EFFECT OF β–ADRENERGIC AGONISTS ON UREA RECYCLING BY CATTLE FED VARYING LEVELS AND FORMS OF NITROGEN SUPPLEMENTATION

by

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Abstract

Two experiments analyzed effects of zilpaterol-HCl and nitrogen supplementation in the form of either dried distiller's grains with solubles (DDGS) or urea fed to steers. In Experiment 1, steers were fed corn-based diets: control (CON; 10.2% CP), urea (UREA; 13.3% CP), or DDGS (14.9% CP). Nitrogen intake differed among treatments (99, 151, and 123 g/d for CON, DDGS, and UREA). Urea-N synthesis tended to be greater for DDGS (118 g/d) than for UREA (86 g/d), which tended to be greater than CON (52 g/d). Urinary urea-N excretion was greater (P<0.03) for DDGS (35.1 g/d) and UREA (28.6 g/d) than for CON (12.7 g/d). Gut entry of urea-N (GER) was numerically greatest for DDGS (83 g/d), intermediate for UREA (57 g/d), and least for CON (39 g/d). Urea-N returned to the ornithine cycle tended to be greater for DDGS (47 g/d) than for UREA (27 g/d) or CON (16 g/d). The percent of microbial N flow derived from recycled urea-N tended (P=0.10) to be greater for DDGS (35%) than for UREA (22%) or CON (17%). The percent of urea production captured by ruminal bacteria was greater (P<0.03) for CON (42%) than for DDGS (25%) or UREA (22%). Experiment 2 diets were identical to those used in Experiment 1. In addition, steers were also fed either 0 or 60 mg/d zilpaterol-HCl. Dietary CP was 9.6, 12.4, and 13.7% for CON, UREA, and DDGS, respectively. Zilpaterol increased (P<0.01) total DMI and N intake; however, zilpaterol did not affect urea entry rate (P=0.80) or GER (P=0.94). Urea entry rate and GER were numerically greater for DDGS than CON and UREA. In conclusion, zilpaterol did not influence urea entry rate or GER. This lack of response in the face of greater N intake was interpreted to suggest that zilpaterol may reduce urea production and GER at constant N intake.

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Completing this thesis marks the completion of a goal I set out to achieve just over two years ago, and it is something that carries an inherent sense of accomplishment. I did not reach this point solely on my own; were it not for the help others, this thesis would have been nearly impossible to complete. It is here that I will attempt to thank all of those who helped me along this journey.

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Dedication

I dedicate this to my wife; the greatest woman I know, and my best friend.

"The quality of a person's life is in direct proportion to their commitment to excellence, regardless of their chosen field of endeavor." -Vince Lombardi

CHAPTER 1 - Literature Review

Introduction

Ruminants recycle N in the form of urea for the purpose of amino acid synthesis. This improves the efficiency of dietary N use; however, metabolizable protein is not typically limiting to growth efficiency or growth rate of ruminants. Ethanol co-products may alter site and extent of N absorption compared to parent grains. Therefore, N recycling in growing ruminants fed ethanol co-products is warranted. Additionally, β-adrenergic agonists, when fed to promote growth of ruminants, are likely to have a significant impact upon N-recycling. It is unlikely that β-adrenergic agonists alter site or extent of N digestion; however, they may impact the amount of urea-N available for recycling to the rumen. This could lead to consequences for animals fed a large proportion of their intake protein as ruminally undegraded intake protein (UIP) or those animals which are slightly deficient in total intake protein. Although improvements have been made recently in quantifying the rate at which ruminants recycle N, more work is still needed with regard to growing ruminants. Until a model can be presented that accurately accounts for the amounts of N recycled by growing ruminants, economic losses due to suboptimal gains or wasteful nitrogenous excretions will persist. Ultimately, the following questions must be answered. How much recycling is associated with current forms of N supplementation in growing ruminants? Can N requirements for growing ruminant be refined? Can alternative forms of dietary N reduce environmental impact and maximize gain efficiency? The purpose of this review is to analyze the responses of growing ruminants to current practices used to deliver dietary N, with an emphasis on use of distiller's grains as a dietary N source.

Purposes of and Responses to N Supplementation

Nitrogen is consumed by ruminants in one of two forms: true protein or non-protein N (NPN). Protein is digested in the rumen by hydrolysis to peptides and then, further, to amino acids. Nonstructural carbohydrate-fermenting bacteria within the rumen may utilize peptides and amino acids for microbial cell protein production. When energy limits microbial growth in the rumen, 66% of the microbial protein in nonstructural carbohydrate-fermenting bacteria comes from peptides or amino acids (Russell et al., 1983). Amino acids that are taken up by bacteria may be degraded to ammonia and a carbon skeleton, which may be used to produce volatile fatty acids (VFA). Non-protein N is traditionally introduced into the rumen as urea, ammonia, nucleic acids, or nitrates. Urea is readily hydrolyzed to release ammonia in the rumen. Ruminal nonstructural carbohydrate-fermenting bacteria derive 34% of their microbial-cell protein from this free ammonia pool within the rumen (Russell et al., 1983). Additionally, bacteria that ferment structural carbohydrate are unable to efficiently utilize peptides and amino acids (Bryant and Robinson, 1961; Allison et al., 1962; Bryant, 1973). Thus, most of the microbial-cell protein produced by these species must be derived from the ammonia pool. Microbial-cell proteins (otherwise stated as bacterial crude protein, BCP) ultimately pass out of the rumen to the duodenum.

According to the NRC (1996), BCP can supply between 50 and 100% of the metabolizable protein (MP) required by beef cattle. Hence, protein in growing cattle diets is often provided mainly to promote optimal digestion of organic matter (OM) by ruminal microflora and to prevent deficiencies in ruminally available N (RAN) rather than to directly increase MP supply to the host. Deficiencies of RAN in a high-energy diet lead to reduced microbial growth. This limits the amount and extent to which the rumen can produce energy-yielding products, such as VFA, from the microbial fermentation of dietary OM.

Through a series of *in vitro* techniques, Belasco (1954a, 1954b) observed the importance of increasing both the amount of N and rate at which N becomes available to ruminal microbes. He observed numeric increases in the percent of cellulose digested with increases in protein provided to the microbes. Moreover, he recognized that the increased cellulose digestion was directly linked to increased microbial activity.

Bryant and Robinson (1962) found that ammonia's contributions to the ruminal microflora's growth and activity was substantial, with nearly all (>80%) of their isolates exhibiting enhanced growth in ammoniated media. It had been suggested prior to their work that nitrogenous sources besides ammonia (i.e., amino acids, peptides) were of limited value to cellulose-digesting bacteria (Bryant and Robinson, 1961; Allison et al., 1962). Through a series of limiting-media techniques, they observed that N limitations extended to cellulolytic microbes and microbes serving other functions. At least 25 percent of the predominant non-cellulose-fermenting microbes were limited in growth by availability of ruminal ammonia. This work showed that the growth of many ruminal microbes both involved in and excluded from the process of ruminal cellulose digestion were limited by ruminally available ammonia. If a predominant fraction of the ruminal microflora is growth limited by RAN, the beneficial fermentation products these microbes produce would be limited as well.

Dietary energy availability is affected by the extent to which ruminal microbial growth is optimized. Ruminal microbial growth can be limited either by the amount of dietary energy or by RAN. The requirement for N is typically dependent on the ruminally-available energy. When N is limiting, ruminal NH_3-N will remain low (<1.4 m*M*). Satter and Slyter (1974) established concentrations of NH_3-N necessary for maximal growth of the ruminal microflora. They conducted a series of experiments that simulated conditions within the rumen with continuous-

culture fermenters that were fed various levels of total N and RAN. They observed that ruminal protein and VFA production were optimized when the NH_3-N content in the fermenters reached a concentration of 1.4 m*M* but suggested that concentrations up to 3.6 m*M* may be needed to provide a safety factor for maximum microbial growth. When microbial ATP was predicted for diets with varying levels of RAN, substantial decreases in ATP were noted under N-limiting conditions. Therefore, Satter and Slyter (1974) concluded that both BCP production and energy availability increased substantially up to the point at which ruminal NH_3-N was equal to 1.4 m*M*. It appears that, as the microbial growth is optimized, so is the ruminally-degradable fraction of dietary structural carbohydrates.

Griswold et al. (2003), using a continuous, dual-flow culture apparatus, studied the effects of both high and low levels of ruminally degraded protein (RDP) provided from either NPN or peptides on microbial efficiencies and digestibilities of DM, OM, and carbohydrate fractions of the diet. These researchers observed significant increases in microbial efficiencies for diets that contained higher amounts of RDP and with NPN inclusion. Furthermore, increases in digestibilities of DM and OM were observed with higher treatment levels of RDP, and NPN inclusion led to increases in digestibilities of DM, OM, ADF, and non-fiber carbohydrate (NFC). Under the conditions in this study, the authors showed that increasing RAN in the form of amino acids and peptides can improve the digestion of both structural carbohydrates and non-structural carbohydrates by microbes. However, with treatments that were higher in RDP but without NPN, only digestibilities of NDF, hemicellulose, and non-structural carbohydrate significantly increased. Most likely, these authors observed limitations in N available to cellulose digesting bacteria because their methods led to limitations in available NH_3-N when no NPN was provided, even with the high level of RDP. Therefore, this work leads us to conclude that with

in vitro techniques, ruminal microbes that are limited by available N will demonstrate increases in efficiency and greater digestibilities of the energetic components of the diet when increasing levels of N are provided up to the requirement of the microbes.

A series of experiments were conducted by Milton et al. (1997b) with the purpose of defining the effects of increasing levels of RAN to growing cattle consuming diets based on dry-rolled corn. They fed British x Continental yearling steers four diets (containing 0, 0.5, 1.0, and 1.5% urea) in which all of the supplemental N in the diets was from urea. Urea inclusion led to a cubic response in dry matter intake (DMI) with DMI tending to be lower for steers consuming 0.5 and 1.5% urea than those consuming 0 or 1% urea. Furthermore, urea supplementation increased ADG (5.9%) and the gain to feed ratio (G:F) (10.2%) when compared to the basal diet. A quadratic response to dietary urea was observed for both HCW and dressing percentage, whereas a linear increase was observed in fat thickness and yield grade. In addition, a tendency ($P = 0.18$) for increased percentage of USDA Choice carcasses was observed with urea inclusion. The authors concluded that there is an increase in dietary energy availability with increasing RAN. Optimal levels of urea for ADG ($r^2 = 0.31$; $P = 0.19$) and G:F ($r^2 = 0.40$; $P = 0.10$) were determined to be 0.9% of dietary DM.

A metabolism experiment conducted by Milton et al. (1997b) with four duodenally- and ruminally-cannulated steers consuming diets similar to earlier experiments was conducted to determine effects of urea supplementation on diet digestibilities. They observed numerical improvements in both apparent- and true-ruminal OM (22% increase; $P = 0.22$) and ruminal starch (33% increase; $P = 0.11$) digestibilities with the lowest level of urea inclusion in the diets when compared to the basal diet. They did not observe increases in digestibility with additions of urea beyond 0.5% of dietary DM. Additionally, the authors observed no differences in microbial

protein supply to the small intestine. Ruminal ammonia concentrations increased when more than 0.5% urea was added to the diets, demonstrating that the greater inclusion levels were in excess of microbial requirements. This led the authors to postulate that the proper amount of RAN for optimal digestion from urea supplementation to the diet was somewhere between 0.5 and 1.0% and that microbial requirements for RAN were exceeded at levels over 1.0% urea.

