similarly, forage intake was not expected to increase with CP supplementation in the current study because NDF intake ranged from 19.6 to 22.9

$g \cdot kg\ BW^{-1} \cdot d^{-1}$. In contrast, NDF intake by unsupplemented controls was approximately 6.4, 5.1, and 8.2 and increased with CP supplementation to a maximum of 14.3, 11.3, and 13.3 $g \cdot kg\ BW^{-1} \cdot d^{-1}$ in the studies of DelCurto et al. (1990), Köster et al. (1996), and Bandyk et al. (2001), respectively. The increase in NDF intake with CP supplementation in the current study is probably a consequence of supplement NDF intake. This is supported by the lack of a CP supplementation effect on straw intake and the high NDF concentration in the NPN supplements (approximately 57%; DM basis).

Similar straw and total DMI for urea and biuret treatments is similar to results from other studies comparing urea and biuret as N supplements to ruminants consuming forage-based diets (Oltjen et al., 1969; Chicco et al., 1971; Bond and Rumsey, 1973). Oltjen et al. (1969) supplemented steers consuming a timothy hay-based diet with urea or biuret. They stated that NPN source had no affect on total DMI. Also, Chicco et al. (1971) supplemented young bulls consuming elephant grass with biuret or urea. They noted that forage intake was not affected by CP supplementation or NPN source. Bond and Rumsey (1973) cited that forage intake by Angus calves was not affected by addition of urea or biuret to a liquid-molasses supplement.

The tendency for forage and total DM and OM intake to be greater for D compared with 2D treatments agrees with the results of Bohnert et al. (2002b). They supplemented lambs with a high- or low-DIP supplement every day, once every 3 d, or once every 6 d. They reported that as SF decreased from daily to once

every 6 d, lambs receiving the high- and low-DIP supplements had an 8 and 19% decrease in forage and 7 and 17% decrease in total DMI, respectively. This was partially attributed to the substitution of supplement intake for forage intake. Also, Bohnert et al. (2002b,c) suggested that infrequent supplementation may have disrupted rumen function for a period of time because of the larger quantity of supplement provided during a supplementation event as SF decreased. This could explain the tendency for depressed total DMI for 2D compared with D treatments in the current study. However, other research has reported no affect of SF on total DMI by ruminants consuming low-quality forage (Krehbiel et al., 1998; Huston et al., 1999a). Krehbiel et al. (1998) supplemented ewes consuming bromegrass hay with soybean meal every 24 or 72 h. They observed that total DMI was increased with CP supplementation while no difference was noted for total DMI between 24- and 72-h supplemented ewes. Also, Huston et al. (1999a) supplemented ewes consuming wheat straw with cottonseed meal daily or once every 7 d and reported that SF did not affect straw or total DMI.

Increased DM, OM, and NDF digestibility with CP supplementation of low-quality forage has been reported in numerous studies (DelCurto et al., 1990; Horney et al., 1996; Bohnert et al., 2002b). This has been attributed to improved N availability by the ruminal microflora which increases ruminal fiber digestion (Campling et al., 1962; Petersen, 1987). If we assume supplement DM digestibility was 70% in the current study, estimated apparent forage digestibility for each treatment was approximately 39, 45, 45, 45, and 42% for the CON, urea D, urea

2D, biuret D, and biuret 2D, respectively. This suggests that forage DM digestibility was increased with CP supplementation and not affected by NPN source or SF.

As observed in the current study, Chicco et al. (1971) and Ammerman et al. (1972) indicated that DM and OM digestibility, respectively, were not affected by NPN source when urea or biuret supplied the majority of supplemental N provided to ruminants consuming low-quality forage. Chicco et al. (1971) reported that DM digestibility of 4% CP forage by young bulls was 51 and 49% for those receiving supplemental biuret or urea, respectively. Similarly, Ammerman et al. (1972) supplemented wethers consuming 2.6% CP forage with soybean meal or soybean meal and NPN mixtures in which urea or biuret supplied 50% of the supplemental N. They stated that supplemental N source had no affect on OM or cellulose digestibility. Also, studies by Coleman and Wyatt (1982), Brandyberry et al. (1992), and Bohnert et al. (2002a) demonstrated that infrequent supplementation of CP to ruminants consuming low-quality forage does not negatively affect DM and (or) OM digestibility. Coleman and Wyatt (1982) supplemented steers consuming 3% CP forage with cottonseed meal daily, once every other day, or once every 4 d. They noted that daily and every other day CP supplementation increased DM and OM digestibility compared with an unsupplemented control and was not affected by SF. Also, Brandyberry supplemented mature beef cows grazing native range (approximately 5% CP) in southeastern Oregon with alfalfa hay or alfalfa pellets every day or every other day. They reported that SF had no affect on forage, NDF,

or total OM digestibility. Similarly, Bohnert et al. (2002a) reported that apparent total tract OM digestibility was not influenced by SF in steers consuming low-quality forage (5% CP) and supplemented with CP daily, once every 3 d, or once every 6 d. Therefore, our results suggest that supplementing urea or biuret daily or every other day to ruminants consuming low-quality is an effective means of increasing nutrient digestibility compared with non-supplemented individuals.

Apparent total tract N digestibility for supplemented wethers was approximately 110% greater than the CON. This is comparable to other results observed with ruminants consuming low-quality forage and provided supplemental CP (Ferrell et al., 1999; Bohnert et al., 2002a,b). Ferrell et al. (1999) supplemented wethers consuming 4% CP bromegrass hay with supplements in which urea, soybean meal, or a 50:50 mix of blood meal and feather meal provided the supplemental N. They cited that apparent total tract N digestibility was approximately 101, 83, and 86% greater compared with the control for urea, soybean meal, and the 50:50 blood meal and feather meal mix, respectively. Similarly, Bohnert et al. (2002a) and Bohnert et al. (2002b) supplemented steers and lambs, respectively, with a high- or low-DIP supplement daily, once every 3 d, or once every 6 d and noted that CP supplementation increased apparent total tract N digestibility by approximately 81% with the steers and 170% with the lambs compared with unsupplemented controls. The greater N digestibility with CP supplementation is most likely because CP supplements are generally more digestible than low-quality forage (N basis) and(or) metabolic fecal N can be a

significant proportion of total fecal N in unsupplemented ruminants (Ferrell et al., 1999). Metabolic fecal N constitutes a greater proportion of total fecal N of unsupplemented ruminants consuming low-quality forage because of their low N intake and relatively constant quantity of metabolic fecal N (5.35 g N/kg DMI; NRC, 1985); therefore, the combination of greater N digestibility in CP supplements and increased metabolic fecal N (as a percentage of N intake) in unsupplemented ruminants results in increased apparent total tract N digestibility with CP supplementation.

Increased urinary and(or) fecal N excretion has been observed with CP supplementation of ruminants consuming low-quality forage compared with unsupplemented individuals (Ammerman et al., 1972; Bohnert et al., 2002b). Ammerman et al. (1972) reported that CP supplementation of wethers consuming 2.6% CP pangolagrass hay resulted in increases in fecal and urinary N excretion of 42 and 372%, respectively, compared with an unsupplemented control. Similarly, Bohnert et al. (2002b) noted that N excretion was increased by 22% in the feces and 307% in the urine of CP supplemented wethers compared with unsupplemented wethers receiving the same basal diet (5.2% CP meadow hay). Increased urinary N excretion with increased N intake has been demonstrated with ruminants (Huntington et al., 1996; Huntington et al., 2001), primarily because of greater excretion of urea-N (Waterlow, 1999; Huntington et al., 2001). Therefore, the increased excretion of urinary N with CP supplementation in the current study is

most likely because of increased N intake and greater urea-N excretion compared with CON.

The lack of an affect of NPN source on urinary and fecal N excretion agrees with other research comparing urea and biuret as CP supplements to low-quality forage (Oltjen et al., 1969; Ammerman et al., 1972). Oltjen et al. (1969) supplemented steers consuming timothy hay-based diets with urea or biuret and reported fecal and urinary N excretion in adapted animals was approximately 25 and 26% and 47 and 47% of N intake for urea and biuret supplemented steers, respectively. Also, Ammerman et al. (1972) reported that when urea or biuret comprised 50% of the total supplemental N provided to lambs consuming low-quality forage (2.6% CP) there was no difference in the quantity of N excreted in the urine or feces. However, Chicco et al. (1971) noted that urinary N excretion was greater for urea-supplemented compared with biuret-supplemented bulls consuming mature, green-chopped elephant grass. They reported no difference in fecal N excretion.

Infrequent supplementation has had little affect on fecal or urinary N excretion, even when CP was provided once every 6 d (Tudor and Morris, 1971; Coleman and Wyatt, 1982; Bohnert et al., 2002b). Tudor and Morris (1971) pulse-dosed solubilized urea once daily, twice daily, three times daily, or twice on alternate days to wethers consuming barley straw and noted that fecal and urinary N excretion were not affected by SF. Also, Coleman and Wyatt (1982) supplemented steers consuming range hay (7.9% CP) with cottonseed meal every

day, once every 2 d, or once every 4 d and reported that SF had no affect on urinary or fecal N excretion. Similarly, Bohnert et al. (2002b) supplemented lambs consuming low-quality meadow hay (5.2% CP) with CP supplements daily, once every 3 d, or once every 6 d and cited no difference in the quantity of N excreted in the feces or urine because of SF.

The increase in N balance and digested N retained observed with CP supplementation in the current study agrees with other research in which supplemental CP was provided to ruminants consuming low-quality forage (Egan, 1965; Ammerman et al., 1972; Bohnert et al., 2002b). In his classical work, Egan (1965) supplemented mature wethers consuming wheat straw with casein (6 g/d of N) and noted that supplementation increased N balance from 0.70 g/d without supplementation to 5.47 g/d with supplemental casein. Also, Ammerman et al. (1972) increased N balance and digested N retained (expressed as a percentage of N intake) in wethers consuming low-quality forage (2.6% CP) and supplemented with soybean meal, urea and soybean meal (50:50 N basis), or biuret and soybean meal (50:50 N basis) compared with wethers receiving forage alone. In a supplementation frequency study, Bohnert et al. (2002b) provided a low- or high-DIP supplement to lambs consuming 5.2% CP hay and increased average N balance by 0.103 and 0.096 $g \cdot kg\ BW^{-1} \cdot d^{-1}$ and average digested N retained by 47 and 43%, respectively, compared with an unsupplemented control. These results suggest that supplemental CP can, at least partially, alleviate the N deficiency that often results when ruminants consume low-quality forage.

Our observation that NPN source did not affect N balance or digested N retained agrees with the work of Oltjen et al. (1969) and Ammerman et al. (1972). Oltjen et al. (1969) reported that N balance was approximately 27% of N intake in steers consuming timothy hay and supplemented with urea or biuret. Also, Ammerman et al. (1972) noted that N balance and digested N retained were negative for unsupplemented wethers but positive for wethers provided supplemental urea and biuret. Additionally, they reported no difference between urea and biuret treatments for N balance and digested N retained (12 and 17% and 19 and 24% of N intake, respectively). Chicco et al. (1971) supplemented young bulls consuming 6.7% CP forage with urea or biuret and increased N balance by 71 and 132%, respectively, compared with an unsupplemented control. In contrast to our results, however, they noted that biuret supplemented bulls had a greater N balance compared with those receiving urea. These results suggest that urea or biuret can be effectively used as a source of supplemental N by ruminants consuming low-quality forage (< 7% CP).

Infrequent supplementation of CP to ruminants consuming low-quality forage has resulted in similar N balance and digested N retained compared with daily supplementation (Romero et al., 1976; Coleman and Wyatt, 1982; Bohnert et al., 2002b). Romero et al. (1976) provided steers consuming 2% CP spear grass hay with urea as an oral drench twice a day, once a day, or once every 2 d. Nitrogen balance was –5.0 g/d for unsupplemented steers and increased to 7.0, 5.7, and 5.7 g/d for steers supplemented twice a day, once a day, and once every 2 d,

respectively. Supplementation frequency did not affect N balance. Similarly, Coleman and Wyatt (1982) supplemented steers consuming range hay with cottonseed meal daily, once every 2 d, or once every 4 d and noted that N balance and digested N retained were not affected by SF. Bohnert et al. (2002b) supplemented wethers consuming 5.2% CP meadow hay with a low- or high-DIP supplement daily, once every 3 d, or once every 6 d and increased N balance compared with an unsupplemented control. However, they reported a linear decrease in N balance as SF decreased. They attributed this to a similar decrease in N intake as SF decreased. This assumption is supported by digested N retained, which averaged approximately 29% for supplemented treatments (–16% for the control) and was not influenced by SF. Therefore, based on the results in the current and aforementioned studies, infrequent supplementation of CP to ruminants consuming low-quality forage has only minimal effects on N balance and digested N retained compared with daily supplementation.

Plasma urea concentration is positively correlated with N intake (Harmeyer and Martens, 1980). This coincides with the 87 and 105% increase in average N intake and plasma urea-N, respectively, that we observed with CP supplementation compared with the CON. Also, other research with ruminants has demonstrated increased plasma urea-N with CP supplementation of low-quality forage (Krehbiel et al., 1998; Ferrell et al., 1999; Bohnert et al., 2002b). However, limited research, with conflicting results, has compared the affect of biuret or urea supplementation on plasma urea-N (Oltjen et al., 1969; Chicco et al., 1971. Oltjen et al. (1969)

supplemented steers consuming timothy hay with urea or biuret and reported that plasma urea-N was greater with biuret compared with urea (6.11 compared with 5.66 mMol/L). Also, Chicco et al. (1971) supplemented young bulls consuming green-chopped elephant grass with urea or biuret and noted that NPN supplementation increased plasma urea-N compared with an unsupplemented control, with no difference because of NPN source. In the current study, urea supplemented lambs had 32% greater plasma urea-N compared with those receiving biuret (5.6 versus 4.3 mMol/L). It is not readily apparent why there are conflicting responses in plasma urea-N with urea and biuret supplementation. However, experimental diets used by Oltjen et al. (1969) were 85% timothy hay and approximately 13.5% CP. Consequently, protein may not have been the first limiting nutrient. This could have masked any supplemental NPN effects on plasma urea-N. Also, Chicco et al. (1971) collected plasma samples from young bulls 2 h after feeding for measurement of urea-N. This may not have been sufficient time after feeding to determine supplemental NPN effects on plasma urea-N. For instance, they reported ruminal NH_3-N was 60 and 24 mg/100 mL rumen fluid for urea and biuret treatments, respectively, 2 h after feeding. Plasma samples taken at a later time may have resulted in NPN effects on plasma urea-N because of the potential for increased NH_3-N absorption from the rumen with urea supplementation that should increase urea-N production by the liver. This could explain the increased plasma urea-N for urea compared with biuret supplementation in the current study (plasma samples collected 2, 4, and 6 h after supplementation).

Decreased plasma urea-N with decreased SF has been reported in other studies with ruminants consuming forage-based diets (Huston et al. 1999a; Bohnert et al., 2002b). The general response observed with infrequent supplementation of CP to ruminants is a larger peak in plasma urea-N following a supplementation event compared with daily supplementation. This normally occurs within 24 h of supplementation and is proportional to the quantity of supplement provided. Plasma urea-N then decreases until the next supplementation event. Huston et al. (1999a) supplemented non-pregnant, Rambouillet ewes consuming oat hay (8% CP) with cottonseed meal daily, every other day, or once every 7 d. They noted that serum urea-N remained relatively constant with daily supplementation, had an every-other-day pattern of high and low with the every other day treatments, and was very high for approximately 2 d following supplementation and then declined over the next 5d with the once every 7 d treatment. Similarly, Bohnert et al. (2002b) supplemented wethers consuming low-quality meadow hay daily, once every 3 d, or once every 6 d with a low- or high-DIP supplement. They reported that plasma urea-N demonstrated a bimodal pattern during the 6 d supplementation period in the lambs supplemented once every 3 d (a moderate peak following each supplementation event), while a large, single peak was observed on the day following supplementation with the wethers receiving supplement once every 6 d. Also, they reported that average plasma urea-N linearly decreased as SF decreased over a 6 d supplementation period. Plasma urea-N was 4.88, 4.31, and 4.36 mMol/L and 5.22, 4.39, and 4.32 mMol/L for supplementation daily, once every 3

d, and once every 6 d with the low- and high-DIP supplements, respectively. Therefore, the decrease in average plasma urea-N observed with the alternate day compared with daily treatments in the current study is because the decline in plasma urea-N on the day only daily supplements were provided was greater than the increase observed on the day all supplements were provided (Figure 2.1).

Experiment 2: Performance Study

Supplementation of CP to beef cows consuming low-quality forage has routinely improved weight and BCS change compared with not providing a CP supplement (Horney et al., 1996; Mathis et al., 1999; Bohnert et al., 2002b). This was also noted in the current study. Precalving (within 14 d of calving) weight gain was increased approximately 230% and precalving BCS was approximately 0.30 units greater with CP supplementation compared with the CON.

Research suggests that biuret supplementation of ruminants consuming low-quality forage results in similar, or slightly improved, performance compared with urea (Raleigh and Turner, 1968; Turner and Raleigh, 1969; Rush et al., 1976). This agrees with the similar cow performance observed in the current study with urea and biuret supplementation. Raleigh and Turner (1968) supplemented yearling heifers grazing crested wheatgrass with biuret, urea, or cottonseed meal. They noted that CP supplementation increased weight gains by approximately 74% compared with an unsupplemented control and was approximately 18% greater with biuret supplementation compared with urea or cottonseed meal. Turner and

Raleigh (1969) supplemented steers provided 8% CP hay with biuret, urea, or cottonseed meal and reported that cottonseed meal supplementation increased gains compared with biuret and urea (.55, .48, and .47 kg/d, respectively). However, no difference was noted between urea and biuret. Also, Rush et al. (1976) conducted a series of experiments comparing urea and biuret as CP supplements to beef cattle consuming forage-based diets. They concluded that biuret was used more effectively as a CP supplement to ruminants compared with urea. They attributed this to slower ruminal degradability and more efficient N utilization with biuret.

Infrequent supplementation of CP to beef cows consuming low-quality forage has been shown to maintain acceptable levels of performance compared with daily supplementation (Huston et al., 1999b; Farmer et al., 2001; Bohnert et al., 2002b). The aforementioned studies used sources of natural protein as CP supplements; therefore, further discussion concerning infrequent supplementation of NPN is warranted given the use of urea and biuret in the current study. However, we are aware of only three studies that have evaluated the impact of infrequent supplementation of urea or biuret on ruminant performance (Thomas and Armitage, 1972; Oltjen et al., 1974; Farmer et al., 2002). Thomas and Armitage (1972) supplemented steer calves (fed approximately 6 kg/d grass hay) daily or every other day with 20% CP supplements in which soybean meal, urea, or biuret provided the source of supplemental CP. They noted, as in the current study, that weight gain was not affected by CP source or SF. Also, Oltjen et al. (1974) supplemented growing steers consuming pangola grass hay with urea, biuret, or

cottonseed meal every day or three times a week. They reported that steer daily gain was not affected by CP source of SF, but was greater for CP supplementation compared with a control (mineral supplement only). This agrees with the data of Farmer et al. (2002). They supplemented pregnant cows (last third of gestation) consuming dormant, tallgrass prairie forage (4% CP) daily or three times per week with 40% CP supplements in which urea provided 0, 15, 30 or 45% of the supplemental DIP (soybean meal provide the remainder of DIP). They reported no difference in cow BCS change because of SF when urea provided 0, 15, or 30% of the supplemental DIP. However, they reported a more negative BCS change when urea provided 45% of the supplemental DIP compared with the other DIP treatments. This was attributed to supplement refusal because of the high urea content. These results support or observation that daily and alternate day supplementation of urea or biuret yielded similar effects on cow performance. In addition, this agrees with the N balance data obtained in Experiment 1 that suggests N retention was improved with CP supplementation and not influenced by SF. Therefore, the lambs and cows in the current study were able to use the supplemental N provided by urea or biuret, even when provided every other day, to maintain BW and improve N status.

IMPLICATIONS

Ruminants appear to have the ability to conserve nitrogen over an extended period, thereby storing it for use between periods of supplementation. Also,

ruminants consuming low-quality forage (< 6% crude protein) can effectively use supplemental non-protein nitrogen to maintain nitrogen status and performance. This suggests that non-protein nitrogen can be an economical alternative to natural protein for use in hand-fed and self-fed supplements to ruminants consuming low-quality forage. However, infrequent supplementation of non-protein nitrogen, especially urea, should be used with caution because of the potential for ammonia toxicity. Nevertheless, biuret appears to be a safer alternative for infrequent supplementation because of its decreased solubility and slower hydrolysis to ammonia compared with urea.

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DAILY AND ALTERNATE DAY SUPPLEMENTATION OF UREA OR BIURET TO RUMINANTS CONSUMING LOW-QUALITY FORAGE: II. EFFECTS ON SITE OF DIGESTION AND MICROBIAL EFFICIENCY IN STEERS

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ABSTRACT

Five steers (491 ± 21 kg BW) were used in an incomplete 5 × 4 Latin square with four 24-d periods to determine the influence of supplemental non-protein N (NPN) source and supplementation frequency (SF) on nutrient intake and site of digestion in steers consuming low-quality forage (4% CP). Treatments included an unsupplemented control and a urea or biuret supplement placed directly into the rumen daily or every other day at 0700. Supplements were calculated to provide 90% of the estimated DIP requirement. Urea and biuret supplements (29% CP) were provided on an isonitrogenous basis. Forage OM intake was not affected ($P > 0.05$) by NPN supplementation, NPN source, or SF. However, total OM and N intake were increased ($P < 0.01$) with supplementation. Duodenal flow of N was greater ($P = 0.04$) with NPN supplementation compared with the control. In addition, duodenal bacterial N flow was increased with NPN supplementation ($P = 0.04$) and for biuret compared with urea ($P < 0.01$). Also, bacterial efficiency (g bacterial N/kg OM truly digested in the rumen) was greater ($P = 0.05$) for biuret compared with urea. Apparent total tract N digestibility was increased with NPN

supplementation ($P < 0.01$) and not affected by NPN source or SF. These results suggest that urea or biuret can be used effectively as a supplemental N source by steers consuming low-quality forage.

Key Words: Urea, Biuret, Forage, Non-protein N, Supplementation, Frequency

INTRODUCTION

Many cattle in the Western United States consume low-quality forage (< 6% CP) from late summer through winter. Research has shown that protein supplementation of low-quality forage can increase cow weight gain and body condition score (Clanton and Zimmerman, 1970; Beaty et al., 1994; Bohnert et al., 2002b), forage intake and digestibility (Kartchner, 1980; Köster et al., 1996), and can improve reproductive performance (Sasser et al., 1988; Wiley et al., 1991). However, this can be an expensive practice. Winter feeding and labor costs in the Intermountain West often total $100 to 200 per cow each year. Infrequent supplementation is a management practice that can decrease labor costs while maintaining acceptable levels of performance (Hunt et al., 1989; Huston et al., 1999b; Bohnert et al., 2002b). In addition, non-protein N (**NPN**) sources are attractive protein replacements because of their low cost per unit of N compared with natural protein.

Urea and biuret are two sources of NPN commonly used in ruminant diets. Data has shown that hydrolysis of urea to ammonia and CO_2 occurs very rapidly,

irrespective of dietary history (Helmer and Bartley, 1971). This can lead to ammonia toxicity if urea is consumed in large quantities within a short time (Raleigh and Wallace, 1963; Helmer and Bartley, 1971; Bartley et al., 1976). In contrast, biuret is less soluble in water and degraded to ammonia at a slower rate compared with urea (Fonnesbeck et al., 1975). As a result, biuret is comparatively non-toxic (Hatfield et al., 1959) and does not decrease supplement palatability like urea (Fonnesbeck et al., 1975; Clanton, 1978); therefore, it can be incorporated into supplements at higher concentrations compared with urea.

Data is limited comparing the effects of urea or biuret supplemented infrequently on forage intake and nutrient digestibility. Consequently, the objective of this research was to compare daily and alternate day supplementation of urea or biuret on the utilization of low-quality forage by steers.

MATERIAL AND METHODS

Five Angus x Hereford steers (491 ± 21 kg) with ruminal and double L-shaped duodenal cannulas (Streeter et al., 1991) were allotted randomly to one of five treatments in an incomplete 5 × 4 Latin square design (Cochran and Cox, 1957) and housed in individual pens (4 × 8 m) within an enclosed barn with continuous lighting. Treatments consisted of an unsupplemented control and urea or biuret supplemented daily (**D**) or every other day (**2D**; **CON** = control, **UD** = urea supplement every day, **U2D** = urea supplement every other day, **BD** = biuret supplement every day, and **B2D** = biuret supplement every other day).