Additional work by Milton et al. (1997b) feeding 100 medium-framed steers was conducted with alfalfa hay, instead of prairie hay, as the roughage source. Five 5 levels of dietary urea were fed (0, 0.35, 0.70, 1.05, and 1.40%). Performance of the steers was less than in their first experiment but followed similar trends. Dry matter intake ($P = 0.10$), ADG ($P < 0.05$), and G:F ($P < 0.05$) were quadratically related to dietary urea level. By regression analysis, the authors predicted the optimum level of urea for both ADG ($r^2 = 0.30$; $P = 0.05$) and G:F ($r^2 = 0.40$; $P = 0.01$) was 0.5% of dietary DM.

These papers show that growing cattle fed a corn-based diet are able to digest greater amounts of OM with the addition of RAN. Moreover, MP supply to the animal was not improved with increasing amounts of RAN, suggesting that the increases in dietary N were needed only for the enhancement of microbial metabolism.

Gleghorn et al. (2004) conducted studies to quantify both the amount and type of protein needed to maximize energy digestion by cattle fed diets based on steam-flaked corn. They observed linear increases in ADG with CP increasing up to 14.5% of dietary DM through 56 d on feed and quadratic responses by 84 and 112 d on feed, with 13% CP yielding maximum gains. No differences were observed among CP sources, but ADG was numerically greatest for cattle fed a diet with urea as the sole supplemental N source, intermediate for a blend of urea and cottonseed meal, and least for cottonseed meal only. Furthermore, G:F linearly increased from

0.182 to 0.185 ($P = 0.03$) for the overall feeding period with increasing levels of urea-N supplementation. These results suggest that increasing RAN promoted greater energy availability from the diets and that urea may be a better source of supplemental CP than cottonseed meal.

Processing of carbohydrate sources in diets for growing ruminants may impact RAN requirements as more energy is available to ruminal microbes. Work by Cooper et al. (2002) determined degradable intake protein (DIP) requirements of finishing cattle fed high-concentrate diets based on either high-moisture (HMC), steam-flaked (SFC), or dry-rolled corn (DRC) by altering urea supplementation. When cattle consumed HMC, there were no differences in DMI among DIP levels ($P = 0.74$), but there were linear increases in ADG as DIP increased. Cattle consuming SFC diets responded quadratically to increasing DIP for both DMI and ADG. Dry mater intake ($P = 0.08$) and ADG ($P = 0.03$) of cattle fed DRC-based diets responded linearly to increasing DIP. An interaction between corn processing method and dietary DIP ($P < 0.01$) was observed for ADG and DMI. Non-linear regression analyses were conducted for G:F for all treatments. Lack of convergence with DRC-based diets led these authors to conclude that the lowest level of DIP (4.8% of DM) used in their study met animal requirements. Conversely, predictions of breakpoints for HMC (dietary DIP 10.2%) and SFC (dietary DIP 7.1%) were estimated. These results lead these researchers to conclude that greater amounts of RAN were needed to maximize ruminal microbial growth as soluble carbohydrates are increased. They also concluded that the primary function of dietary DIP is to allow for increases in energetic byproducts of fermentation rather than to increase the amount of N available to the animal.

Many high-concentrate finishing diets have the potential to supply adequate amounts of MP to growing cattle (Milton et al., 1997a; Vasconcelos et al., 2008); however, unsupplemented

diets are generally deficient in RAN for microbial growth due to the limited DIP content of most grains. Shain et al. (1998) showed that growing cattle consuming corn-based diets with varying levels of urea (0.0, 0.88, 1.34, and 1.96% of DM) were most efficient when urea was provided at 0.88% of diet DM (DIP = 6.4% of DM). Subsequent to their feeding and growth observations, an experiment was conducted by these authors to observe the metabolic effects of their treatments. The treatment with the lowest level of RAN (no supplemental urea) yielded low (< 1.4 m*M*) concentrations of ruminal NH_3-N. The 0.88%-urea treatment yielded ruminal concentrations of NH_3-N (2.78 m*M*) that were above the requirements observed by Satter and Slyter (1974) for optimal microbial growth, and accordingly growth was not negatively affected. Most likely, the 0.88%-urea treatment did not significantly limit ruminal microbial growth, diet fermentation, or production of energy-yielding endproducts. Moreover, the increases in efficiency and rate of gain along with low levels of ruminal NH_3-N for treatments without urea supplementation support the concept that growth of the ruminal microbes was limited by restriction of RAN and ultimately limited the digestion of dietary energy, as suggested by Burroughs et al. (1975), when no supplemental N was provided.

Zinn et al. (2003) evaluated digestive function and growth performance using steam-flaked barley as the primary source of carbohydrate and varying levels of urea (0, 0.4, 0.8, 1.2% of DM) to adjust dietary DIP. Contrary to other reports, these authors did not observe any increases in ruminal OM or ADF digestibilities; however, steam-flaked barley comprised 85.1% (11.8% CP) of the total CP of the basal diet (10.5% CP). With a true-ruminal N availability of 72.5%, the resulting 7.3% DIP in the basal diet may have sufficed for optimal digestion of these diets. Similar to others, linear improvements in ruminal starch digestion were observed with increases in dietary urea; however, increasing dietary urea did not increase flow of microbial N

to the duodenum. Total-tract digestibilities of starch and OM increased linearly with dietary urea levels, as did ADG. Most likely, RAN was sufficient in all diets that had supplemental urea. This work supported the idea that increasing N to the rumen does not necessarily increase MP supply to the animal.

Chizzotti et al. (2008) investigated the effects of increasing RAN in the form of NPN on performance, digestibility, and microbial efficiency. They reported no improvements in microbial N efficiencies as RAN was increased above that of their basal diet. Ruminally-degraded OM and ruminally-degraded carbohydrate responded quadratically as ruminally-degradable protein increased, with the two intermediate treatment levels being greater (8.52 and 9.24% of DM) than the treatment levels that had the largest (10.14% of DM) and smallest (8.19% of DM) amounts of ruminally-degraded protein. Ammonia-N flow from the rumen and plasma urea concentration increased linearly but no differences were observed in microbial-N flow from the rumen. Treatment levels within these experiments supplied a considerable excess of RAN. Excesses in RAN explained the observed increases in both NH_3-N leaving the rumen and plasma urea concentrations. Lack of benefit to microbial-N efficiency is most likely due to the fact that the basal diet probably met the microbial requirements for RAN. As the ruminal NH_3-N levels usually exceeded 10 m*M* during the 8-h after feeding, it is likely that the ruminal microbes' N requirements for optimal growth were satisfied and that additions of RAN provided excesses of N. Chizzotti et al. (2008) established that the NPN fractions of the diet could comprise up to 46.5% of the total CP without inhibition of animal performance.

The Increasing Use of Ethanol Co-Products in Diets for Growing Cattle

Fermentation of various cereal grains for the production of ethanol has been conducted throughout recorded history. One coproduct of the production of ethanol, dried distiller's grains

with solubles (DDGS), was recognized as a possible nutrient source for livestock at the beginning of the 20th century (Henry, 1900). More recently, a combination of legislation (EISA, 2007) and economic trends led to unprecedented growth in fermentation of cereal grains (most commonly corn) for the production of fuel ethanol. Recent reports (RFA, 2009) show that annual production of ethanol in the United States rose to approximately 3.4×10^{10} L, with approximately 82% of this production resulting from the dry-milling process. Fermentation of 100 kg of corn grain via the dry-milling process typically yields 40.2 L of ethanol and 32.3 kg of DDGS (Schingoethe, 2006). Therefore, approximately 2.2×10^{10} kg of DDGS was produced in 2008. By 2010, production is expected to rise to 2.6×10^{10} kg of DDGS (CAST, 2006). Furthermore, the Energy Independence and Security Act of 2007 mandates that 1.4×10^{11} L of renewable fuels be produced annually by 2022. This would be expected to result in nearly 9.2×10^{10} kg of DDGS, if 82% of the mandated ethanol production were produced through dry-milling of corn grain.

The use of DDGS and other coproducts as nutritional supplements for cattle diets has increased concurrently with ethanol production. Recently, Vasconcelos and Galyean (2007) surveyed 29 nutritionists who reported that an average of 82.8% of their clients were feeding grain coproducts. Additionally, over 68% of the respondents indicated that distiller's grains (either wet or dried) were the primary coproduct used, with an average DM inclusion rate of 16.5%. It was estimated that the responses accounted for more than 69% of all cattle on feed in 2007. These responses did not mean that 82.8% of all cattle on feed in 2007 consumed grain coproducts; however, it indicated that, among those cattle on feed serviced by nutritionists, both the total amount consumed and the levels of daily inclusion in cattle diets have risen to unprecedented levels.

A recent survey conducted by the USDA-NASS (2007) contacted a random sampling of cattle-feeding operations in 12 Midwestern states that had a minimum of 50 cattle. Thirty-six percent of respondents replied that they included ethanol coproducts in their diets. An additional 34% claimed that they were not currently feeding coproducts but had considered including them in their diets. The most common reason for not using coproducts were availability (35% of respondents) and issues related to infrastructure and handling (22% of respondents). An unimportant factor (2% of respondents) in use of coproducts was concern about nutritional value.

Ethanol coproducts play a significant role in the nutrition of growing cattle in this country. Cattle feeders apparently have little concern about the nutritional value of coproducts. It may be inferred that the number of cattle feeders that use ethanol coproducts will rise as production of ethanol continues to increase and coproducts become more available.

Ethanol Coproducts as a Supplemental N Source

Production of fuel ethanol relies heavily on the fermentation of cereal grains. Ethanol production is maximized via the dry-milling process. A major advantage of dry-milling compared to other means of ethanol production is the capability to ferment a variety of cereal grains (i.e., corn, sorghum, wheat, barley, or mixtures of grains). The most commonly fermented substrate is corn. Stock et al. (2000) reviewed the most common procedures for the production of ethanol via the dry-milling procedure. Typically, the dry-milling process involves grinding the grain and then using yeast (most commonly *Saccharomyces cerevisiae*) to ferment the mash to produce alcohol. Large corn particles can be removed from the liquid fraction prior to distillation or they may be left to go through the distillation column; however, leaving the larger particles generally results in greater volume yields of alcohol per weight unit of the fermented substrate.

After distillation, the resulting whole stillage (5 to 10% DM) is processed via centrifugation or pressing to remove the larger particles.

The larger particles separated from the whole stillage can be marketed as wet distiller's grains (WDG) or, after drying, marketed as dried distiller's grains (DDG). The liquid fraction of whole stillage is commonly known as thin stillage and is composed of yeast cells and fine grain particles. Thin stillage is marketed in 1 of 3 ways: it may be concentrated through drying and then marketed as condensed distiller's solubles (CDS); it may be dried along with DDG to produce dried distillers' grains with solubles (DDGS); or it may be added to WDG and marketed as wet distiller's grains plus solubles (WDGS).

The average composition of corn is: 61.0% starch; 3.8% corn oil (fat); 8.0% protein; 11.2% fiber; and 16.0% moisture (Davis, 2001). The procedure for producing ethanol from corn grain removes the starch component through the fermentation process, which leads to the remaining components becoming concentrated approximately three-fold. These concentrated components comprise the coproduct known as distiller's grains with solubles (DDGS). The NRC (1996) lists DDGS as 10.3% fat, 29.5% CP, and 46.0% NDF (DM basis). Due to the high concentration of CP and increased amounts of UIP, DDGS has drawn interest as a supplemental N source for growing cattle. Increasing availability of DDGS led researchers to investigate its potential as an energy source, and these results have been reviewed (Klopfenstein et al., 2008). This review will focus mainly on DDGS for supplementing N to growing cattle.