Supplemented treatments were formulated to provide 90% of the estimated degradable intake protein (**DIP**) requirement assuming a microbial efficiency of 11% (NRC, 1996). The urea and biuret treatments received the same amount of total supplemental N over a 2-day period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day compared with D treatments. Urea and biuret intake was approximately .069, .138, .085, and .170 g/kg BW on each supplementation day for UD, U2D, BD, and B2D, respectively. Estimates of undegradable intake protein (**UIP**) and DIP were based on in situ degradability using techniques similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for hard fescue straw and supplements, respectively. The amount of CP supplied by each supplement was approximately 0.04% of BW/d (averaged over a 2-day period). Protein supplements were placed directly into the rumen via the ruminal cannula at 0700 for supplemented treatments. Steers had continuous access to fresh water and chopped (4 - 8 cm) hard fescue (*Festuca trachyphylla*) straw. Nutrient content of the hard fescue straw and protein supplements is listed in Table 3.1. Forage was provided daily at 120% of the previous 5 d average intake in two equal portions (0715 and 1900), with feed refusals from the previous day determined before feeding. A trace mineralized salt mix was available free choice (7.3% Ca, 7.2% P, 27.8% Na, 23.1% Cl, 1.5% K, 1.7 % Mg, 0.5% S, 2307 ppm Mn, 3034 ppm Fe, 1340 ppm Cu, 3202 ppm Zn, 32 ppm Co, 78 ppm I, 90 ppm Se, 79 IU/kg vitamin E, and 397 kIU/kg vitamin A). In addition, an intramuscular injection of vitamins A, D, and E (500,000, 50,000, and

Table 3.1: Supplement composition and feedstuff nutrient content

Item	Hard Fescue Straw	Urea Supplement[a]	Biuret Supplement[a]
Urea %	-	5.3	-
Biuret %	-	-	6.1
Soybean Hulls %	-	91.0	90.2
Dried Molasses %	-	3.7	3.7
Nutrient Composition			
CP, % DM	4.0	28.9	29.0
DIP[b], %CP	76.0	83.0	84.2
OM, % DM	94.3	90.8	92.7
NDF, % DM	77.4	60.1	56.3
ADF, %DM	41.2	39.7	39.1

[a] Pelleted supplements were provided by ADM Alliance Nutrition, Inc., Quincy, IL.
[b] Degradable intake protein. Estimates are based on dacron bag degradabilities. Techniques were similar to those described by Mass et al. (1999) and Bohnert et al. (1998) for straw and supplements, respectively.

1500 IU of Vitamins A, D, and E, respectively; Vitamin E-AD 300; AgriLabs; St. Joseph, MO) was administered to each steer at the onset of the trial to safeguard against deficiency. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Oregon State University.

Experimental periods were 24 d, with 10 d of diet adaptation and 14 d of sampling. Intake was measured beginning d 11 and concluding d 22. On d 13 and 18, treatment effects on ruminal particulate fill were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on a day all supplements were provided and on a day only daily supplements were provided. Total ruminal contents were weighed, mixed by hand, and sub-sampled in triplicate (approximately 400 g). The remaining ruminal contents were replaced immediately into the animal. Ruminal samples were weighed, dried in a forced-air oven (55°C; 96 h), reweighed in order to calculate DM, ground to pass a 1-mm screen in a Wiley mill, and composited within period and day by steer. A more complete description of these procedures is provided in a companion paper (Currier et al., 2002b). Ruminal bacteria were isolated from ruminal contents on d 13. A 2-kg sample was weighed into a container and 1 L of cold (4°C) 0.9% (wt/vol) NaCl was added. This mixture was well mixed by hand and homogenized (Waring blender; Waring Products, New Hartford, CT) at high speed for 1 min and strained through four layers of cheesecloth. The bacteria were then separated from protozoa and feed particles by centrifugation (800 x g for 20 min). The resulting supernatant was collected and stored (-20° C) for later isolation of ruminal bacteria. The

supernatant was thawed, put into 250-mL bottles, and centrifuged (10,000 x g for 15 min, 4° C) to pellet bacteria. The resulting supernatant was decanted and discarded. The pellet was resuspended using distilled water and centrifuged (10,000 x g for 15 min, 4° C). This step was repeated once and the bacteria were frozen (-20° C), lyophilized, ground with a mortar and pestle, and composited by treatment.

Gelatin capsules containing 9 g of chromic oxide were dosed intra-ruminally at 0700 and 1900 on d 14 to 24 for use as an indigestible marker of digesta flow. Samples of hard fescue straw and CP supplements were collected on d 11 to 22 and orts were collected on d 12 to 23. Samples of feed and orts were dried at 55°C for 48 h. On d 19 to 24, approximately 200 g of duodenal digesta was collected at 0800, 1200, 1600, and 2000. Sub-samples (75 g) were composited by steer and stored (-20°C). Composited duodenal samples were lyophilized. Feces was collected on d 19 to 24. Steers were fitted with harnesses and fecal bags on d 19 (0730). Fecal bags were weighed and emptied twice daily at 0730 and 1630. The feces collected at 1630 were stored individually by steer in a sealed 50-gallon polyethylene bag for mixing with the 0730 collection the following morning (24 h fecal collection). Feces was manually mixed and a 2.5% sub-sample (wet weight) obtained, dried for 96 h at 55°C, re-weighed for DM, and composited by steer within period. Dried samples of hay, orts, and feces were ground through a Wiley mill (1-mm screen). Duodenal samples were ground through a 1-mm screen

using a Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) because of limited sample size.

Ground samples of hard fescue straw and CP supplements were composited by period and daily orts composited by steer (within period) on an equal weight basis (5% as-fed). Feed, orts, duodenal digesta, and feces were analyzed for DM and OM (AOAC, 1990), N (Leco CN-2000, Leco Corporation, St. Joseph, MI), and NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Samples of duodenal digesta and feces were prepared as described by Williams et al. (1962) for analysis of Cr using atomic absorption spectroscopy (air/acetylene flame; Model 351 AA/AE Spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA). Duodenal Cr concentration was used in conjunction with nutrient concentration to determine duodenal nutrient flow (Merchen, 1988). Recovery of dosed Cr in the feces averaged 105 ± 1%.

The purine content of duodenal digesta and ruminal bacteria was determined using the technique of Zinn and Owens (1986) as modified by Makkar and Becker (1999). Total flow of bacterial N at the duodenum was estimated by dividing the average bacterial N:purine ratio of harvested bacteria by the N:purine ratio of the duodenal digesta and multiplying the quotient by the total N flow at the duodenum.

STATISTICAL ANALYSIS

Data were analyzed as an incomplete 5 × 4 Latin square (Cochran and Cox, 1957) using the GLM procedure of SAS (1996). The model included period, steer, and treatment. Because the treatment structure consisted of a 2 × 2 factorial plus a negative control, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were: 1) CON vs. CP supplementation; 2) urea vs. biuret; 3) D vs. 2D supplementation; 4) NPN source × supplementation frequency (SF).

RESULTS

Intake of straw DM and OM was not affected ($P > 0.33$) by NPN supplementation or NPN source (Table 3.2). However, total DM and OM intake was increased ($P < 0.01$) with NPN supplementation compared with the control and tended ($P = 0.08$) to be greater for D compared with 2D treatments. Also, NDF intake was increased ($P < 0.01$) with NPN supplementation with no difference because of NPN source; however, D treatments tended ($P = 0.09$) to have greater NDF intake compared with 2D treatments.

Apparent and true (corrected for bacterial OM) OM disapearance from the stomach were not affected by NPN supplementation, NPN source, or SF ($P > 0.22$; Table 3.2). Also, NDF disappearance from the stomach was not altered by NPN supplementation or for urea compared with biuret ($P > 0.56$). However, NDF disappearance tended ($P = 0.08$) to be greater for D compared with 2D treatments.

Table 3.2: Effects of non-protein nitrogen (NPN) source and supplementation frequency on steer dry matter, organic matter, and neutral detergent fiber intake and organic matter and neutral detergent fiber disappearance in steers

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source × SF
Daily DM Intake, g/kg BW										
Straw	17.1	17.4	17.0	17.9	17.2	0.3	0.37	0.34	0.08	0.56
Supplement[d]	0.0	1.3	1.3	1.4	1.4					
Total	17.1	18.8	18.4	19.2	18.5	0.3	<0.001	0.32	0.08	0.57
Daily OM Intake, g/kg BW										
Straw	16.1	16.5	16.1	16.9	16.2	0.3	0.37	0.34	0.08	0.56
Supplement[e]	0.0	1.2	1.2	1.3	1.3					
Total	16.1	17.7	17.3	18.1	17.4	0.3	0.009	0.29	0.08	0.57
Daily NDF Intake, g/kg BW	13.2	14.3	14.0	14.6	14.0	0.2	0.004	0.55	0.09	0.56
Daily OM dissapperance from stomach										
Apparent, % OM intake	38.5	41.5	38.4	39.2	37.3	2.3	0.80	0.48	0.31	0.80
True, % of OM intake[f]	55.1	56.7	55.2	57.7	54.8	1.7	0.62	0.86	0.23	0.69
Daily NDF disappearance from stomach, % of NDF intake	55.9	60.1	55.6	59.1	54.7	2.3	0.57	0.69	0.08	0.98
Daily duodenal OM flow, g/kg BW	9.9	10.4	10.6	11.0	10.9	0.4	0.08	0.23	0.84	0.59
Daily OM disappearance from intestines,										
g/kg BW	2.5	2.2	2.8	3.0	2.8	0.4	0.61	0.31	0.55	0.34
% of duodenal OM flow	24.7	20.9	26.2	25.8	25.2	2.4	0.94	0.46	0.36	0.26
% of OM intake	15.3	12.3	16.2	16.3	16.3	2.1	0.99	0.35	0.37	0.38
Apparent total tract OM disappearance, %	53.7	53.8	54.6	55.5	53.7	0.5	0.31	0.52	0.38	0.04

Continued Table 3.2: Effects of non-protein nitrogen (NPN) source and supplementation frequency on steer dry matter, organic matter, and neutral detergent fiber intake and organic matter and neutral detergent fiber disappearance in steers

[a] CON = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source × SF = interaction of NPN source vs supplementation frequency.
[d] UD received 1.3 g/kg BW daily; U2D received 2.6 g/kg BW every other day; BD received 1.4 g/kg BW daily; B2D received 2.8 g/kg BW every other day.
[e] UD received 1.2 g/kg BW daily; U2D received 2.4 g/kg BW every other day; BD received 1.3 g/kg BW daily; B2D received 2.6 g/kg BW every other day.
[f] Corrected for bacterial OM.

Daily duodenal OM flow (g/kg BW) tended to increase ($P = 0.08$) with NPN supplementation (Table 3.2), but was not affected by NPN source or SF ($P > 0.22$). Daily intestinal disappearance of OM (expressed as g/kg BW; percentage of duodenal flow; percentage of OM intake) was not affected ($P > 0.25$) by NPN supplementation, NPN source or SF. In addition, NPN supplementation did not affect apparent total tract OM digestion ($P = 0.31$). However, we did observe an NPN source by SF interaction ($P = 0.04$) for apparent total tract OM digestion because OM disappearance decreased as SF decreased with biuret while no difference was observed between urea D and 2D treatments.

Daily N intake was increased ($P < 0.01$) with NPN supplementation and was greater ($P = 0.03$) for D versus 2D supplementation (Table 3.3). Also, duodenal N flow increased ($P = 0.04$) with NPN supplementation, with no difference because of NPN source or SF ($P > 0.11$). Bacterial N at the duodenum (g/kg BW) increased ($P = 0.04$) with NPN supplementation and for biuret ($P < 0.01$) compared with urea. However, duodenal bacterial N (expressed as a percent of total duodenal N), and non-bacterial N (g/kg BW), were not affected by NPN supplementation or SF ($P > 0.21$). Biuret had greater ($P < 0.01$) bacterial N flow at the duodenum (expressed as a percent of total duodenal N) than urea, while urea had greater ($P < 0.01$) non-bacterial N flow at the duodenum compared with biuret.

Mean bacterial N for all treatments was 7.17% (DM basis) and the overall average bacterial N: purine ratio was 1.16 (Table 3.3). Apparent bacterial N synthesis (g bacterial N/kg OM apparently digested in the rumen) tended ($P = 0.07$)

Table 3.3: Effects of non-protein nitrogen (NPN) source and supplementation frequency on nitrogen intake and disappearance in steers

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source × SF
Daily N Intake, g/kg BW	0.109	0.173	0.171	0.178	0.172	0.002	<0.001	0.06	0.03	0.28
Daily N flow at duodenum, g/kg BW	0.282	0.295	0.310	0.331	0.310	0.010	0.04	0.12	0.79	0.13
Daily bacterial N at duodenum, g/kg BW	.213	.208	.229	.271	.251	.010	0.04	0.002	0.92	0.07
Daily bacterial N at duodenum, % of total duodenal N	76.3	71.2	74.1	81.7	81.0	1.4	0.67	<0.001	0.43	0.22
Daily non-bacterial N at duodenum, g/kg BW	.069	.087	.081	.060	.059	.004	0.53	<0.001	0.33	0.50
Bacterial N, % of DM	7.14	6.96	7.13	7.17	7.44					
Bacterial N:purine ratio	1.16	1.23	1.19	1.09	1.11					
Bacterial N synthesis										
g of N/kg of OMAD[d]	34.5	28.2	35.3	39.8	40.9	4.1	0.74	0.07	0.35	0.48
g of N/kg of OMTD[e]	24.1	20.8	24.2	26.2	26.9	1.8	0.84	0.05	0.28	0.46
Daily N disappearance from stomach										
Apparent, % of N intake	-160.4	-69.9	-80.8	-86.6	-80.6	8	<0.001	0.33	0.77	0.32
True, % of N intake[f]	37.5	50.9	53.0	65.0	66.1	4.1	0.002	0.01	0.71	0.90
True, g/kg BW[f]	.040	.086	.090	.118	.113	.005	<0.001	<0.001	0.97	0.39
Daily N disappearance from intestines										
g/kg BW	0.197	0.196	0.216	0.230	0.210	0.010	0.19	0.19	0.99	0.07
% of intake	181.9	112.5	126.0	130.2	121.9	7.4	<0.001	0.39	0.74	0.18
% of duodenal flow	69.7	66.3	69.7	69.2	67.3	0.9	0.17	0.77	0.39	0.02
ATTN disappearance, %[g]	21.6	42.6	45.2	43.5	41.3	1.2	<0.001	0.25	0.86	0.07

Continued Table 3.3: Effects of non-protein nitrogen (NPN) source and supplementation frequency on nitrogen intake and disappearance in steers

[a] CON = control; UD = urea supplement every day; U2D = urea supplement every other day; BD = biuret supplement every day; B2D = biuret supplement every other day.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source × SF = interaction of NPN source vs supplementation frequency.
[d] OMAD = apparent OM disappearance from stomach.
[e] OMTD = true OM disappearance from stomach (corrected for bacterial OM).
[f] Corrected for bacterial N.
[g] ATTN = apparent total tract N disappearance.

to be greater for biuret compared with urea and was not affected by SF ($P > 0.34$). Likewise, true bacterial efficiency (corrected for bacterial N) was not affected by NPN supplementation or SF ($P > 0.27$) but was greater ($P = 0.05$) for biuret compared with urea.

Apparent N disappearance from the stomach (as a percentage of N intake) was more negative for the CON compared with NPN treatments ($P < 0.01$; Table 3.3). Also, true N disappearance from the stomach (corrected for bacterial N) was greater with NPN supplementation ($P < 0.01$) and for biuret compared with urea ($P < 0.01$).

Intestinal N disappearance (g/kg BW) was not affected ($P > 0.06$) by NPN supplementation, NPN source, or SF. Interestingly, intestinal N disappearance was greater ($P < 0.01$) for the CON compared with NPN supplemented treatments when expressed as a percentage of N intake. Crude protein supplementation did not influence disappearance of N from the intestine compared with the CON when expressed as a percentage of duodenal N flow ($P > 0.16$). However, we did note an NPN source by SF interaction ($P = 0.02$). This was because N disappearance increased as SF decreased with urea, while no change in disappearance was noted with biuret as SF decreased. In addition, apparent total tract N disappearance increased ($P < 0.01$) with NPN supplementation (Table 3.3).

DISCUSSION

This is the first research of which we are aware that has compared the effects of supplemental urea or biuret on microbial protein production and duodenal flow and intestinal disappearance of nutrients in ruminants consuming low-quality forage (<6% CP). Additionally, we provided NPN supplements on a D or 2D basis to better represent the infrequent consumption often observed with commercial NPN-based self-fed supplements. This data should add to our understanding of N metabolism in ruminants consuming N deficient, forage-based diets. Also, it provides ruminant nutritionists with information that can be used in evaluating NPN supplements for use by livestock operations that rely on low-quality forage as a primary feed source.

Intake of low-quality forage by ruminants has been reported to increase with CP supplementation (DelCurto et al., 1990; Köster et al., 1996; Bandyk et al., 2001). However, this response was not observed in the current study or in a companion paper in which wethers consumed similar hard fescue straw and identical NPN treatments (Currier et al., 2002a). Currier et al. (2002a) noted that forage DM and OM intake was not affected by NPN supplementation or NPN source compared with an unsupplemented control. They attributed the lack of a response to NDF intake, which was 19.6, 22.9, 21.3, 22.8, and 20.8 $g \cdot kg\ BW^{-1} \cdot d^{-1}$ for the control, UD, U2D, BD, and B2D, respectively. This appears valid based on the suggestion by Mertens (1985; 1994) that forage DM intake is maximized when NDF intake is approximately 12.5 $g \cdot kg\ BW^{-1} \cdot d^{-1}$. Other research with ruminants

consuming low-quality forage has supported this suggestion (Ferrell et al., 1999; Bohnert et al., 2002a,b). Ferrell et al. (1999) supplemented lambs consuming low-quality forage and reported that CP supplementation did not increase forage intake when NDF intake was 13.0 g · kg $BW^{-1} \cdot d^{-1}$ without supplemental CP. They suggested that an increase in forage intake with CP supplementation can be expected if NDF intake is low (below approximately 12.5 g · kg $BW^{-1} \cdot d^{-1}$) but not if NDF intake is high (above approximately 12.5 g · kg $BW^{-1} \cdot d^{-1}$). Bohnert et al. (2002a,b) supplemented steers and lambs consuming 5% CP meadow hay with a low- or high-DIP supplement and reported that forage intake was not increased with CP supplementation. They cited NDF intake was 13.0 and 13.9 g · kg $BW^{-1} \cdot d^{-1}$ without supplemental CP for lambs and steers, respectively, and averaged 14.5 and 13.2 g · kg $BW^{-1} \cdot d^{-1}$ and 15 and 15.1 g · kg $BW^{-1} \cdot d^{-1}$ for lambs and steers provided low- and high-DIP supplements, respectively. Furthermore, NDF intake in unsupplemented controls was approximately 6.4, 5.1, and 8.2 g · kg $BW^{-1} \cdot d^{-1}$ in the studies of DelCurto et al. (1990), Köster et al. (1996), and Bandyk et al. (2001) and increased to 14.3, 11.3, and 13.3 g · kg $BW^{-1} \cdot d^{-1}$ with CP supplementation. Therefore, based on these results and the high NDF intake observed in the current study, we did not anticipate an increase in forage intake with CP supplementation. Relatedly, the increase in NDF intake with CP supplementation in the current study is most likely because of the high NDF concentration in the supplements (approximately 57%; DM basis). This is supported by the lack of a difference in straw intake with CP supplementation.

In contrast to forage intake, total DM and OM intake increased with NPN supplementation, indicating total nutrient intake was increased. This agrees with numerous studies that have noted CP supplementation of ruminants consuming low-quality forage increases total DMI (Ferrell et al., 1999; Bohnert et al., 2002a,b). Also, no difference in straw and(or) total DM and OM intake because of NPN source is comparable to results from other studies comparing urea and biuret as CP supplements to ruminants consuming forage-based diets (Oltjen et al., 1969; Chicco et al., 1971; Currier et al., 2002a). Oltjen et al. (1969) supplemented steers consuming a timothy hay-based diet with urea or biuret and cited NPN source had no affect on total DMI. Chicco et al. (1971) supplemented young bulls consuming 6.7% CP forage with biuret or urea and reported forage intake was not affected by NPN source. Also, Currier et al. (2002a) noted that forage and total DM and OM intake was not affected by NPN source in lambs consuming the same straw and treatments used in the current study.

The tendency for daily supplementation to increase forage and total DM and OM intake compared with supplementation every other day is comparable to results reported in a companion paper (Currier et al., 2002a) and by Bohnert et al. (2002b). Currier et al. (2002a) supplemented wethers consuming 4.3% CP hard fescue straw with urea or biuret D or 2D and reported that D supplemented wethers tended to have greater (approximately 8%) forage and total DM and OM intake compared with those receiving a supplement 2D. Also, Bohnert et al. (2002b) supplemented lambs with a high- or low-DIP supplement every day, once every 3 d, or once every

6 d. They reported forage and total DMI decreased 8 and 19% and 7 and 17% as SF decreased from daily to once every 6 d for lambs receiving the high and low-DIP supplements, respectively. In contrast, Krehbiel et al. (1998) supplemented ewes fed bromegrass hay with soybean meal every 24 or 72 h and noted no difference in total DMI between 24- and 72-h supplemented ewes. Also, Huston et al. (1999a) supplemented ewes consuming wheat straw with cottonseed meal daily or once every 7 d and reported SF did not affect straw or total DMI.

It is not readily apparent why 2D supplementation tended to decrease intake compared with D supplementation in the current study. It is possible that the greater quantity of supplement provided on the 2D treatments may have substituted for forage intake, thereby decreasing forage and total DMI. Furthermore, Bohnert et al. (2002b,c) suggested that infrequent supplementation may disrupt rumen function for a period of time because of the larger quantity of supplement provided during a supplementation event. This is supported in the current study by the tendency for ruminal NDF digestibility to be greater for D compared with 2D treatments. However, ruminal fermentation characteristics (rumen liquid fill, liquid dilution rate, pH) reported in a companion paper are not consistent with this suggestion (Currier et al., 2002b). Also, the lack of a SF effect on apparent and true (corrected for bacterial OM) ruminal OM digestion suggests that ruminal fermentation was not negatively affected by 2D supplementation.

Our observation that apparent and(or) true (corrected for bacterial OM) OM and NDF disappearance from the stomach was not affected by CP supplementation

or NPN source agrees with the statement of Galyean and Owens (1991) that supplemental CP source (NPN, natural protein, DIP, or UIP) has little to no affect on site of digestion of low-quality forage. Also, other studies support our results that CP supplementation did not increase ruminal OM disappearance (Spragg et al., 1986; Bohnert et al., 2002a). Spragg et al. (1986) reported apparent ruminal OM digestibility by heifers fed alkali-treated oat straw alone or supplemented with cottonseed meal was 44 and 41% of OM intake, respectively. Also, Bohnert et al. (2002a) supplemented steers consuming meadow hay (5.3% CP) with low- or high-DIP supplements daily, once every 3 d, or once every 6 d, and did not affect apparent or true (corrected for bacterial OM) OM or NDF disappearance from the stomach compared with an unsupplemented control. However, these results contrast with those of Lintzenich et al. (1995) and Köster et al. (1996). Lintzenich et al. (1995) supplemented steers consuming dormant bluestem-range forage with alfalfa (hay, sun-cured pellets, or dehydrated pellets) and cited that CP supplementation increased true ruminal OM digestibility compared with an unsupplemented control. Similarly, Köster et al. (1996) supplemented cows consuming dormant, tallgrass-prairie forage with increasing amounts of casein and increased true ruminal OM and NDF digestibility compared with an unsupplemented control. The hays used by Lintzenich et al. (1995) and Köster et al. (1996) were very poor quality (1.9 and 2.8% CP, respectively) compared with the hard fescue straw in the current study (4% CP) and the forages used in the aforementioned studies of Spragg et al. (1986) and Bohnert et al. (2002a; 11 and

5% CP, respectively). Therefore, ruminal N was probably much more deficient and could have been more limiting to ruminal digestion. Ruminal NH_3-N concentrations in unsupplemented cattle support this hypothesis. They were 0.16, 0.24, 6.90, and 0.80 m*M* in the studies of Lintzenich et al. (1995), Köster et al. (1996), Spragg et al. (1986), and Bohnert et al. (2002a), respectively. Ruminal NH_3-N in the current study was 1.48 m*M* (Currier et al., 2002b). Consequently, CP supplementation may have had a more positive effect on ruminal digestion in the studies of Lintzenich et al. (1995) and Köster et al. (1996).

The tendency for duodenal OM flow to increase with CP supplementation coincides with increased total OM intake. In addition, other research has reported similar results with CP supplementation of forage-based diets (Spragg et al., 1986; Bohnert et al., 2002a). Spragg et al. (1986) reported that the quantity of OM leaving the abomasum was greater for heifers consuming alkali-treated oat straw and supplemented with cottonseed meal compared with an unsupplemented control. This was also directly related to total OM intake, which increased with supplementation. Similarly, Bohnert et al. (2002a) noted that total OM intake and duodenal OM flow increased with CP supplementation of steers fed 5% CP meadow hay.

The lack of a CP supplementation effect on intestinal OM disappearance contrasts with results reported by Bohnert et al. (2002a). They supplemented steers fed low-quality forage with low- or high-DIP supplements daily, once every 3 d, or once every 6 d and reported that CP supplementation increased intestinal OM

disappearance compared with an unsupplemented control. A possible explanation for the conflicting results cited above for intestinal OM disappearance is that we noted a tendency ($P = 0.08$) to increase duodenal OM flow with CP supplementation while Bohnert et al. (2002a) significantly ($P < 0.001$) increased duodenal OM flow. Also, duodenal OM flow increased with CP supplementation in the current study by approximately 8% while Bohnert et al. (2002a) reported an increase of 25% compared an unsupplemented control. Therefore, Bohnert et al. (2002a) may have had a greater chance of measuring an increase in intestinal OM disappearance. Also, Bohnert et al. (2002a) noted, as in the current study, no affect of CP source or SF on intestinal OM disappearance.