The most prominent nitrogenous compound in corn grain is the alcohol-soluble protein known as zein. Zein is remains largely intact during the dry-milling process and is known to be deficient in lysine and tryptophan. The amount of zein within corn grain is variable (Showalter and Carr, 1922) and is directly related to the overall amount of N in the grain (Mitchell et al.,

1952) and maturity of the grain (Hansen et al., 1946). Several reports have indicated that no more than 40% of zein is digested by ruminal microbes (McDonald, 1954; Annison, 1956; Ely et al., 1967; Little et al., 1968) and that ruminal carbohydrate availability has little effect on ruminal degradation of zein (Ely et al., 1967).

Preliminary evaluations of DDGS as a feedstuff for growing cattle diets were conducted by Horn and Beeson (1969). Their initial experiment was conducted by feeding two sets of identical twin Angus steers via a paired feeding technique through which they obtained equal intakes of two isonitrogenous and isocaloric treatment diets (11% CP). They supplemented a relatively low (5% of DM) level of DDGS to their basal diet at the expense of both dry rolled corn (DRC) and urea. The inclusion of DDGS led to increased dietary N retention: 9.07% retention with the basal diet and 16.16% retention with the addition of DDGS ($P < 0.01$). Additionally, the percentage of absorbed N that was retained was greater with the addition of DDGS (23.27%) than for the basal treatment (12.69%, $P = 0.01$).

Horn and Beeson (1969) conducted a second trial where they individually fed diets similar to their first experiment to 4 Hereford steers for *ad libitum* intake. They observed that dietary N retained was greater for the DDGS treatment (18.36%) when compared to the basal diet (11.23%, $P < 0.01$) and that absorbed N retained was greater for the DDGS treatment (25.77%) than for the basal diet (14.94%, $P < 0.01$). These authors showed also that the inclusion of DDGS as a supplemental N source in combination with urea yielded greater efficiencies in the use of N by cattle compared to supplemental N being supplied by urea alone.

Chen et al. (1977) compared the effects of varying levels of DDGS or CDS alone (either centrifuged or pressed) and found evidence that supported the earlier work of Horn and Beeson (1969). They conducted 5 studies measuring differences in N balance of steers consuming diets

with urea and either centrifuged CDS (CCDS), screened distiller's solubles (SDS), centrifuged processed distiller's grains with solubles (CDGS), or pressed distiller's grains with solubles (SDGS). Digested N retention of steers consuming CCDS (0.5% of DM) were not different from controls, but authors noted that SDS was associated with greater digested N retention (42.0%) when compared to controls (39.2%). There were only slight numerical increases in dietary N retained and digested N retained associated with feeding CDGS (2.5% of DM); however, dietary N retained (37.7%) and percentage of digested N retained (56.0%) increased compared to controls (32.6 and 49.9%, respectively) when SDGS was included in steer diets (5% of DM). Chen et al. (1977) demonstrated that inclusion of DGS at low levels in combination with urea led to greater N efficiencies in cattle. In addition, the work of these authors provided evidence that processing techniques influenced the value of various coproducts as N sources.

Early work by Satter et al. (1977) established the rate of ruminal protein degradation for dry-milling coproducts. They fed sheep (fitted with duodenal re-entrant cannulas) diets formulated to be isofermentable with soybean meal (SBM), DDG, or DDGS as the primary N source and measured flows of N to the small intestine. Total N flow to the duodenum was numerically greatest for DDG (32.1 g/d), intermediate for DDGS (30.8 g/d), and least for SBM (24.2 g/d). Non-NH_3 N reaching the duodenum was numerically greatest for DDG (31.4 g/d), intermediate for DDGS (29.9 g/d), and least for SBM (22.3 g/d). Conversely, ruminal NH_3-N was numerically greatest for SBM (1.9 g/d), intermediate for DDGS (1.0 g/d), and least for DDG (0.8 g/d). This work established that relatively large proportions of the protein fraction in distiller's grains escaped ruminally degradation compared to SBM, and that DDGS and DDG differ from one another in the amount of N that escapes ruminal degradation.

Merchen et al. (1979) conducted a series of studies to determine the amount of protein bypassing ruminal digestion when brewers' dried grains (BDG) were fed to cattle. Abomasally-cannulated cattle were fed 1 of 2 isocaloric diets that were supplemented with either urea or BDG and formulated to contain 11.5% CP. Merchen et al. (1979) determined that 61% of the N from the BDG escaped ruminal degradation and concluded that BDG protein was similar to zein in ruminal digestibility.

Merchen et al. (1979) conducted a second experiment in which they supplemented 3 isocaloric and isonitrogenous diets fed to abomasally-cannulated cattle. The supplemental N was provided by urea, a combination of urea and soybean meal, or a combination of urea and BDG. The combinations of N sources were designed such that one-third of the supplemental N was from urea and that two-thirds was from plant N. These researchers reported no differences in either solid or liquid flow rates; however, the total amount of N reaching the abomasum was greatest for those treatments which included plant protein. Among plant proteins, BDG provided the greatest duodenal N flow. Moreover, the BDG-urea combination resulted in the greatest (95.8 g/d) abomasal N flow, the soybean meal-urea combination was intermediate (76.0 g/d), and the urea-only supplement was least (57.2 g/d). The BDG had a ruminal bypass value of 48.1%, whereas the soybean meal had a ruminal bypass value of only 24.1%.

The results of Merchen et al. (1979) indicated that the amount of N in BDG that escapes ruminal degradation is high. The treatments in which N was supplemented mainly by BDG led to the least microbial N reaching the abomasum. This indicated that microbial growth was limited by the low availability of RAN in BDG.

Firkins et al. (1984) investigated the rate of N degradation of ethanol coproducts (both WDG and DDG) compared to other coproducts from grain processing that are readily degraded

in the rumen. The first experiment employed a combination of both *in vitro* and *in vivo* analyses to measure the readily soluble N fraction of WDG, DDG, wet corn gluten feed (WCGF), and dry corn gluten feed (DCGF). They used a modified Burrough's mineral mix (MBMM) to estimate N solubility. The rate of disappearance of the slowly ruminally degradable fraction of these coproducts was determined by suspending dacron bags in the rumens of 4 steers and removing them after 2, 4, 6, 8, and 96 h of incubation. Nitrogen remaining after 96 h was considered to be insoluble in the rumen and assumed to escape ruminal degradation under normal conditions. The data resulting from the 2, 4, 6, and 8 h incubations was used to estimate the rate of ruminal N degradation by regressing the natural logarithm of N remaining (% of original sample) in the bags against incubation time. These researchers reported that acid detergent insoluble N was greater for WDG (15.6% of N) and DDG (12.3% of N) compared to WCGF (3.0% of N) and DCGF (3.2% of N). The WDG had greater (99.5% of N) insoluble N in the MBMM than the DDG (94.4% of N), which in turn was greater than WCGF and DCGF (58.7 and 46.3 % of N, respectively). Conversely, the rate and extent of N disappearance *in situ* was not different between WDG and DDG (average = 4.1%/h); these treatments were less than WCGF and DCGF, which had an average rate of degradation of 9.2%/h.

Firkins et al. (1984) conducted a second study to track the amount of N escaping rumen degradation by feeding isonitrogenous rations to Angus-Hereford steers fitted with both rumen and duodenal cannulas that were supplemented with either urea, WDG, DDG, WCGF, or DCGF. They used a dual phase marker system which allowed them to estimate duodenal passage of both solid and liquid digesta. They observed a tendency for less total N reaching the duodenum and less NH_3-N reaching the duodenum for WCGF (9.0 g/d) and DCGF (8.6 g/d) compared to WDG (11.3 g/d) and DDG (11.1 g/d). Additionally, non-NH_3-nonbacterial N reaching the duodenum

was least for the urea diet (20.9 g/d), intermediate for the gluten feed (GF) diets (37.0 g/d), and greatest for the distiller's grains (DG) diets (69.4 g/d). Therefore, the amount of escape protein was greatest for DG (50.5%), intermediate for GF (20%), and least for urea (0%). These authors noted also that BCP production was less for DG (787.2 g/d) compared to urea (925.6 g/d) and DCGF (945.6 g/d).

Firkins et al. (1984) demonstrated that the N components of DG are less readily degraded in the rumen than urea and GF. Additionally, less BCP was produced by DG than urea or DCGF.

Firkins et al. (1986) studied protein degradability of DDG and DCGF and made comparisons of the site and extent of digestion within the gastrointestinal tract of cattle. Four steers fitted with ruminal, duodenal, and ileal cannulas were fed iso-nitrogenous diets (13.5% CP) that contained either DCGF or DDG as the supplemental N source. Rates of ruminal N degradation of each supplemental N source were estimated using a dacron bag technique. Nitrogen disappearance from the dacron bags was greater ($P < 0.05$) for DCGF (7.98%/h) than for DDG (3.11%/h). Additionally, ruminal ammonia concentrations were significantly less for DDG (6.6 mg/dL) than for DCGF (9.8 mg/dL). Apparent ruminal OM digestion as a percentage of intake and apparent ruminal OM digestion as a percentage of total digestion were greater ($P < 0.05$) for DCGF (45.5 and 65.2%) than for DDG (40.1 and 58.7%). Conversely, the apparent OM digestion in the small intestine was significantly greater for DDG (34.0% of total digestion) than DCGF (29.0% of total digestion). No differences among treatments were noted with respect to apparent OM digestion within the large intestine or the total tract. Duodenal flows of NH_3-N were greater for DCGF than for DDG and greater amounts of non-NH_3-N were observed for DDG than for DCGF, indicating treatment differences in site of digestion. Apparent non-NH_3-N

digestion in the small intestine was greater ($P < 0.05$) for DDG than DCGF, and there were no treatment differences in large intestine or total-tract digestibilities.

This work showed that DG were less degraded in the rumen than other N supplements in cattle diets. In addition, this data showed less OM digestion in the rumen with diets that included DG, perhaps because of lower ruminal ammonia concentrations. Even though a larger fraction of the DG N escaped ruminal degradation, similar quantities of MP were supplied to the host animal when compared to more readily degraded N sources.

Conclusion

Ruminally-available N is fed to cattle in order to achieve maximal fermentation of feedstuffs and, thus, to achieve maximal production of energy-yielding end products. Coproducts of fuel ethanol manufacture (distiller's grains) have become increasingly available. Subsequently, their inclusion in diets of growing cattle has risen to levels previously unknown in the U.S. cattle-feeding industry. Distiller's grains are useful to supply MP to growing cattle; however, they are less susceptible to ruminal degradation than other protein supplements. It has been suggested that lesser ruminal protein degradation may limit microbial growth and fermentation in certain diets.

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CHAPTER 2 - Effect of Nitrogen Supplementation on Urea Kinetics and Microbial Use of Recycled Urea in Steers Consuming Corn-Based Diets[1]

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Abstract

We studied effects of supplementing N as dried distiller's grains with solubles (DDGS) or urea to steers consuming corn-based diets. Six ruminally- and duodenally-cannulated steers (244 kg) were used in 2 concurrent 3 × 3 Latin squares and fed 1 of 3 corn-based diets: control (CON; 10.2% CP), urea (UREA; 13.3% CP), or DDGS (14.9% CP). Periods were 14 d with 9 d for adaption and 5 d for collection of urine and feces. Urinary $^{15}N^{15}N$-urea, resulting from venous infusions of $^{15}N^{15}N$-urea, was used to measure urea kinetics. Dry matter intake (6.0 kg/d) was not affected by treatment, but N intake differed (99, 151, and 123 g/d for CON, DDGS, and UREA). Urea-N synthesis was greater ($P = 0.09$) for DDGS (118 g/d) than for UREA (86 g/d), which in turn was greater than CON (52 g/d). Urea-N excreted in the urine was greater ($P < 0.03$) for DDGS (35.1 g/d) and UREA (28.6 g/d) than for CON (12.7 g/d). Gut entry of urea-N was numerically greatest for DDGS (83 g/d), intermediate for UREA (57 g/d), and least for CON (39 g/d). The amount of urea-N returned to the ornithine cycle was greatest ($P = 0.09$) for DDGS (47 g/d), least for CON (16 g/d), and intermediate for UREA (27 g/d). The fraction of recycled urea-N that was apparently used for anabolism tended ($P = 0.14$) to be greater for CON (0.56) than for DDGS (0.31) or UREA (0.45) but no differences were observed among treatments in the amount of urea-N utilized for anabolism ($P = 0.66$). The percent of total microbial N flow to the duodenum derived from recycled urea-N tended ($P = 0.10$) to be greater for DDGS (35%) than for UREA (22%) or CON (17%). The percent of urea production that was captured by ruminal bacteria was greater ($P < 0.03$) for CON (42%) than for DDGS (25%) or UREA (22%). Urea kinetics in cattle fed grain-based diets were largely related to the amount of N consumed.

Introduction

Nitrogen absorbed post-ruminally by cattle may be made available to ruminal microbes via N recycling. Recycled N can be incorporated N into microbially-synthesized amino acids, which may be absorbed by cattle and used for metabolic processes such as anabolism. This is an advantage when dietary protein levels are low or when ruminally-available N is limited by poor ruminal protein degradation. Recent work (Huntington, 1989; Archibeque et al., 2001, 2002; Marini and Van Amburgh, 2003; Huntington et al., 2008; Wickersham et al., 2008a, 2008b, 2009a, 2009b) has been conducted to better quantify the capability of cattle to recycle N.

Accurate prediction of the amount of recycled N reaching the rumen is important because of the variety of supplemental protein sources fed to cattle. A survey of consulting feedlot nutritionists by Vasconcelos and Galyean (2007) reported increasing use of coproducts from ethanol production (82.8% of all clients reported using grain coproducts in finishing diets). Zein is the primary protein in coproducts of ethanol production from corn and has been shown to be about only 40% available to ruminal microbes (McDonald, 1954; Annison, 1956; Ely et al., 1967; Little et al., 1968). Thus, N recycling may be of greater relative importance when distiller's grains are used to supplement N to cattle.

Little work has quantified urea recycling in cattle fed high-concentrate diets. The goal of our study was to better predict the amount of N recycled by growing cattle fed corn-based diets supplemented with dried distillers' grains with solubles and to quantify use of recycled N by ruminal microbes.

Materials and Methods

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

Six ruminally- and duodenally-fistulated steers (initial BW = 244 ± 33 kg) of British breeding were used in 2 concurrent 3 × 3 Latin squares with the treatment sequence reversed between squares to balance for carryover effects. Treatments were 3 corn-based diets (Table 1): control (CON; 10.2% CP), urea (UREA; 13.3% CP), or dried distiller's grains with solubles (DDGS; 14.9% CP). Treatments delivered 3 different levels of CP, which resulted from inclusion rates of urea and DDGS that were similar to those used commonly in corn-based diets fed to finishing cattle (Galyean, 1996; Vasconcelos and Galyean, 2007). The supplemental DDGS was from a single source (Dakota Gold; POET Nutrition, Sioux Falls, SD). Dried distiller's grains with solubles was selected as a supplemental protein source because of its relatively high content of undegradable intake protein (UIP). Urea was selected as a supplemental N source that is completely ruminally degradable.

Experimental periods were 14 d long; each period consisted of 10 d for adaptation to treatment diets and 4 d for sample collection. Steers were housed in metabolism crates continuously to allow for total collection of urine and feces. Steers were allowed ad libitum access to water and fed twice daily in equal amounts at 0500 and 1700 h. Intakes were determined to be near ad libitum intake for each individual steer prior to the experiment. Five grams of Cr_2O_3 was manually mixed into diets at each feeding (10 g/d) starting on d 7 and continuing through the end of the period to serve as an indigestible marker of nutrient flow to the duodenum.

A clean urine collection vessel containing 900 mL of 10% (wt/wt) H_2SO_4 was placed under each steer at 0530 h on d 10 through d 13 of each period. Blood (10 mL) was collected by jugular venipuncture into heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) 4 h after feeding (0900 h) on d 10. Samples were placed in ice water immediately after collection and centrifuged at 1,200 × *g* for 15 min within 1 h of collection. Plasma was isolated and frozen for later analysis of plasma urea-N, glucose, creatinine, and amino acid concentration.

A temporary indwelling catheter was placed into an ear vein for infusion of $^{15}N^{15}N$-urea. Indwelling jugular catheters were utilized to deliver the continuous infusions in 2 steers during period 2 and in 3 steers during period 3 due to an inability to place ear catheters. The $^{15}N^{15}N$-urea solution was prepared using sterile techniques in a laminar-flow hood by combining 3.6 g of $^{15}N^{15}N$-urea (99%, Medical Isotopes, Inc., Pelham, NH) with 1 L of sterile saline solution (0.9% NaCl). The solution was passed through a 0.22-μm filter (Sterivex, Millipore Corporation, Billerica, MA) into a sterilized glass container. A sterilized rubber septum was crimped onto the container after filtration and the solution was stored at 4°C until use. The $^{15}N^{15}N$-urea solution was prepared immediately prior to the initial infusion for each period.

Sterile saline solution was infused continuously after catheters were placed until 0530 h on d 11 of each period. Continuous infusion of the $^{15}N^{15}N$-urea solution (4.16 mL/h) began at that time and continued through the end of each period. The infusion of the $^{15}N^{15}N$-urea solution delivered 0.48 mmol of urea-N/h via a programmable syringe pump (BS-9000 Multi-Phaser, Braintree Scientific, Inc., Braintree, MA).

Diets were sampled (100 g/d) as they were weighed and were frozen (-20°C). If any orts were present, they were removed at 0455 h daily, weighed, and frozen (-20°C). Collection vessels for urine and feces were removed at 0530 h daily and weighed. Urine samples were

mixed thoroughly and then 1% of daily output was sampled and frozen. At the same time, a representative portion of urine was mixed with 0.05 *M* H_2SO_4 (1 part urine with 4 parts H_2SO_4) such that the final solution weight was equal to 1% of the daily urinary output and frozen for analysis of urinary purine derivatives and creatinine. Fecal samples were mixed thoroughly by hand and 5% was sampled and frozen. Samples of feces and urine from d 10 through d 13 were pooled by steer and used to measure N balance. Feed and ort samples collected from d 9 through d 12 corresponded to urine and fecal samples collected from d 10 through 13. Urine and fecal samples used for ^{15}N determinations were collected from d 10 for measuring background ^{15}N and from d 13 for measuring enriched levels of ^{15}N. Urine (100 mL) and wet feces (470 mL) were sampled and subsequently frozen (-20°C) for analysis of ^{15}N enrichments. At the same times, 20 mL of urine was diluted with 80 mL of 0.05 *M* H_2SO_4 and frozen (-20°C) for analysis of ^{15}N enrichment of purine derivatives.

On d 14 of each period, ruminal bacterial samples were collected for measurement of ^{15}N enrichment. Approximately 400 mL of ruminal digesta was collected from the dorsal and ventral rumen through the ruminal cannula 1, 3, 5, 7, 9, and 11 h after feeding. The digesta was immediately strained through 4 layers of cheesecloth and the liquid portion was analyzed for pH. Immediately, 10 mL of strained ruminal fluid was mixed with 1 mL of 6 *M* HCl and frozen at -20°C for analysis of ruminal NH_3. Another 8 mL of the strained ruminal fluid was mixed with 2 mL of 25% (wt/wt) metaphosphoric acid and frozen at -20°C for analysis of ruminal VFA. Remaining strained ruminal fluid and ruminal contents were blended (1 min; NuBlend, Waring Commercial, Torrington, CT) with 0.5 L of saline solution (0.9% NaCl) to isolate ruminal bacteria. After blending, the liquid fraction isolated by filtration through 4 layers of cheesecloth was immediately frozen (-20°C) and the remaining particulate matter was replaced in the rumen.

On d 14, approximately 300 mL of duodenal digesta was collected from the duodenal cannula 1, 3, 5, 7, 9 and 11 h after feeding and frozen (-20°C).

Laboratory Analyses

Within period, feed samples were pooled across day on an equal weight basis. Ort and fecal samples were composited by steer within period. Feed and ort samples and subsamples of feces were dried at 55°C in a forced-air oven for 72 h, air-equilibrated for 24 h, and weighed to determine partial DM. Duodenal digesta samples were freeze-dried. Once dried, all samples were ground to pass a 1-mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific USA, Swedesboro, NJ). The DM of feed, ort, fecal, and duodenal samples was determined by drying for 24 h at 105°C in a forced-air oven. The OM was determined by ashing for 8 h in a muffle oven at 450°C. The N content of feed, ort, duodenal digesta, wet feces, and urine samples was determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation St. Joseph, MI) and CP was calculated as N × 6.25. Chromium concentration of fecal and duodenal samples was determined by atomic absorption after preparation of samples as described by Williams et al. (1962). Ruminal bacteria were isolated by thawing samples of ruminal contents and then centrifuging samples at 500 × *g* for 20 min. Supernatants were centrifuged at 20,000 × *g* for 20 min to form a bacterial pellet. The pellet was resuspended with saline (0.9% NaCl) and centrifuged again at 20,000 × *g* for 20 min. The bacterial pellets were frozen and freeze-dried.

Concentrations of allantoin, uric acid, and creatinine were determined in pooled (d 10 to 13) urine samples using reverse-phase HPLC (adapted from Shingfield and Offer, 1999). Samples were analyzed on a Hewlett-Packard 1050 Ti-Series liquid chromatography system (Hewlett-Packard, Palo Alto, CA) equipped with a ultra-violet/visible detector set at 218 nm

(Acutect 500 UV/VIS, Thermo Fisher Scientific Inc, Waltham, MA) and autosampler (AS 1000 SpectraSYSTEM, Thermo Fisher Scientific Inc, Waltham, MA). Separation of the sample components was achieved using a 5 μm Discovery BIO Wide Pore C_{18} column (250 × 4.6 mm i.d.; Sigma-Aldrich, St. Louis, MO) with a 5 μm Discovery BIO Wide Pore C_{18} (20 × 4.6 mm i.d.; Sigma-Aldrich, St. Louis, MO) guard column. The mobile phase was prepared by dissolving 1.01 g of sodium 1-heptane sulfonic acid and 0.86 g of ammonium phosphate into 1 L of deionized H_2O with 35 mL of methanol and 70 μL of triethylamine added. The pH was adjusted to 3.2 with HCl, and the entire solution was filtered (0.45 μm MAGNA-R, MSI, Westboro, MA) and degassed with He. Urine samples were diluted to be within the linear range of the standards (20/1) with a diluent that was prepared by dissolving 0.86 g ammonium phosphate and 1.01 g sodium 1-heptane sulfonic acid into 1 L of H_2O (pH adjusted to 2.1 with HCl). Diluted samples were filtered (0.45 μm Syringe Filter Fisherbrand, Fisher Scientific, Pittsburgh, PA) and stored at 4°C. Sample injection volume was 5 μL. Chromatography at room temperature (approximately 24°C) was achieved at a flow-rate of 0.5 mL/min (10 min), then 1.5 mL/min (29 min), and then 0.5 mL/min (1 min) with a total run time of 40 min.