In contrast to our results, Bohnert et al. (2002a) and Currier et al. (2002a; companion paper) increased apparent total tract OM disappearance with CP supplementation of ruminants consuming low-quality forage. Also, both studies reported OM disappearance was not affected by CP source or SF while we noted an NPN source by SF interaction. However, other researchers have reported results similar to ours for total tract OM digestibility (Romero et al., 1976; Köster et al., 1996; Mathis et al., 2000). Romero et al. (1976) provided supplemental urea to steers consuming 2.2% CP forage and noted that OM digestibility was not affected by CP supplementation. Also, Köster et al. (1996) provided increasing levels of casein to cows fed dormant tallgrass-prairie forage (1.9% CP) and did not increase total tract OM digestibility compared with an unsupplemented control. Similarly, Mathis et al. (2000) provided increasing levels of casein to steers fed bermudagrass

(8.2% CP) or bromegrass (5.9% CP) hay and reported supplementation had no affect on total tract OM digestibility.

It is not clear why, as SF decreased, apparent total tract OM disappearance decreased with biuret but was not different for urea D and 2D. Bohnert et al. (2002b) reported a similar response with lambs consuming low-quality forage (5% CP). They provided a low- or high-DIP supplement daily, once every 3 d, or once every 6 d and noted a CP degradability by SF interaction in which apparent total tract OM digestibility increased as SF decreased with the low-DIP supplement and decreased as SF decreased with the high-DIP supplement. In another study, Farmer et al. (2001) supplemented steers fed dormant tallgrass-prairie hay with a 43% CP supplement 7 d/wk, 5 d/wk, 3 d/wk, or 2 d/wk and reported that total tract OM digestibility decreased as SF decreased. Also, Romero et al. (1976) provided urea once a day, twice a day, or every other day to steers fed a low-quality forage and did not affect total tract OM digestibility. The reason for inconsistent results for total tract OM digestibility with infrequent supplementation is not clear. Bohnert et al. (2002b) suggested that infrequent supplementation of a ruminally degradable protein source could disrupt ruminal fermentation, thereby decreasing OM digestion. However, this does not appear relevant to the current study because both supplements were comprised of approximately 90% soybean hulls and contained similar quantities of DIP (Table 3.1).

The approximately 60% increase in N intake with NPN supplementation was expected because of treatment design. However, the decrease in N intake as

SF decreased contradicts results reported in a companion paper (Currier et al., 2002a). Currier et al. (2002a) supplemented wethers fed a similar basal diet with the same treatments used in the current study and reported no affect of NPN source or SF on N intake. The reason for the greater N intake with daily compared with alternate day supplementation is because of differences in forage N intake. This is because N supplements were manually dosed on an equal N basis ($g \cdot kg\ BW^{-1} \cdot d^{-1}$) directly into the rumen; therefore, supplement N intake was equal for all supplemented treatments over a 2-day period. Similarly, Bohnert et al. (2002b) provided CP supplements to lambs on an isonitrogenous basis over a 6-day period and reported that N intake by lambs supplemented daily, once every 3 d, or once every 6 d decreased linearly as SF decreased. They also reported a linear decrease in forage intake as SF decreased.

Increased duodenal flow of total and bacterial N with CP supplementation of ruminants consuming low-quality forage has been reported in numerous studies (Hannah et al., 1991; Köster et al., 1996; Bohnert et al., 2002a). Also, research involving urea supplementation of ruminants consuming forage-based diets has been reported to increase bacterial N production, or bacterial numbers, compared with no supplementation (Hsu et al., 1991; Bowen et al., 1998). However, increasing the proportion of supplemental DIP provided by urea (at the expense of casein DIP) has not affected microbial N production in ruminants fed dormant tallgrass-prairie forage (Köster et al., 1996). Also, Kropp et al. (1977) noted that production of microbial N in steers consuming low-quality roughage (cottonseed

hulls) was not altered when urea provided 75, 100, or 115% of the steers' estimated CP requirement. Interestingly, biuret supplementation increased duodenal bacterial N flow, expressed as g/kg BW and as a percentage of total duodenal N, by almost 20 and 12%, respectively, compared with urea. Therefore, because NPN source did not have an affect on the total flow of duodenal N while biuret increased duodenal bacterial N flow compared with urea, duodenal non-bacterial N flow was greater with urea-supplemented steers compared with those receiving biuret. Also, it is of interest to note that bacterial N flow at the duodenum averaged almost 150% of N intake for all steers, indicating that N recycling played a large role in steer N metabolism.

We are aware of no research that has compared the effects of supplemental urea and biuret on bacterial N production. However, Oltjen et al. (1969) reported that the average number of total bacteria in rumen fluid was 422.3 and 439.2 $\times 10^8$ cells/mL for steers fed timothy hay and supplemented with urea or biuret, respectively. This was not a significant difference, but agrees numerically with our observation of increased bacterial N production for biuret compared with urea. It is possible that the slower and more sustained release of ruminal NH_3-N often observed with biuret compared with urea (NRC, 1976; Bartle et al., 1998) may have allowed for a ruminal environment that supported increased bacterial growth. The slower and more prolonged production of ruminal NH_3-N with biuret compared with urea is especially evident with 2D supplementation in the current study (Currier et al., 2002b). Currier et al. (2002b) reported that ruminal NH_3-N at

3, 6, and 9 h after supplementation on the day all supplements were provided was approximately 12, 6, and 3 m*M* and 5, 5, and 5 m*M* for urea and biuret 2D treatments, respectively.

Bacterial N averaged approximately 76% of total duodenal N for all treatments, emphasizing the importance of bacterial protein to the N status of ruminants consuming low-quality forage. Merchen and Bourquin (1994) noted in their review that bacterial N has ranged from 47 to 81% of total duodenal N in ruminants consuming low-quality forage; therefore, our values are at the high end of this range. This was not unexpected considering that the majority of the supplemental N was in the form of NPN and the hard fescue straw had a low N and high DIP content (Table 3.1). Therefore, bacterial N should be a high proportion of total duodenal N flow. Also, Clark et al. (1992) noted in their comprehensive review that microbial protein averaged 7.7% N (DM basis), ranging from 4.8 to 10.6%, while the average N:purine ratio was 1.06 and ranged from 0.61 to 2.13. Our results for average bacterial N (7.2% N; range of 7.0 to 7.4%) and N:purine ratio (1.16; range of 1.09 to 1.23) are well within this range.

Our observation that CP supplementation did not affect apparent or true (corrected for bacterial OM) bacterial efficiency, expressed as g bacterial N/kg OM ruminally digested, agrees with other studies with ruminants consuming low-quality forage (Krysl et al., 1989; Lintzenich et al., 1995; Bowen et al., 1998). Krysl et al. (1989) provided soybean meal or steam-flaked grain to steers grazing blue grama rangeland and reported that rumen microbial efficiency (g bacterial

N/kg OM truly digested) was not influenced by supplementation. Similarly, Lintzenich et al. (1995) noted that true microbial efficiency in steers fed dormant bluestem-range forage was not affected by CP supplementation. Also, Bowen et al. (1998) supplemented heifers consuming 2% CP hay with 8, 16, 32, or 64 g/d of urea and did not affect estimated ruminal bacterial efficiency (g bacterial N/kg DMI). However, these results contradict other studies in which CP supplementation increased rumen microbial efficiency in ruminants consuming low-quality forage (Köster et al., 1996; Bohnert et al., 2002a). Köster et al. (1996) provided 0, 180, 360, 540 or 720 g/d of DIP in the form of casein to beef cows fed dormant, tallgrass-prairie forage and linearly increased true microbial efficiency as supplemental DIP increased. Likewise, Bohnert et al. (2002a) provided a low- or high-DIP supplement to steers consuming 5% CP meadow hay and reported that supplementation increased the quantity of bacterial N/kg of OM truly digested in the rumen compared with an unsupplemented control. A possible reason for the increased bacterial efficiency with CP supplementation in the studies of Köster et al. (1996) and Bohnert et al. (2002a) is increased rumen liquid dilution rate. Rumen liquid dilution rate increased linearly as DIP supplementation increased in the study of Köster et al. (1996) and was approximately 16% greater for supplemented treatments compared with an unsupplemented control in the study of Bohnert et al. (2002a,c). In contrast, CP supplementation did not increase rumen liquid dilution rate in the current study (Currier et al., 2002b) or in the studies of

Krysl et al. (1989) and Lintzenich et al. (1995). Bowen et al. (1998) did not report rumen liquid dilution rate.

It is unclear why apparent bacterial efficiency tended to increase and true bacterial efficiency was increased with biuret compared with urea supplementation. Forage intake, supplement intake, and ruminal OM digestion were similar between NPN sources, as were rumen liquid and particulate passage rates (Currier et al., 2002b). It is possible that adaptation to biuret by ruminal bacteria allowed for a species composition shift that could have been more efficient in the use of NH_3-N and(or) OM from low-quality forage. However, we have no data to support this proposition. Also, as discussed above for bacterial N production, it is possible that the slower and more sustained release of ruminal NH_3-N often observed with biuret compared with urea may have allowed for a ruminal environment that increased bacterial efficiency.

The negative apparent ruminal N disappearance reported for all treatments indicates that N recycling played a major role in ruminal N metabolism (Bunting et al., 1989). This was especially evident with the CON in which apparent N disappearance was –160% of N intake. This is almost double the values noted with NPN supplementation. Negative values for apparent ruminal N disappearance have been reported in many studies with ruminants consuming low-quality forage (Hannah et al., 1991; Lintzenich et al., 1995; Köster et al., 1996; Bohnert et al., 2002a). In contrast to apparent ruminal N disappearance, true ruminal N disappearance (corrected for bacterial N) was approximately 57 and 154% greater

with NPN supplementation compared with the control when expressed as a percentage of N intake and g/kg BW, respectively. This is indicative of the high DIP content in the NPN supplements. Also, biuret supplementation resulted in approximately 26% greater true ruminal N disappearance, expressed as a percentage of N intake, while the total quantity of N that truly disappeared from the rumen (g/kg BW) was 31% greater compared with urea. This coincides directly with our observation of increased duodenal bacterial N flow and bacterial efficiency for biuret compared with urea supplementation.

Interestingly, we noted no difference in the quantity of total N (g/kg BW) that disappeared from the intestines of supplemented and unsupplemented steers. Also, each of the treatment means for intestinal N disappearance were greater than 100% of N intake, reaffirming the important role that N recycling played in N metabolism. The greater intestinal N disappearance, as a percentage of N intake, with the CON indicates that N recycling played a larger role in the N metabolism of unsupplemented steers compared with those receiving supplemental N. This agrees with our observation that duodenal bacterial N flow (g/kg BW) was approximately 195% of N intake with the CON compared with an average of 138% for NPN supplementation. The increase in intestinal N disappearance (as a percentage of duodenal N flow) with urea as SF decreased, compared with no affect with biuret, suggests that the digestibility of N flowing to the small intestine was increased with U2D compared with UD. However, a more probable explanation is that the U2D treatment had a greater quantity of NH_3-N flowing to the small intestine,

specifically on the day of supplementation, compared with UD. This would be consistent with the greater ruminal NH_3-N concentration with U2D compared with UD on the day all supplements were provided (Currier et al., 2002b). A portion of this NH_3-N could have flowed to the small intestine where it would have been absorbed, resulting in increased intestinal N digestibility.

Bohnert et al. (2002a) reported that SF had no affect on intestinal N disappearance. They supplemented steers consuming low-quality forage (5% CP) with a low- or high-DIP supplement daily, once every 3 d, or once every 6 d and reported that intestinal N disappearance, reported as a percentage of N intake or duodenal N flow, was not affected by SF. These results are consistent with our observations that intestinal N disappearance (g/kg BW and as a percentage of intake) was not affected by NPN source or SF. Also, this supports results noted with wethers and cows in a companion paper (Currier et al., 2002a). Currier et al. (2002a) reported that D or 2D supplementation of NPN to ruminants consuming low-quality forage resulted in similar N balance, N retention, and animal performance. Therefore, our results suggest that every-other-day supplementation of NPN to ruminants consuming low-quality forage does not affect intestinal N disappearance compared with daily supplementation.