Dried bacterial, duodenal, and fecal samples were analyzed for ^{15}N enrichment via a stable isotope elemental analyzer (ThermoFinnigan Delta Plus, Thermo Electron Corporation, Waltham, MA). Ruminal VFA were determined by GLC as described by Vanzant and Cochran (1994). Colorimetric determinations of ruminal ammonia (Broderick and Kang, 1980) and plasma urea (Marsh et al., 1965) were completed with an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL). Starch concentrations of feed, orts, and feces were determined using the procedures of Herrera-Saldana and Huber (1989) with glucose measurement according to Gochmann and Schmitz (1972).

Urinary urea and ammonia concentrations were determined colormetrically using an AutoAnalyzer (Technicon Analyzer II) according to the methods of Marsh et al. (1965) and Broderick and Kang (1980). Measurement of ^{15}N enrichment of urinary urea was conducted using an adaptation of the techniques of Wickersham et al. (2009b). Ammonia was removed from the samples by pipetting urine (30 μmol of urea) onto a column (Poly-Prep Chromatograph Columns 0.8×4 cm, Bio-Rad Laboratories, Hercules, CA) containing 2 mL of a strong cation exchange resin (Dowex 50W-X8, 100 to 200 mesh, H^+ form, Sigma Chemical, St. Louis, MO). The subsequent effluent was discarded. The column was then rinsed twice with double deionized water (10 mL/rinse) and the effluent discarded. A final rinse (10 mL) of double deionized water was applied to the column and the effluent was collected and analyzed for urea (Marsh et al., 1965) and ammonia (Broderick and Kang, 1980). After it was determined that no ammonia was present, a volume containing 3 μmol of urea was pipetted into an Exetainer tube (Labco International, Houston, TX) and the total volume was brought to 4 mL with double deionized water and the solution was frozen (-20°C). Sodium hypobromite was prepared according to the procedures of Sprinson and Rittenberg (1949). Bromine was dissolved with vigorous stirring (50 g, 99.5%, Fisher Scientific, Pittsburgh, PA) into 100 mL of 40% (wt/wt) sodium hydroxide, previously cooled to 0°C in an ice bath, and then the solution was brought to a final volume of 156 mL with 40% (wt/wt) sodium hydroxide cooled to 0°C. Samples were allowed to thaw at room temperature (approximately 24°C) prior to performing the Hoffman degradation. Ultra-high purity He was bubbled through the samples for approximately 5 min and then samples were immediately frozen in liquid N_2. Sodium hypobromite (0.3 mL; previously bubbled with ultra-high purity He) was pipetted into the Exetainer tube and the tube was immediately capped with a Hungate stopper (13 mm; Bellco Glass Inc, Vineland, NJ). A vacuum pump (pressure of less

than 50 mtorr) was used to remove gas from the tube and ultra-high purity He was added; this process was repeated 5 times. After the final addition of He, the sample was removed from the liquid N_2 and allowed to thaw at room temperature. The sample was placed in a water bath (60^{o}C) for 5 min, vortexed, and then placed back in the water bath for an additional 10 min in order to speed the Hoffman degradation. Samples were analyzed using a stable isotope gas bench (ThermoFinnigan Delta Plus) for $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$.

Purine derivatives in urine were analyzed for total ^{15}N enrichment using a stable isotope elemental analyzer (ThermoFinnigan Delta Plus). Purine derivatives were isolated from the diluted urine samples using a modification of the methods of Chen et al. (1998). Urine (6 mL) was combined with 3 mL of 6 *M* ammonia hydroxide and vortexed. This solution was pipetted over a column (Poly-Prep Chromatograph Columns 0.8×4 cm, Bio-Rad Laboratories, Hercules, CA) containing 2 mL of an anion exchange resin (Dowex 1×8 chloride form, 100 to 200 mesh, Sigma Chemical, St. Louis, MO) and rinsed with 12 mL of double deionized water. The effluent was discarded. A final rinse of the columns was performed with 4 mL of 0.1 *M* HCl and the effluent was collected for analysis. Twenty μL of 40% (wt/wt) NaOH was added to the final effluent and the samples were vortexed for 10 s. Samples were then pipetted to deliver 0.1 mg of N into microcentrifuge tubes (Fisherbrand, Premium Flat Top Microcentrifuge Tubes, Fisher Scientific, Pittsburgh, PA) and dried at 90^{o}C within a dry block heater (Pierce Reacti-Therm III Heating Module, Thermo Fisher Scientific, Rockford, IL) for 6 h. The dried purine derivatives were resolubilized in 150 μL of double-deionized water and vortexed. The solution was transferred into pressed tin capsules (5×9 mm), which were placed into a 96-well microtiter plate and dried at 63^{o}C for 4 h in a dehydrator (American Harvest, Snackmaster Dehydrator Model 2200/FD-30, Chaska, MN).

Plasma urea (Marsh et al. 1965), plasma creatinine (Chasson et al., 1961), and plasma glucose (Gochman and Schmitz, 1972) were measured with an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL). Plasma amino acids were analyzed using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) with a flame ionization detector in combination with a GC-FID free amino acid analysis kit (EZ:faast Kit, Phenomenex, Torrance, CA).

Calculations

Urea kinetics were calculated according to the methods described by Lobley et al. (2000). Duodenal flows were calculated by dividing the fecal output of Cr by the Cr concentration of duodenal digesta. Bacterial and duodenal ^{15}N enrichments were calculated as ^{15}N/total N and corrected for values in the background fecal samples. Bacterial N flow was calculated by multiplying duodenal N flow by the ratio of duodenal ^{15}N enrichment to bacterial ^{15}N enrichment. The flow of bacterial N derived from recycled urea-N (Wickersham et al., 2009b) was calculated by multiplying bacterial N flow by the ratio of bacterial ^{15}N enrichment to ^{15}N enrichment of urinary urea (calculated as one-half of the $^{14}N^{15}N$-urea enrichment plus the $^{15}N^{15}N$-urea enrichment). Duodenal flow of ruminally-undegraded intake N was calculated by subtracting microbial N flow from total duodenal N flow. Microbial N supply was also calculated from urinary excretion of purine derivatives using the procedures of Chen and Gomes (1992). Ruminal microbial capture of recycled N was alternatively calculated by substituting bacterial-N enrichments with urinary purine-derivative enrichments, assuming that microbial enrichments were the same as enrichments for urinary purine derivatives (Hristov et al., 2005),

and by replacing measured bacterial N flow with estimates of bacterial N derived from urinary excretion of purine derivatives (Chen and Gomes, 1992).

Statistical Analysis

All data from one steer from all periods were removed because this steer did not exhibit normal digestive function. This steer had low ruminal (-1%) and total tract (71%) DM digestions, high ruminal pH (6.4), and low ruminal VFA concentrations (69 m*M*) when compared to other steers in this experiment. Data related to duodenal flow for one steer fed the control diet in a single period were excluded due to an apparent marker failure (negative ruminal digestion). All observations from one animal fed the control diet in one period were excluded due to negative ruminal DM digestion as well as unusual urea kinetics (very large urea entry rates).

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For variables without repeated sampling, terms in the model included treatment and period with steer included as a random effect. Model terms for fermentation profile variables were treatment, period, hour, and hour × treatment with steer included as a random term. The repeated term was hour, with steer × period serving as the subject. Compound symmetry was used for the covariance structure. For comparisons of methods, the model included period, treatment, method, and treatment × method and with steer and steer × period × treatment included as random terms. The LSMEANS option was used to calculate treatment means. Significance among treatments was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$. Means were separated using pair-wise *t*-tests when *F*-tests were significant.

Results and Discussion

Intake, digestibility, and nutrient flow

Dry matter intake (DMI; 6.04 ± 0.27 kg/d; Table 2) and organic matter intake (OMI; 5.77 ± 0.25 kg/d; Table 2) did not differ among treatments ($P \geq 0.18$ and 0.21, respectively) but they were numerically 7 and 6% less, respectively, when steers consumed UREA. Total starch intake (Table 2) was decreased by DDGS in the diet ($P = 0.05$), which was expected because the DDGS contained less starch than the corn it replaced. Calculated ME intakes (NRC, 1996) were 18.4, 17.0, and 18.3 Mcal/d for CON, UREA, and DDGS, respectively.

Ruminal digestibilities of DM and OM did not differ among treatments (Table 2). Total tract digestibility of OM (Table 2) was greater ($P = 0.09$) for UREA when compared to DDGS and CON. Additionally, total tract digestion of DM (Table 2) followed the same pattern as OM and was numerically greatest for UREA. These increases in DM and OM digestibilities may be explained by urea-N stimulating microbial fermentation, although ruminal digestion of DM and OM were not different among treatments ($P = 0.81$ and 0.78, respectively). In addition, the numerically lower DMI for UREA may have contributed to the increases in total tract digestibilities. Ruminal-apparent digestibility of dietary N was less ($P = 0.06$) for CON than for DDGS or UREA.

Starch digestion tended ($P = 0.11$; Table 2) to be greatest (96.6% of intake) for DDGS. This also coincided with numerically greater MP supply, and it is possible that the greater amounts of undegraded-intake protein (UIP) flowing to the hindgut increased pancreatic amylase activity (Richards et al., 2003). Lower intakes of starch for DDGS may also have contributed to the trend toward increased digestibility.

Total tract digestion of N was different ($P < 0.01$; Table 2) among treatments, which was expected due to differences in dietary N concentrations. As dietary N increases, endogenous fecal losses represent a smaller proportion of intake.

As expected, N intake increased with increasing N concentration in the diet ($P < 0.01$; Table 2); steers consumed the greatest amount of N when fed DDGS, the least amount of N with CON, and UREA was intermediate. Additionally, UIP (Table 3) was numerically greatest for DDGS, although microbial N flowing to the duodenum and microbial efficiency did not differ among treatments.

Increases in N intakes led to increased N outputs. Fecal N output (Table 2) was least ($P < 0.01$) for CON, intermediate for UREA, and greatest for DDGS. Urinary N excretion (Table 2) was greater ($P = 0.02$) for DDGS and UREA than for CON, but DDGS and UREA did not differ from one another. Nitrogen retention (Table 2) was greatest ($P = 0.02$) when steers were fed DDGS, and UREA was numerically greater than CON. These treatment effects on N retention may have been a response to an increasing MP supply. When Wessels and Titgemeyer (1997) limit fed steers of similar BW (254 kg) to gain 1 kg/d with increasing levels of CP and MP, they observed linear increases in N retention with increasing CP and MP. Gleghorn et al. (2004) fed cattle of slightly heavier initial BW (357 kg or 305 kg) diets based on steam-flaked corn over the course of 2 experiments. They observed an increase in average daily gains (ADG) during the initial 56 d on feed as N inclusion (and presumably MP) increased, regardless of the N source or degradable intake protein (DIP):UIP. Cole et al. (2006) fed heavier steers (315 kg), and N retention during the initial 112 d and the final 56 d on feed was not improved when they fed levels of CP as high as those provided by our DDGS treatment. Perhaps N retention by our steers was improved by increases in MP because of their relatively young age and light BW.

Ruminal NH_3 concentration was greatest ($P = 0.05$) for UREA, and DDGS was numerically greater than CON (Table 6). Although the diet × time interaction was not significant for ruminal NH_3, concentrations were elevated at times near feeding for UREA and DDGS, but not for CON (Figure 1). Ruminal pH was not different among treatments (Table 6), but there was a treatment × time interaction ($P = 0.02$; Figure 2). Zinn et al. (2003) observed that increases in dietary urea resulted in increases in ruminal pH 1 h after feeding, which may explain in part the treatment × time interaction we observed. It is also possible that a slower rate of feed intake for steers fed UREA led to the more constant ruminal pH for that treatment. Ruminal concentrations of acetate, propionate, and butyrate did not differ among treatments (Table 6).

Urea kinetics

Urea kinetics are described in Table 4. Urea entry rate was greater ($P = 0.09$) for DDGS than CON, whereas UREA was not different than either DDGS or CON. Gut entry of urea (GER) did not differ among treatments ($P = 0.25$), but there were large numerical differences that corresponded to the pattern of urea entry rate. The treatment that yielded the numerically greatest bacterial capture of endogenously produced urea was DDGS (82.8 g/d), whereas CON (38.9 g/d) was least, and UREA (57.2 g/d) intermediate. Amounts of urea-N eliminated via urine, returned to the ornithine cycle following gut entry, and lost to feces following gut entry all followed the same treatment pattern as urea entry rate. Urinary urea-N excretions were greater ($P = 0.03$) for both DDGS and UREA than for CON. The amount of urea-N returned to the ornithine cycle and re-incorporated into urea tended to be greatest ($P = 0.09$) for DDGS, least for CON, and intermediate for UREA, which did not significantly differ from either DDGS or CON.

We did not observe statistical differences among treatments in urea-N losses to the feces; amounts were small, representing less than 4 g/d.

Treatment differences in urea returned to the ornithine cycle expressed as proportion of either urea entry rate or of GER were similar to those for the amount returned to the ornithine cycle, though less significant. The amount of urea-N lost in the urine expressed as a proportion of the total urea entry rate did not differ among treatments ($P = 0.25$) but was numerically less for CON (0.27) when compared to DDGS (0.35) or UREA (0.36). The proportion of urea-N which entered the GIT and was subsequently returned to the ornithine cycle when expressed as a proportion of urea entry rate was numerically greatest for DDGS (0.39), least for CON (0.27), and intermediate for UREA (0.31). Urea-N returning to the ornithine cycle as a fraction of GER tended ($P = 0.11$) to be greatest for DDGS (0.63), least for CON (0.37), and intermediate for UREA (0.49). Urea-N lost in the feces when measured as a proportion of GER was greatest ($P = 0.02$) for CON and least for DDGS and UREA. Interestingly, urea-N utilized for anabolism (fraction of GER) tended ($P = 0.14$) to be least for DDGS (0.31), intermediate for UREA (0.45), and greatest for CON (0.56).

Reynolds et al. (1991) measured arterial-venous fluxes of urea across the portal drained viscera (PDV) and liver of heifers consuming isonitrogenous concentrate-based diets containing approximately 17% CP and with BW and DMI similar to our steers. Urea-N appearance across the liver was 166 g N/d. This was slightly greater than our urea entry rates for DDGS, probably due to the greater N intakes in their study. Reynolds et al. (1991) also observed that 68.8 g/d of urea-N disappeared across the PDV; PDV flux of urea would be similar to our measures of GER, although PDV flux does not include salivary transfers of urea-N that are part of GER. Theurer et al. (2002) reported that steers fed concentrate-based diets had negligible salivary contributions to

gut entry of urea (approximately 2% of hepatic ureagenesis). Thus, it may be assumed that little transfer of urea-N from saliva occurred in the experiment of Reynolds et al. (1991). Moreover, Reynolds et al. (1991) reported flux of urea-N across the total splanchnic tissues was close to the difference between hepatic urea production and urea-N lost in the urine. Calculating salivary urea-N transfer to the gut as the difference between urinary urea-N excretion and total splanchnic flux, urea-N transfer from saliva to the rumen would have been 1.4 g/d or less than 1% of total N intake (Reynolds et al., 1991). In comparing the PDV flux of urea-N to our measure of GER, Reynolds et al. (1991) reported a measure that was intermediate between our observations for DDGS and UREA.

Plasma urea-N concentrations (PUN) did not differ among treatments but increased numerically with N intake (Table 5). Others have reported that PUN was closely related to protein intake (Somers, 1961; Preston et al., 1965). Additionally, urea eliminated in the urine was greater ($P = 0.03$) for DDGS and UREA than CON. Cocimano and Leng (1967) observed that as PUN increased in lambs so did the amount of urea excreted. Moreover, these authors reported that the relationship between PUN and urea excretion rates followed a sigmoidal curve whereby minimal amounts of urea were excreted via the urine at low N intakes (dietary CP $\leq$ 9.0% of DM) and maximum levels were reached at high N intakes, when sheep were limited by their capacity to eliminate urea to the urine. This suggested that, when N intake was restricted, ruminants conserved and recycled urea most efficiently. Our PUN would be among the smaller values reported by Cocimano and Leng (1967). According to their work, our PUN should have led to only small differences among treatments in urea excreted in the urine. Conversely, we observed significant increases in urinary urea with only small increase in PUN. If urea excretion in urine and PUN by cattle has a pattern similar to that of sheep, it is shifted such that urinary

urea excretion is more responsive to changes in PUN. More work is needed to better quantify the relationship between PUN and urinary urea excretion by cattle.

Guerino et al. (1991) measured the effects of increasing UIP supply on urea-N fluxes in cattle consuming concentrate-based diets. They observed increases in urinary urea-N losses, PDV absorption, hepatic ureagenesis, and total splanchnic release that corresponded to increasing amounts of abomasally-infused casein as a source of UIP. These data support the relationships that we observed in urea entry rate, urinary urea-N losses, and GER.

Huntington et al. (1996) fed mature steers 11 different diets of various forage-to-concentrate ratios and measured differences in nutrient fluxes across splanchnic tissues. When cattle consumed diets relatively similar to our, levels of hepatic urea release were similar to CON and urinary urea-N excretions were similar to UREA.

Wickersham et al. (2008b) used methods similar to ours to measure the effects of increasing and oscillating levels of DIP on urea kinetics in steers consuming prairie hay that was deficient in N. These authors reported that urea entry rate was similar to that for CON and UREA in our study when supplementing casein post-ruminally at either 61 or 183 mg of N/kg BW daily. They reported much lower urinary urea-N excretions at these N intakes compared with our study. This was likely caused by inherently greater endogenous N recycling, which is characteristic of low-quality forage diets (Huntington et al., 1996). It may also have been supported by greater salivary transfer of urea-N for their forage diet than for our corn-based diet. Their data also reported slightly greater GER when compared to CON and UREA in our study and this was probably related to greater salivary urea transfer to the gut.

Ruminal microbial capture of recycled urea-N is related to microbial N needs and to amounts of recycled urea-N. Wickersham et al. (2009b) observed that urea entry rate and GER

increased linearly as increased amounts of UIP were provided. Additionally, as urea entry rate and GER increased in response to dietary UIP supplementation, amount of GER captured by ruminal microbes increased. Also, Wickersham et al. (2008a) measured increases in urea entry rate and GER as DIP supplementation increased, and amount of recycled urea-N captured by ruminal microbes increased with DIP supplementation. However, increases in urea entry rate and GER were greater for supplementation with UIP (Wickersham et al., 2009b) than with DIP (Wickersham et al., 2008a). We observed that urea entry rate expressed as percent of N intake was numerically greater for DDGS (83%) than UREA (73%). These numerical differences agree with greater increases in urea entry rate and GER when UIP is provided than when DIP is provided (Wickersham et al., 2008a, 2009b). Moreover, Wickersham et al. (2009b) observed that an increasing proportion of microbial N was derived from recycled urea-N when UIP was supplemented, but no difference in the proportion of microbial N from recycled urea-N was observed in response to DIP supplementation (Wickersham et al., 2008a). We observed that microbial capture of recycled urea-N, expressed as a proportion of total microbial N flow, was greater ($P = 0.10$) for DDGS than UREA, which agrees with the results of Wickersham et al. (2008a, 2009b) and suggests that recycled N is more important for ruminal microbes when UIP rather than DIP is provided to cattle; this greater importance is reflective of both greater GER and less RAN when UIP rather than DIP is supplemented.

Plasma Amino Acids

Few differences were observed during our study in plasma amino acids (Table 5). Plasma Gln was less ($P = 0.08$) for DDGS than for UREA or CON. Although numerous factors can alter plasma Gln concentration, one possibility may be that plasma Gln was reduced by hepatic deamidation for subsequent incorporation into urea. Ornithine was greatest ($P = 0.06$) for UREA

(74 m*M*), least for CON (52 m*M*), and intermediate for DDGS (63 m*M*), which did not differ from either UREA or CON. These measurements coincided with the calculated levels of dietary DIP. Ornithine, much like Gln, plays a significant role in ureagenesis. Ornithine is the final product that arises from arginase activity during release of urea, and it is also the initial substrate required for ornithine transcarbamoylase to synthesize citrulline within the mitochondria.

Microbial Capture of Endogenously Produced Urea

Capture of recycled urea-N by ruminal microbes was measured using methods described by Wickersham et al. (2009a). Recycled urea-N captured by ruminal microbes (Table 4) was not different among treatments ($P = 0.28$) but was numerically greater for DDGS (30 g/d) than for CON (17 g/d) or UREA (18 g/d). Total amount of urea-N captured by ruminal microbes depends on the quantity of urea-N recycled to the rumen and on the fractional capture of that recycled urea-N. Ruminal microbial capture of recycled N as a percentage of urea entry rate was greatest ($P = 0.03$) for CON (42%) when compared to both DDGS and UREA; DDGS and UREA did not differ (25 and 22%, respectively). Microbial capture of recycled N as a percentage of GER tended to be greatest ($P = 0.11$) for CON (61%), intermediate for DDGS (43%), and least for UREA (33%).

Efficiency with which ruminal microbes capture recycled urea-N is related both to the proportion of GER that is recycled to the rumen and to the availability of competing N sources in the rumen. The ability of ruminal microbes to capture this recycled urea-N increases as the proportion of GER that is returned to the rumen increases. In addition, the ruminal microbes are more dependent upon recycling mechanisms to meet their needs for N as RAN becomes more limiting.

Steers fed DDGS captured ($P = 0.10$) a greater proportion of their microbial N from recycled urea-N (35%) than did steers fed UREA (22%) or CON (17%). Efficiency of recycled-urea capture by ruminal microbes (i.e., microbial capture as a percentage of GER) appeared to be related to ruminal NH_3; as ruminal ammonia increased, the efficiency of recycled-N capture by microbes decreased among our treatments. Wickersham et al. (2009b) reported similar increases in the amount of recycled urea-N captured by ruminal microbes with increasing levels of UIP intake, which corresponded to increasing urea entry rate and GER. Moreover, they reported that the efficiency of microbial capture of recycled N was greater when RAN was less.