Increased apparent total tract N disappearance with CP supplementation has been reported in other studies with ruminants consuming low-quality forage (Ferrell et al., 1999; Bohnert et al., 2002a,b). Also, these results concur with those reported for apparent total tract N disappearance in a companion study (Currier et

al., 2002a). Currier et al. (2002a) reported that CP supplementation increased apparent total tract N disappearance by approximately 112% in wethers consuming the same basal forage and NPN supplements used in the current study, while we noted CP supplementation increased total tract N disappearance by 100% compared with the CON. In addition, NPN source and SF did not affect apparent total tract N disappearance in the current study or in a companion paper (Currier et al., 2002a). This in in agreement with other research that has reported no affect of SF on total tract N disappearance in ruminants consuming forage-based diets (Coleman and Wyatt, 1982; Brown et al., 1996; Bohnert et al., 2002a,b).

IMPLICATIONS

Daily and alternate day supplementation of urea or biuret to ruminants consuming low-quality forage does not adversely affect forage intake, nutrient digestibility, site of digestion, or microbial efficiency compared with unsupplemented animals. Ruminants consuming low-quality forage appear to efficiently use urea and biuret as sources of supplemental nitrogen. Consequently, alternate day supplementation of non-protein nitrogen may provide beef producers with a management alternative to decrease supplementation costs and improve economic returns.

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DAILY AND ALTERNATE DAY SUPPLEMENTATION OF UREA OR BIURET TO RUMINANTS CONSUMING LOW-QUALITY FORAGE: III. EFFECTS ON RUMINAL FERMENTATION CHARACTERISTICS IN STEERS

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ABSTRACT

Five ruminally and duodenally cannulated steers (491 ± 21 kg BW) were used in an incomplete 5 × 4 Latin square with four 24-d periods to determine the influence of supplemental non-protein N (NPN) source and supplementation frequency (SF) on DMI and site of digestion in steers consuming low-quality forage (4% CP). Treatments (TRT) included an unsupplemented control (CON) and a urea or biuret supplement placed directly into the rumen daily (D) or every other day (2D) at 0700. Supplements were calculated to provide 90% of the estimated DIP requirement, with urea and biuret supplements (29% CP) provided on an isonitrogenous basis. Forage was provided at 120% of the previous 5 d average intake in two equal portions at 0715 and 1900. Ruminal fluid was collected 0, 3, 6, 9, 12, and 24 h after supplementation on a day of and a day before supplementation for all TRT. Ruminal NH_3-N increased ($P < 0.04$) with CP supplementation the day all supplements were provided and the day only daily supplements were provided compared with the CON. However, an NPN source × SF interaction ($P = 0.03$) on the day all supplements were provided indicated NH_3-

N increased at a greater rate for urea as SF decreased compared with biuret. Ruminal NH_3-N on the day only daily supplements were provided was greater (P = 0.02) for D compared with 2D. On the day all supplements were provided, D treatments increased (P = 0.05) ruminal indigestible acid detergent fiber passage rate and ruminal liquid volume compared with 2D treatments. These results suggest that urea or biuret can be used effectively as a supplemental N source by steers consuming low-quality forage without adversely affecting ruminal fermentation, even when provided every other day. Also, biuret should be safer and more useful as a CP supplement when offered infrequently to ruminants because of its slower ruminal degradation to NH_3-N compared with urea.

Key Words: Urea, Biuret, Forage, Non-protein N, Supplementation, Frequency

INTRODUCTION

Ruminants from late summer through winter typically consume low-quality forage (<6% CP) that results in low levels of ruminal NH_3-N that hinder microbial protein synthesis and ruminal fermentation (Funk et al., 1987; Köster et al., 1996). Supplementation with degradable intake protein (**DIP**) has been shown to increase ruminal NH_3-N in ruminants consuming low-quality forage (Köster et al., 1996; Bohnert et al., 2002c). Ruminal NH_3-N has been estimated to provide 40 to 100% of the N used for the production of microbial protein (Stern and Hoover, 1979), the

primary source of protein for ruminants consuming low-quality forages (Köster et al., 1996; Bohnert et al., 2002a).

Decreasing the frequency of CP supplementation to ruminants consuming low-quality forage has been shown to result in acceptable levels of performance (Huston et al., 1999; Bohnert et al., 2002b) with only minimal impacts on nutrient intake and digestibility (Beaty et al., 1994; Köster et al., 1996; Bohnert et al., 2002a). This supports the hypothesis of Cocimano and Leng (1967) that N recycling may support ruminal fermentation between supplementation events.

Non-protein N (**NPN**) supplements are attractive alternatives to most natural protein supplements, because of their low cost per unit of N. However, the rapid hydrolysis of urea to NH_3-N can result in NH_3 toxicity if consumed in large quantities in a short period of time (Raleigh and Wallace, 1963; Bartley et al., 1976). Biuret is comparatively non-toxic because it is less soluble in water and is ruminally degraded to NH_3-N at a slower rate compared with urea (Fonnesbeck et al., 1975; Hatfield et al., 1959). However, data is limited concerning ruminal fermentation in response to infrequent supplementation of NPN, with none comparing infrequent supplementation of urea and biuret. The objective of this research was to compare daily and alternate day supplementation of urea or biuret to steers consuming low-quality forage.

MATERIALS AND METHODS

A full description of experimental procedures (excluding ruminal fermentation measurement and analysis) and diet composition is given in a companion paper (Currier et al., 2002a). Briefly, five cannulated (ruminal and duodenal) beef steers (491 ± 21 kg) were allotted randomly to one of five treatments in an incomplete 5 × 4 Latin square design (Cochran and Cox, 1957) and housed in individual pens (4 × 8 m) within an enclosed barn with continuous lighting. Treatments consisted of an unsupplemented control and urea or biuret supplemented daily (**D**) or every other day (**2D**; **CON** = control, **UD** = urea supplement every day, **U2D** = urea supplement every other day, **BD** = biuret supplement every day, and **B2D** = biuret supplement every other day). Supplemented treatments were formulated to provide 90% of the estimated degradable intake protein requirement assuming a microbial efficiency of 11% (NRC, 1996). The urea and biuret treatments received the same amount of total supplemental N over a 2-d period; therefore, the 2D treatments received double the quantity of supplemental N on their respective supplementation day compared with D treatments.

Experimental periods were 24 d, with 10 d of diet adaptation and 14 d of sampling. On d 13 and 18, treatment effects on ruminal DM and indigestible ADF (**IADF**) fill were determined by manually removing reticulorumen contents 4 h after feeding. This allowed sampling on the day all supplements were provided and the day only daily supplements were provided, respectively. Total ruminal contents

were weighed, mixed by hand, and sub-sampled in triplicate (approximately 400g). The remaining ruminal contents were replaced immediately into the animal. Ruminal samples were weighed, dried in a force-air oven (55°C; 96 h), reweighed for DM, ground to pass a 1-mm screen in a Wiley mill, and composited within period and day by steer.

On d 19 and 24, each steer was intra-ruminally pulse-dosed with 5 g of Co-EDTA in a 150-ml aqueous solution (Uden et al., 1980) at 0700 (the time supplements were provided). As described above for ruminal evacuations, this allowed sampling on the day all supplements were provided and the day only daily supplements were provided. The Co marker was administered throughout the rumen using a stainless steel probe with a perforated tip. Ruminal fluid (approximately 100 mL) was collected by suction strainer (Raun and Burroughs, 1962; 19-mm diameter, 1.6-mm mesh) immediately prior to dosing and at 3, 6, 9, 12, and 24 h post-dosing. Ruminal fluid pH was measured immediately after collection (Orion SA 520, Boston, MA). Twenty milliliters were stored (-20°C) for later analysis of Co concentration and 5 mL acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and stored (-20°C) for subsequent analysis of VFA and NH_3-N. Frozen (-20°C) ruminal samples were prepared for analysis by thawing, centrifuging (15,000 x g for 10 min for VFA and NH_3-N; 2,000 x g for 20 min for Co), and collecting the supernatant. Cobalt concentration in ruminal fluid was analyzed by atomic absorption using an air/acetylene flame (Model 351 AA/AE Spectrophotometer, Instrumentation Laboratory, Inc., Lexington, MA). Ruminal

liquid fill and liquid dilution rate were estimated by regressing the natural logarithm of Co concentration against sampling time as described by Warner and Stacy (1968). Volatile fatty acids were analyzed as described by Horney et al. (1996) and NH_3-N by a modification (sodium salicylate substituted for phenol) of the procedure described by Broderick and Kang (1980) using a UV/VIS spectrophotometer (Spectronic 710 Spectrophotometer, Bausch & Lomb, Inc., Rochester, NY).

Ground samples of hard fescue straw and CP supplements were composited by period and daily orts composited by steer (within period) on an equal weight basis (5% as-fed). Feed, orts, and ruminal particulate were analyzed for DM and OM (AOAC, 1990), and, except for ruminal particulate, NDF (Robertson and Van Soest, 1981) and ADF (Goering and Van Soest, 1970) using procedures modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Feed, orts, ruminal particulate, and fecal samples (from Currier et al., 2002a) were analyzed for IADF as described by Bohnert et al. (2002c). Fecal recovery of IADF was 87.0 ± 1.0%. Digesta kinetics techniques described by Van Soest (1982) were used to determine IADF passage by dividing IADF intake by the quantity of IADF in the rumen 4 h after feeding.

STATISTICAL ANALYSIS

Ruminal liquid fill, liquid dilution rate, DM fill, IADF fill, and IADF passage rate were analyzed as an incomplete 5 × 4 Latin square using the GLM

procedure of SAS (1996). The model included period, steer, and treatment. Because the treatment structure consisted of a 2 × 2 factorial plus a negative control, orthogonal contrasts were used to partition specific treatment effects. Contrast statements were: 1) CON vs CP supplementation; 2) urea vs biuret; 3) D vs 2D supplementation; 4) NPN source × supplementation frequency (**SF**).

Ruminal pH and NH_3-N data were collected at the fixed times after feeding on the day all supplements were provided and the day only daily supplements were provided (d 19 and 24, respectively), were analyzed using the REPEATED statement with the MIXED procedure of SAS (1996). The model included steer, period, treatment, time, and treatment × time. In addition, steer × period × treatment was used to specify variation between steers (using the RANDOM statement). Steer × period × treatment was used as the SUBJECT and autoregression used as the covariance structure. The same contrasts noted above were used to partition the treatment sums of squares.

RESULTS

On the day all supplements were provided, ruminal DM fill and IADF fill were not affected ($P > 0.13$) by CP supplementation, NPN source, or SF (Table 4.1). However, ruminal IADF passage rate was greater ($P = 0.05$) for D treatments compared with 2D treatments, with no difference ($P > 0.19$) because of CP supplementation or NPN source.

Table 4.1: Effects of non-protein nitrogen (NPN) source and supplementation frequency on ruminal DM fill, indigestible acid detergent fiber (IADF) fill, liquid fill, and liquid and IADF passage rates in steers fed hard fescue straw

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source x SF
Day all supplements provided										
DM fill, g/kg BW	33.9	35.3	35.3	34.0	36.4	0.8	0.14	0.87	0.16	0.16
IADF fill, g/kg BW	8.40	8.76	8.76	8.61	8.82	0.19	0.16	0.81	0.62	0.58
IADF passage, %/h	1.62	1.62	1.58	1.71	1.59	0.03	0.99	0.20	0.05	0.22
Liquid fill, mL/kg BW	209	228	194	248	223	13	0.35	0.09	0.05	0.72
Liquid dilution rate, %/h	7.8	7.6	8.1	7.3	7.6	0.3	0.71	0.19	0.15	0.79
Day only daily supplements provided										
DM fill, g/kg BW	35.7	37.2	35.1	37.6	35.6	1.0	0.59	0.67	0.09	0.93
IADF fill, g/kg BW	8.80	9.34	8.86	9.53	8.94	0.24	0.21	0.59	0.06	0.81
IADF passage, %/h	1.55	1.52	1.57	1.53	1.56	0.04	0.89	0.96	0.39	0.76
Liquid fill, mL/kg BW	207	222	226	232	221	10	0.14	0.77	0.70	0.46
Liquid dilution rate, %/h	8.8	8.5	8.7	8.7	9.0	0.3	0.93	0.46	0.45	0.92

[a] CON = control; UD = urea supplement provided every day; U2D = urea supplement provided every other day; BD = biuret supplement provided every day; B2D = biuret supplement provided every other day.

[b] n = 4.

[c] Con vs Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alternate D = daily vs alternate day supplementation; NPN Source x SF = interaction of NPN source vs supplementation frequency.

Ruminal liquid fill was greater ($P = 0.05$) for D treatments when compared with 2D treatments, while no difference ($P = 0.35$) was noted because of CP supplementation on the day all supplements were provided (Table 4.1). However, there was a tendency ($P = 0.09$) for biuret treatments to have higher ruminal liquid fill than urea treatments. Ruminal liquid dilution rate was not affected ($P > 0.14$) by CP supplementation, NPN source, or SF on the day all supplements were provided.