Methods for Analyzing Microbial Capture

Chen and Gomes (1992) provide a simple approach to predicting microbial N flow from urinary purine derivative excretion, obviating the need to use cannulated cattle. They described microbial N flow to the duodenum (g N/d) as 0.727 × microbial purines absorbed (mmol/d), where absorption of microbial purines was calculated as a function of excretion of urinary purine derivatives and metabolic BW. We observed that predictions based on equations of Chen and Gomes (1992) led to values that were 30% greater ($P < 0.01$) than measured microbial N flow to the duodenum (Table 3). Differences likely arose from microbial purine:N ratios in our study that differed from the average values used by Chen and Gomes (1992). For cattle consuming diets similar to ours, microbial N flow to the duodenum (g/d) would be more accurately predicted as 0.558 × microbial purines absorbed (mmol/d).

Marini and Van Amburgh (2003) used ^{15}N enrichments of plasma urea and bacteria in conjunction with urinary excretions of purine derivatives in order to estimate microbial capture of recycled urea-N. We directly measured microbial capture of recycled urea-N using the methods of Wickersham et al. (2009b), and we also estimated microbial capture of recycled urea-

N using methods similar to those reported by Marini and Van Amburgh (2003), except we used the ^{15}N enrichment of urinary urea and of urinary purine derivatives to determine the proportion of microbial N derived from recycled urea. When we calculated microbial capture of recycled N using the approach similar to that of Marini and Van Amburgh (2003), we observed values averaging 35% less ($P < 0.01$) than the measured values for the amount of recycled urea-N contributing to duodenal microbial N flow. The difference reflected that the equation of Chen and Gomes (1992) over-predicted microbial N by 30% (as discussed above) and that the ^{15}N enrichment of purine derivatives averaged only 63% of that of rumen bacteria. By modifying the equation of Chen and Gomes (1992) as discussed above and by multiplying the ^{15}N enrichment of purine derivatives by 1.58 (to account for the lower enrichment in urinary purine derivatives than in ruminal bacteria), microbial capture of recycled urea-N could be adequately predicted (i.e., no differences between measured and predicted values, $P = 0.76$) We suggest caution when applying these values to other experiments, unless experimental methods and conditions mimic closely those presented herein.

Hristov et al. (2005) observed that when ^{15}N was ruminally infused for 8 d, ^{15}N enrichment of urinary purine derivatives was similar to that of ruminal bacteria. The observations of Hristov et al. (2005) differed from those of other workers (Gonzalez-Ronquillo et al., 2003; Orellana Boero et al., 2001) whose measures of enrichment of urinary purine derivatives were less than those of duodenal purine bases. This difference may have arisen because labels were provided for either 3 (Gonzalez-Ronquillo et al., 2003) or 4 d (Orellana Boero et al., 2001), which did not allow enough time for the purine derivatives in urine to become equally enriched. Although Wickersham et al. (2009b) reported that ^{15}N enrichments of ruminal microbes reached a plateau after 48 h of infusion in cattle consuming low-quality

forages, we observed differences ($P < 0.01$; Table 4) between ^{15}N enrichments of ruminal microbes (0.068 atom % excess) and of the urinary purine derivatives (0.043 atom % excess), suggesting that 2 d of adaptation do not allow equilibration of these 2 pools. It is also possible that the enriched urinary purine derivatives of microbial origin may have been diluted by unlabelled purines of endogenous origin, although this is doubtful as Hristov et al. (2005) reported that urinary purine derivatives were predominantly of microbial origin (93.4%). Using the equations of Chen and Gomes (1992), endogenous purine derivatives represented 15% of total urine purine derivatives. Additional work is required to verify the appropriate length of adaption to obtain equal enrichments of ^{15}N in urinary purine derivatives and ruminal microbes.

Conclusions

Urea entry rate and GER were related to N intake in cattle consuming corn-based diets. Efficiency of microbial capture of recycled N (as a fraction of either urea entry rate or GER) increased as ruminal NH_3 decreased.

Increasing the accuracy with which we can estimate urea recycling and subsequent microbial capture of recycled N in cattle consuming corn-based diets will allow for more precise formulations of diets and for reductions in wasteful nitrogenous excretions.

The ability to predict microbial capture of recycled N in non-cannulated cattle will reduce the cost of obtaining these data and thereby increase the number of observations upon which predictions of microbial capture of recycled N can be based. However, adaptations longer than those used in our experiment will be required for the methods to be fully valid. Advances in the measurement of microbial capture of recycled N in cattle, in combination with less invasive measures of urea kinetics (i.e. infusion of double labeled ^{15}N urea), may allow for robust predictive models of urea recycling.

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Table 1. Composition of corn-based diets fed to steers (% of DM)

	Dietary treatment		
Item	Control	Urea	DDGS
Ingredient			
Dry-rolled corn	82.5	81.5	62.5
Dried distiller's grains with solubles	---	---	20.0
Alfalfa hay, late bloom	10.0	10.0	10.0
Cane molasses	6.0	6.0	6.0
Urea	---	1.0	---
Limestone	1.0	1.0	1.0
Salt	0.3	0.3	0.3
Mineral and vitamin premix[1]	0.2	0.2	0.2
Chemical Composition			
DM[2]	84.4	85.0	86.0
OM[2]	95.7	95.7	94.7
CP[2]	10.2	13.3	14.9
Starch[2]	57.1	57.1	46.2
Degradable intake protein[3]	5.4	8.4	6.5
ME,[3] Mcal/kg	2.97	2.94	2.97
Neutral detergent fiber[3]	12.7	12.6	20.1

[1]Provided to diets (DM basis) 50 ppm Mn, 50 ppm Zn, 10 ppm Cu, 0.5 ppm I, 0.2 ppm Se, 11.8 ppm thiamin, 1,860 IU of vitamin A/kg, 233 IU of vitamin D/kg, and 25 IU of vitamin E/kg.
[2]Based on laboratory analyses.
[3]Calculated using the tabular values of NRC (1996).

Table 2. Effect of N supplementation on intake, digestion, and N balance in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea

	Diets					
Item	Control	DDGS	UREA	SEM[1]	SED	*P*[2]
No. of observations	4	5	5			
Intake, kg/d						
DM	6.18	6.17	5.77	0.60	0.24	0.18
OM	5.93	5.86	5.52	0.57	0.23	0.21
Starch	3.46[a]	2.92[b]	3.34[a]	0.30	0.17	0.05
Ruminal digestion,[3] %						
DM	40.6	37.5	42.7	8.4	9.2	0.81
OM	47.2	41.9	46.9	8.5	9.1	0.78
Starch	74.5	74.5	77.4	7.3	5.2	0.77
N	-56.9[a]	-19.9[b]	-20.1[b]	10.7	11.5	0.06
Total tract digestion, %						
DM	78.8	79.1	80.5	1.6	0.74	0.11
OM	79.7[a]	80.0[a]	81.6[b]	1.6	0.76	0.09
Starch	93.7	96.6	95.0	1.6	1.1	0.11
N	69.0[a]	75.8[b]	74.1[c]	1.7	0.51	<0.01
Nitrogen, g/d						
Intake	99.1[a]	150.7[b]	122.5[c]	11.9	6.2	<0.01
Fecal	31.2[a]	37.1[b]	32.6[a]	5.1	1.9	0.05
Urinary	25.8[a]	46.6[b]	42.4[b]	4.7	5.1	0.02
Ammonia	0.8	1.3	1.2	0.22	0.24	0.16
Total purine derivatives	8.5	10.0	9.0	1.0	0.72	0.19
Allantoin	7.3	8.7	7.7	0.90	0.70	0.19
Uric acid	1.2	1.3	1.2	0.13	0.044	0.75
Creatinine	3.9	4.2	4.1	0.27	0.15	0.34
Retained	41.1[a]	67.1[b]	47.4[a]	6.6	6.7	0.02

[1]When SEM or SE of the difference differ among treatments, the largest value is reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]One observation for Control was determined to be an outlier (studentized residual ≥ 3), which caused n = 3 for duodenal flows.
[a,b,c]Means in same row not bearing a common superscript letter differ ($P \leq 0.10$)

Table 3. Effect of N supplementation on nutrient flows to the hindgut and microbial efficiencies in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea

	Diets					
Item	Control	DDGS	UREA	SEM[1]	SED[1]	*P*[2]
No. of observations	4	5	5			
Nutrient flows to the duodenum,[3] g/d						
Total N	159	181	150	25	19	0.24
Microbial N[4]	95	84	83	16	20	0.81
Predicted microbial N[4,5]	112	132	116	17	12	0.26
Undegraded intake protein	51	97	67	18	18	0.12
% of N intake	50	64	53	13	15	0.60
Microbial efficiency[3]						
g N/kg OM truly fermented	25.5	27.2	27.6	6.2	7.7	0.96

[1]When SEM or SE of the difference differ, the largest value is reported.
[2]Due to low degrees of freedom, significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]One observation for a steer receiving CON was determined to be an outlier (studentized residual ≥ 3), which caused n=3 for duodenal flows.
[4]Measured bacterial N and predicted microbial N differed ($P < 0.01$).
[5]Values predicted from urinary purine derivative excretion based on the equations of Chen and Gomes (1992).

Table 4. Effect of nitrogen supplementation on urea kinetics and ruminal microbial capture of urea-N in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea

	Diets					
Item	Control	DDGS	UREA	SEM[1]	SED[1]	*P*[2]
No. of observations	4	5	5			
Urea kinetics, g N/d						
Urea-N entry rate (UER)	51.8[a]	117.9[b]	85.9[ab]	17.0	23.1	0.09
Urinary urea-N elimination (UUE)	12.7[a]	35.1[b]	28.6[b]	4.5	5.6	0.03
GIT entry rate (GER)	38.9	82.8	57.2	17.1	23.2	0.25
Returned to ornithine cycle (ROC)	16.0[a]	46.8[b]	26.5[ab]	8.2	11.1	0.09
Urea-N utilized for anabolism (UUA)	20.1	32.0	27.6	9.3	12.7	0.66
Urea-N excreted in feces (UFE)	2.8	4.0	3.1	0.9	0.8	0.35
Fractional urea kinetics						
UUE/UER (u)	0.271	0.350	0.359	0.037	0.050	0.25
GER/UER	0.729	0.650	0.641	0.037	0.050	0.25
ROC/UER (ρ)	0.274	0.390	0.307	0.040	0.054	0.18
ROC/GER (r)	0.374	0.630	0.492	0.071	0.096	0.11
UUA/GER (a)	0.559	0.309	0.452	0.075	0.103	0.14
UFE/GER (f)	0.075[a]	0.061[b]	0.057[b]	0.012	0.004	0.02
Ruminal microbial capture of recycled N[3]						
g N/d	17	30	18	6.4	8.1	0.28
% of total microbial N	17[a]	35[b]	22[a]	5.3	6.7	0.10
% of UER	42[a]	25[b]	22[b]	4.4	4.7	0.03
% of GER	61	43	33	8.7	9.9	0.11
Bacterial ^{15}N enrichment[4]	0.088[a]	0.064[b]	0.051[b]	0.0070	0.0080	0.01
Urinary PD ^{15}N enrichment[4]	0.057[a]	0.036[b]	0.038[b]	0.0067	0.0038	<0.01

[1]When SEM or SE of the difference differed among treatments, the largest value is reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]One observation for a steer receiving CON was determined to be an outlier (studentized residual ≥ 3), which caused n=3 for duodenal flows.
[4]Reported in atom percent excess, enrichments of ^{15}N in bacteria and in urinary purine derivatives differed ($P < 0.01$).
[a,b,c]Means in same row not bearing a common superscript letter differ ($P \leq 0.10$).