On the day only daily supplements were provided, ruminal DM fill tended ($P = 0.09$) to be greater for D compared to 2D treatments (Table 4.1). Similarly, ruminal IADF fill tended ($P = 0.06$) to be greater for D when compared with 2D treatments. There were no differences ($P > 0.20$) for ruminal DM fill or IADF fill because of CP supplementation or NPN source. Also, there was no affect ($P > 0.13$) of CP supplementation, NPN source, or SF on ruminal IADF passage rate, liquid fill, or liquid dilution rate on the day only daily supplements were provided.

Treatment x time interactions ($P < 0.01$) were noted for ruminal NH_3-N on the day all supplements were provided and on the day only daily supplements were provided. However, after considering the nature of the interactions, we concluded that discussing treatment means while providing the treatment x time figures would aid in the interpretation and discussion of the data. No treatment x time interactions ($P > 0.10$) were present for ruminal pH data. Therefore, overall treatment means are discussed.

On the day all supplements were provided, ruminal NH_3-N increased ($P < 0.01$; Table 4.2; Figure 4.1) with CP supplementation. In addition, a NPN source x SF interaction ($P = 0.03$) occurred, indicating ruminal NH_3-N increased at a greater rate, and magnitude, with urea supplementation as SF decreased compared with biuret supplementation. Ruminal pH was not affected ($P > 0.21$) by CP supplementation, NPN source, or SF on the day all supplements were provided.

On the day only daily supplements were provided, ruminal NH_3-N was increased ($P = 0.03$) with CP supplementation (Table 4.2; Figure 4.1). In addition, ruminal NH_3-N was greater for D compared with 2D treatments ($P = 0.02$) but not affected ($P = 0.41$) by NPN source. Ruminal pH was not affected ($P > 0.47$) by CP supplementation, NPN source, or SF.

DISCUSSION

In our review of research concerning NPN supplementation of ruminants consuming low-quality forage, we are aware of limited data that has compared the effects of urea and biuret on ruminal fermentation (Oltjen et al., 1969; Chicco et al., 1971; Bond and Rumsey, 1973; Löest et al., 2001) and none that has compared the effects of infrequent supplementation of urea and biuret on ruminal fermentation. Therefore, this research will add to our understanding of ruminal fermentation and N metabolism in ruminants consuming low-quality forage while providing ruminant nutritionists and beef cattle producers with information they can use in

Table 4.2: Effects of non-protein nitrogen (NPN) source and supplementation frequency on steer ruminal fermentation characteristics on the day all supplements were provided and the day only daily supplements were provided

	Treatment[a]						P-Value[c]			
Item	CON	UD	U2D	BD	B2D	SEM[b]	Con vs Supp	Urea vs Biuret	Daily vs Alternate D	NPN Source x SF
Day all supplements provided										
Ammonia N, mM	1.36	2.57	4.47	2.72	3.30	0.24	<0.001	0.07	0.001	0.03
pH	6.45	6.49	6.50	6.54	6.50	0.04	0.22	0.49	0.75	0.56
Day only daily supplements provided										
Ammonia N, mM	1.59	2.78	1.80	2.88	2.18	0.27	0.03	0.41	0.02	0.61
pH	6.48	6.53	6.54	6.47	6.53	0.05	0.50	0.52	0.48	0.68

[a] CON = control; UD = urea supplement provided every day; U2D = urea supplement provided every other day; BD = biuret supplement provided every day; B2D = biuret supplement provided every other day.
[b] n = 4.
[c] Con vs Supp = control vs supplemented treatments; Urea vs Biuret = urea vs biuret treatments; Daily vs Alt. Day = daily vs alternate day supplementation; NPN Source x SF = interaction of NPN source vs supplementation frequency.

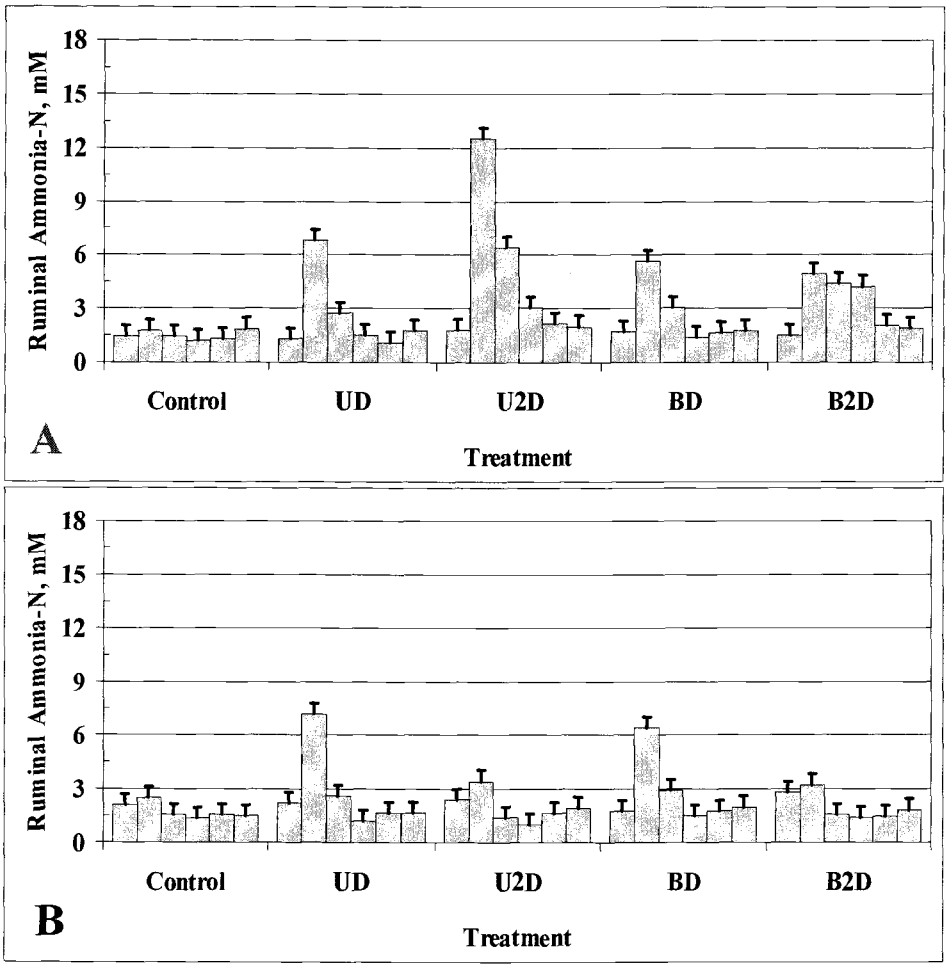

Figure 4.1: Effects of non-protein nitrogen source and supplementation frequency on steer ruminal ammonia N the day all supplements were provided (A) and the day only daily supplements were provided (B). Columns from left to right for each treatment represent 0, 3, 6, 9, 12, and 24 h post-feeding, respectively. Treatments were: Control; UD = urea supplement provided every day; U2D = urea supplement provided every other day; BD = biuret supplement provided every day; and B2D = biuret supplement provided every other day. Treatment x time interactions for A and B are ($P < 0.0001$). SEM for A and B are 0.61 and 0.39, respectively.

developing safe and effective supplementation strategies for use with NPN-based supplements

Variable results have been reported for ruminal liquid and particulate fill and passage rates with CP supplementation of ruminants consuming low-quality forage. Most research has resulted in increased (DelCurto et al., 1990; Hannah et al., 1991; Köster et al., 1996), or no difference in (Caton et al., 1988; Krysl et al., 1989; Beaty et al., 1994), liquid and particulate fill and(or) passage rates. Faichney (1993) reported in his review that rate of passage in ruminants is affected by dietary factors including intake. He suggested that, generally, increased intake is associated with increased passage rate. This may explain much of the inconsistency noted with ruminal liquid and particulate dynamics in CP supplemented ruminants consuming low-quality forage. DelCurto et al. (1990) provided a 12, 28, or 41% CP supplement to steers consuming dormant, tallgrass-prairie forage and noted that CP supplementation increased forage intake (BW basis), ruminal IADF fill, ruminal IADF flow (g/h), ruminal liquid fill, and ruminal liquid dilution rate compared with an unsupplemented control. Similarly, Hannah et al. (1991) reported that supplementing steers fed dormant, bluestem-range forage with a 27% CP concentrate or 18% CP alfalfa pellets increased forage DMI, ruminal IADF fill, ruminal IADF flow (g/h), and ruminal liquid fill and dilution rate compared with non-supplemented steers. Also, Köster et al. (1996) provided increasing quantities of DIP (in the form of casein) to cows consuming dormant, tallgrass-prairie forage and linearly increased ruminal liquid fill and dilution rate

while noting a comparable increase in forage OM intake. In contrast to the aforementioned studies, Caton et al. (1988) supplemented steers grazing blue grama rangeland with a 46% CP supplement and reported that supplementation did not affect ($P > 0.05$) ruminal liquid dilution rate, particulate passage rate, or forage OM intake compared with an unsupplemented control. Furthermore, Krysl et al. (1989) supplemented steers grazing forage similar to that used by Caton et al. (1988) with soybean meal and noted that forage OM intake, ruminal particulate passage rate, ruminal liquid dilution rate, and ruminal liquid fill were not different compared with unsupplemented steers. Consequently, our observation that CP supplementation did not affect ruminal DM, IADF, and liquid dynamics compared with the CON on the day all supplements were provided and the day only daily supplements were provided coincides with the lack of a CP supplementation effect on forage DM and OM intake reported in a companion paper (Currier et al., 2002b).

We are aware of no other research that has compared the effects of urea and biuret on ruminal liquid and particulate dynamics in ruminants consuming low-quality forage. However, other studies have evaluated ruminal fill and dilution rate as a result of increasing the proportion of total supplemental N provided by urea (Kropp et al., 1977; Köster et al., 1997). Kropp et al. (1977) substituted urea for 0, 25, 50, or 75% of the total supplemental N provided by soybean meal to steers fed 3% CP forage and noted that increasing the proportion of supplemental N provided by urea did not affect ruminal liquid dilution rate. Also, Köster et al. (1997)

supplemented steers consuming dormant, tallgrass-prairie forage with supplements in which urea provided 0, 25, 50, 75, or 100% of the supplemental N, with casein providing the remainder of the supplemental N, and did not alter ruminal DM fill, liquid fill, or liquid dilution rate as the proportion of urea increased. These results coincide with our observation that NPN source did not affect ruminal DM fill, particulate fill and passage rate, and liquid fill and dilution rate on the day all supplements were provided and the day only daily supplements were provided. Therefore, it appears that urea and biuret elicit similar affects on ruminal fluid and particulate dynamics in ruminants consuming low-quality forage.

We are aware of three studies that have evaluated the affects of SF on ruminal particulate and liquid dynamics in ruminants consuming low-quality forage (Beaty et al., 1994; Farmer et al., 2001; Bohnert et al., 2002c). Our observation that ruminal IADF passage rate and liquid fill decreased as SF decreased on the day all supplements were provided disagrees with the results of Bohnert et al. (2002c). They supplemented steers fed low-quality meadow hay with low-DIP (40% DIP; CP basis) or high-DIP (82% DIP; CP basis) supplements daily, once every 3 d, or once every 6 d and noted that IADF passage rate was not affected by SF. It is probable that the decreased IADF passage rate as SF decreased on the day all supplements were provided in the current study is related to the tendency for forage and total OM intake to decrease as SF decreased (Currier et al., 2002b). In addition, this could explain the tendency for IADF fill to decrease as SF decreased on the day only daily supplements were provided. Also, Bohnert et al. (2002c)

noted that ruminal liquid fill increased as SF decreased from daily to once every 6 d with the high-DIP supplement but was not altered with the low-DIP supplement. They suggested that, on the day all supplements were provided, increased ruminal liquid fill as SF decreased with the high-DIP supplement might have been because of a disruption in rumen function caused by the large quantity of ruminally degradable supplement provided during a supplementation event for the infrequently supplemented groups compared with the daily treatment. This appears reasonable because infrequent supplementation of the low-DIP supplement (low rumen degradability) did not affect ruminal liquid fill on the day all supplements were provided. Furthermore, they noted that as SF of the high-DIP supplement decreased from daily to once every 6 d, liquid dilution rate decreased approximately 18% on the day all supplements were provided and approximately 28% on the day only daily supplements were provided. Like results were reported by Farmer et al. (2001). They provided an approximately 60% DIP (CP basis) supplement 7 d/wk, 5 d/wk, 3 d/wk, or 2 d/wk to steers fed dormant tallgrass-prairie hay and noted that ruminal liquid passage rate decreased as SF decreased on the day all supplements were provided. These results suggest that providing a large quantity of a highly rumen degradable supplement infrequently (≥ once every 3 d) to ruminants consuming low-quality forage may disrupt rumen function (liquid and particulate fill and passage rates). However, our results suggest that alternate day supplementation of urea or biuret to ruminants consuming low-quality forage has

minimal effects on ruminal liquid and particulate dynamics compared with daily supplementation.

Increased ruminal NH_3-N with CP supplementation of ruminants consuming low-quality forage has been demonstrated in numerous studies (Caton et al., 1988; Köster et al., 1996; Weder et al., 1999). This agrees with our observation that CP supplementation increased ruminal NH_3-N by approximately 240% on the day all supplements were provided and by 152% on the day only daily supplements were provided compared with the CON. Bohnert et al. (2002c) reported similar results with steers fed 5% CP meadow hay and provided a low- or high-DIP supplement daily, once every 3 d, or once every 6 d. They noted that ruminal NH_3-N was increased, on average, by 267 and 173% on the day all supplements were provided and the day only daily supplements were provided, respectively, compared with an unsupplemented control.

The NPN source by SF interaction observed for ruminal NH_3-N on the day all supplements were provided coincides with the ruminal CP degradability by SF interaction reported by Bohnert et al. (2002c). They noted that ruminal NH_3-N increased at a greater rate, and peaked at an elevated concentration, as SF decreased with a high-DIP supplement compared with a low-DIP supplement on the day all supplements were provided. In the current study, peak ruminal NH_3-N on the day all supplements were provided increased from approximately 7 m*M* with UD to almost 13 m*M* with U2D compared with peaks of approximately 5 m*M* for BD and B2D (Figure 4.1). This is indicative of the lower ruminal solubility and slower

enzymatic hydrolysis associated with biuret compared with urea (Fonnesbeck et al., 1975). In addition, other research has demonstrated higher ruminal NH_3-N concentrations for urea supplementation compared with biuret (Chicco et al., 1971; Bartle et al., 1998; Löest et al., 2001). Chicco et al. (1971) supplemented young bulls fed 7% CP forage with urea or biuret and reported that urea increased ruminal NH_3-N by 148% compared with biuret. Also, Bartle et al. (1998) supplemented steers consuming a soybean hulls-based diet with isonitrogenous quantities of urea, biuret, or soybean meal and noted that urea increased NH_3-N concentration at 30, 60, 90, 180, and 360 min after supplementation compared with biuret or soybean meal. Similarly, Löest et al. (2001) used cooked molasses blocks, in which urea or biuret provided the main source of supplemental N, as CP supplements to steers consuming 5.5% CP prairie hay. They reported that ruminal NH_3-N was greater 2, 4, and 8 h after supplementation for urea compared with biuret-based blocks. These results can be interpreted to suggest that ruminal hydrolysis of biuret to NH_3-N is slower than hydrolysis of urea to NH_3-N. Therefore, biuret should be safer than urea when supplemented infrequently to ruminants. Also, early work with biuret suggested that an adaptation period is required to allow ruminal microorganisms to develop adequate biuretolytic activity (Schröder and Gilchrist, 1969; Clemens and Johnson, 1973a; Johnson and Clemens, 1973) and this activity is rapidly lost when biuret supplementation is halted (Clemens and Johnson, 1973b). However, research reported here and in two companion papers suggests that adequate biuretolytic activity can be obtained after at least 18 d of

supplementation and is not lost with every other day supplementation. This is based on our observation that ruminal NH_3-N concentration, N balance and cow performance (Currier et al., 2002a), and rumen microbial protein production (Currier et al., 2002b) were comparable to urea supplementation and not affected by SF.

Ruminal pH averaged approximately 6.5 for all treatments on the day all supplements were provided and on the day only daily supplements were provided. Also, ruminal pH never fell below 6.3 (data not shown) and should have been sufficient to support adequate fiber digestion (Yokoyama and Johnson, 1988). This is supported by results reported in a companion paper that ruminal OM and NDF disappearance were not affected by CP supplementation, NPN source, or SF (Currier et al., 2002b).

IMPLICATIONS

Daily and alternate day supplementation of non-protein nitrogen can be an effective means of providing supplemental nitrogen to ruminants consuming low-quality forage (<6% crude protein). Steers were able to maintain adequate ruminal fiber digestion, particulate passage, and fluid dynamics when supplemented daily or every other day with urea or biuret. Biuret should be a safer and more useful crude protein supplement when offered infrequently to ruminants because of its slower ruminal degradation to ammonia nitrogen and lower probability of causing ammonia toxicity compared with urea. Alternate day supplementation of non-

protein nitrogen can provide ruminant livestock producers with a management alternative that decreases crude protein supplementation costs and improves economic sustainability while maintaining performance similar to daily supplementation.

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CONCLUSION

Ruminants consuming low-quality forage (< 6% crude protein) can effectively use supplemental non-protein nitrogen. In addition, daily and alternate day supplementation of non-protein nitrogen results in similar efficiencies of nitrogen use and cow performance, with both being more positive compared with an unsupplemented control. Also, daily or alternate day supplementation of urea or biuret to ruminants consuming low-quality forage does not adversely affect forage intake, nutrient digestibility, site of digestion, or microbial efficiency compared with unsupplemented steers. Steers were able to maintain adequate ruminal fiber digestion, particulate passage, and fluid dynamics when supplemented daily or every other day with urea or biuret. Ruminants appear to have the ability to conserve nitrogen over an extended period, thereby storing it for use between supplementation events. Infrequent supplementation of non-protein nitrogen, primarily with urea, should be used with caution because of the potential for ammonia toxicity. However, biuret should be a safer and more useful crude protein supplement when offered infrequently to ruminants because of its decreased solubility and slower ruminal degradation and hydrolysis to ammonia nitrogen compared with urea. Therefore, alternate day supplementation of non-protein nitrogen can provide ruminant livestock producers with a management alternative that decreases crude protein supplementation costs and improves economic

sustainability while maintaining performance similar to that of daily supplementation.

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APPENDIX

Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	Avg. Change BCS	Reference
Grass pasture plus liquid supplement (Winter)	Negative CON Molasses Molasses +Biuret Molasses + Biuret/Salt Molasses +Urea	Calves	12	84	-.14 -.05 .07 .02 -.11		Templeton et al., (1970)
Prairie hay and winter range	Negative CON Soybean meal Dry Urea Dry Biuret Liquid Urea Liquid Molasses	Cows	56	84	-1.06 -.86 -.94 -1.09 -1.14 -1.39		Rush and Totusek (1976)
Tallgrass prairie (winter)	Negative CON Positive CON Feed-grade Biuret Biuret Urea	Cows	257	90	-1.36 -1.16 -1.26 -1.29 -1.41	-2.58 -2.00 -2.39 -2.48 -2.90	Rush et al., (1976)
Tallgrass prairie (winter) (Plus a 43%CP Suplement)	Sup 2 times/week Sup 3 times/week Sup 5 times/week Sup 7 times/week	Cows	120	63	-1.18 -1.07 -1.07 -0.95	-.73 -.63 -.75 -.66	Farmer et al., (2000)

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Timothy Hay (6.4% CP)	Negative CON Soybean Meal Urea Biuret	Calves	8	70	0.76 1.20 1.35 1.20	Campbell et al., (1963)
Fatting Ration (20% Wheat Straw)	Negative CON(12%CP) Soybean Meal Urea Biuret	Steers	32	112	0.58 0.59 0.59 0.59	Mies et al., (1967)
Fatting Ration (20% Wheat Straw)	Negative CON(10%CP) Soybean Meal Urea Biuret	Steers	40	112	0.17 0.46 0.33 0.37	Mies et al., (1967)
(1) Crested Wheat (2) Crested Wheat+Barley	Negative CON (1) Negative CON (2) Biuret+Barley (1) Biuret+Barley (2) Urea+Barley (1) Urea+Barley (2) CSM (1) CSM (2)	Heifers	40	100	0.35 0.58 0.68 0.77 0.57 0.68 0.58 0.77	Raleigh and Turner, (1968)

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Corn Silage	Negative CON Urea/Feeding Biuret/Feeding Urea/Ensiling Biuret/Ensiling	Calves	54	99	0.53 0.78 0.69 0.77 0.69	Meiske et al., (1969)
Hay / Barley (20%CP)	CSM Urea Biuret	Steer Calves	54	113	0.55 0.47 0.48	Turner and Raleigh, (1969)
(1) High Roughage (2) Low Roughage	CSM (1) CSM (2) Biuret (1) Biuret (2) CSM+Biuret (1) CSM+Biuret (2)	Steer Calves	36	112	0.64 0.53 0.59 0.51 0.64 0.57	Turner and Raleigh, (1969)
Meadow Hay (1) High Energy (2) Low Energy	Biuret (1) Biuret (2) Urea (1) Urea (2) CSM (1) CSM (2)	Cow-calf	84	12/18-8/2	0.73 0.74 0.74 0.76 0.77 0.75	Turner et al., (1970)

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Meadow Hay (1) High Energy (2) Low Energy	Biuret (1) Biuret (2) Biuret+CSM (1) Biuret+CSM (2) CSM (1) CSM (2)	Cow-calf	102	12/2-7/29	0.78 0.75 0.77 0.78 0.80 0.80	Turner et al., (1970)
Cottonseed Hulls (Salt limiter)	CSM Hand fed CSM Self fed Biuret Hand fed Biuret Self fed	Steer Calves	48	35	0.72 0.83 0.73 0.40	Templeton, (1972)
Grass Hay (8.8% CP)	Alfalfa Biuret	Heifers	200	173	0.47 0.35	Nichols et al., (1972)
Timothy Hay (4.3% CP)	Negative CON Liq. Molasses Liq. Molasses + Urea Liq. Molasses + Biuret	Cows	88	118	-.01 -.10 -.11 0.06	Bond and Rumsey, (1973)

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Timothy Hay (4.3% CP)	Negative CON Liq. Molasses Liq. Molasses + Urea Liq. Molasses + Biuret	Yearlings	34	84	0.52 0.56 0.53 0.46	Bond and Rumsey, (1973)
Timothy Hay (4.3% CP)	Negative CON Liq. Molasses Liq. Molasses + Urea Liq. Molasses + Biuret	Calves	30	112	0.26 0.29 0.28 0.36	Bond and Rumsey, (1973)
Native Range	Negative CON Molasses Urea Urea Biuret Biuret Starch-Urea Starch-Urea Clay-Urea Clay-Urea Wheat Straw Wheat Straw	Calves		112-130	-.20 0.20 0.16 0.05 0.13 0.14 0.15 0.15 0.12 0.10 0.16 0.12	Clanton, (1978)

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Native Range	Negative CON	Calves		112-130	-.05	Clanton, (1978)
	SBM				0.24	
	SBM-Corn-Urea				0.24	
	SBM-Corn-Urea				0.15	
	SBM-Corn-Biuret				0.21	
	SBM-Corn-Biuret				0.20	
	SBM-Alfalfa				0.25	
	SBM-Alfalfa-Urea				0.23	
	SBM-Alfalfa-Urea				0.19	
	SBM-Alfalfa-Biuret				0.21	
	SBM-Alfalfa-Biuret				0.24	
Wheat Straw (7.13% CP)	Negative CON	Lambs	50	73	0.08	Meiske et al., (1955)
	Urea				0.14	
	Biuret				0.13	
	Crude Biuret				0.12	
	SBM				0.14	
Corn-Cob ration	Dehy-Alfalfa-Urea	Ewe Lambs	60	87	0.20	Karr et al., (1964)
	Dehy-Alfalfa-Biuret				0.21	
	SBM				0.19	

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Cracked Corn	SBM SBM+Alfalfa Urea Urea+Alfalfa Biuret Biuret+Alfalfa	Lambs	144	78	0.14 0.20 0.08 0.18 0.10 0.20	Karr et al., (1965)
Corn Silage	Negative CON Urea/Ensile Urea Biuret/Ensile Biuret SBM	Lambs	72	42	0.14 0.17 0.17 0.19 0.17 0.20	Karr et al., (1965b)
Corn	SBM fed 1/d SBM fed 2/d Urea fed 1/d Urea fed 2/d Biuret fed 1/d Biuret fed 2/d	Lambs	60	71	0.18 0.20 0.20 0.21 0.17 0.20	Bolsen et al., (1968)

Continued Table 5.1: An Evaluation of Urea and Biuret Supplementation on Performance in Ruminants

Basal Diet	CP Treatments	Animal Type	No. Animals	Days on Test	Avg. Daily Gain (kg)	References
Winter Range	SBM + 12.5% Biuret + 25.0% Biuret + 50.0% Biuret + 12.5% Urea + 25.0% Urea + 50.5% Urea	Gestating Ewes	30	93	0.04 0.03 0.04 0.03 0.04 0.04 0.04	Van Horn et al., (1969)