Table 5. Effect of nitrogen supplementation on plasma metabolite concentrations in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea

	Diets					
Item	Control	DDGS	UREA	SEM[1]	SED[1]	*P*[2]
No. of observations	4	5	5			
Urea-N, m*M*	2.9	3.9	3.8	0.83	0.85	0.48
Glucose, m*M*	5.4	5.7	5.4	0.18	0.21	0.30
Creatinine, μ*M*	69.4	68.6	72.3	4.0	2.3	0.26
Amino acids,[3] μ*M*						
Ala	193	189	180	20	28	0.88
Gly	290	200	208	29	39	0.14
Val	160	189	168	20	29	0.59
Leu	124	169	163	20	27	0.30
Ile	63	72	71	7	10	0.60
Thr	55	52	58	11	12	0.86
Ser	72	40	54	13	18	0.30
Pro	73	80	80	8	9	0.72
Asn	28	30	33	2	3	0.31
Asp	10	10	10	1	1	0.99
Met	21	24	23	2	2	0.41
Glu	119	126	129	15	11	0.68
Phe	54	61	61	7	8	0.61
Gln	108[a]	59[b]	126[a]	22	25	0.08
Orn	52[a]	63[ab]	74[b]	7	6	0.06
Lys	58	52	63	7	9	0.43
Tyr	53	56	61	7	7	0.53
Trp	31	23	26	4	6	0.46

[1]When SEM or SE of the difference differed among treatments, the largest value was reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]Reliable analysis was not obtained for 1 observation for a steer receiving urea, which caused n = 4 for that treatment.
[a,b,c]Means in same row not bearing a common superscript letter differ ($P \leq 0.10$).

Table 6. Effect of N supplementation on ruminal fermentation characteristics in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea

	Diets					
Item	Control	DDGS	UREA	SEM[1]	SED[1]	*P*[2]
No. of observations	4	5	5			
Ruminal fermentation characteristics						
pH	5.59	5.64	5.67	0.16	0.17	0.89
Ammonia, m*M*	0.2[a]	0.8[a]	2.9[b]	0.9	1.2	0.05
Acetate, m*M*	43.2	40.9	44.2	3.4	4.5	0.74
Propionate, m*M*	44.3	48.0	49.1	6.4	5.5	0.65
Butyrate, m*M*	8.3	6.1	7.0	1.4	1.7	0.45
Isobutyrate, m*M*	1.1	0.8	0.9	0.11	0.14	0.14
Valerate, m*M*	4.2[a]	2.3[b]	3.2[c]	0.61	0.49	<0.01
Isovalerate, m*M*	2.0	1.1	1.1	0.48	0.62	0.25

[1]When SEM or SE of the difference differ, the largest value is reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[a,b,c]Means in same row not bearing a common superscript letter differ ($P \leq 0.10$).

Figure 1. Effect of nitrogen postprandial ruminal NH_3 concentrations in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea. SEM = 1.1. Diet × time, *P* = 0.62.

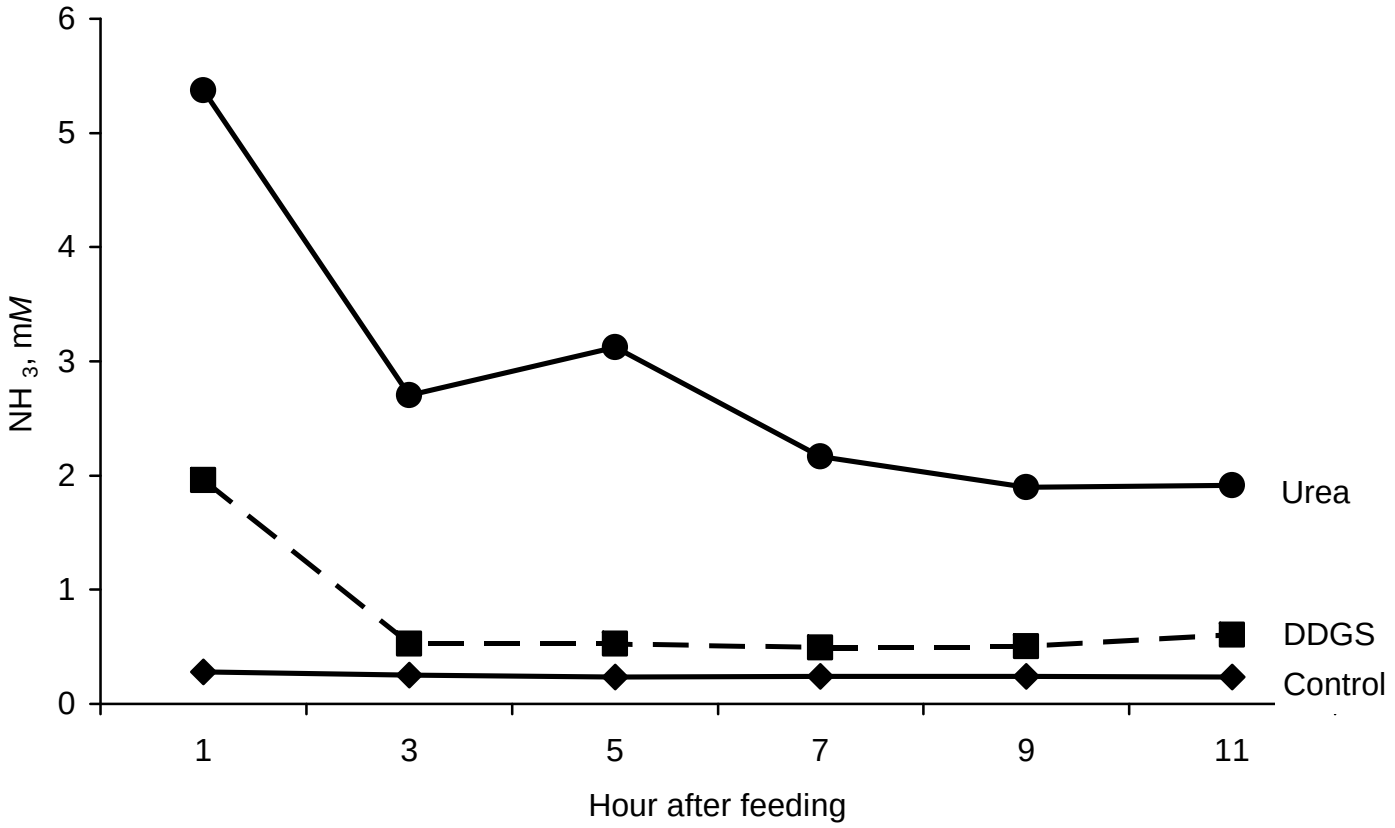
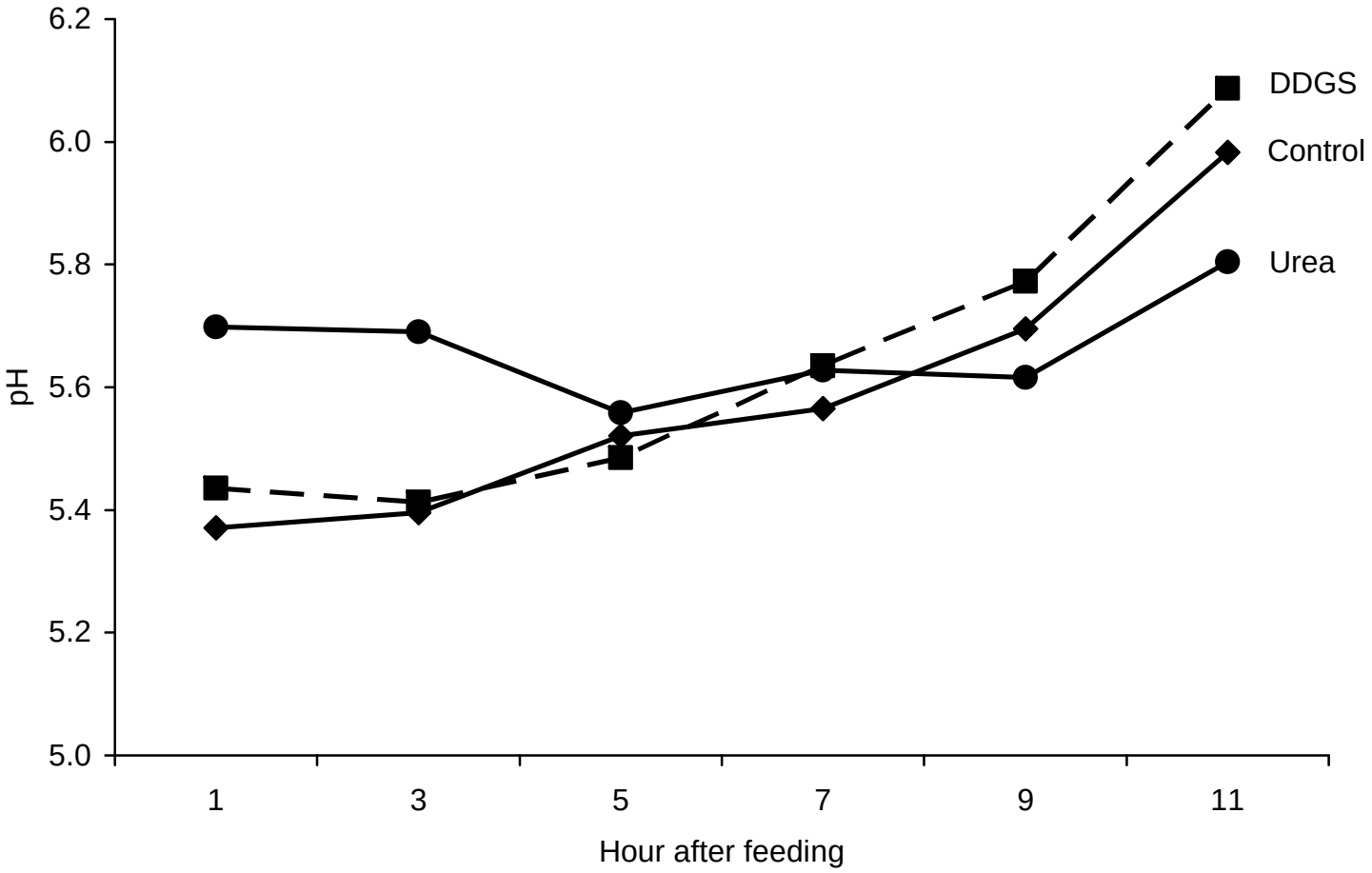


Figure 2. Effects of nitrogen supplementation on ruminal pH in steers consuming corn-based diets supplemented with no protein (Control), with dried distiller's grains with solubles (DDGS), or with urea. SEM = 0.18. Diet × time, *P* < 0.01.



CHAPTER 3 - Effect of Nitrogen Supplementation and Zilpaterol-HCl on Urea Kinetics and Microbial Use of Recycled Urea in Steers Consuming Corn-Based Diets[1]

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Abstract

We studied the effects of zilpaterol-HCl on steers consuming corn-based diets where N supplementation was provided by either dried distiller's grains with solubles (DDGS) or urea. Two sets of 6 steers (average BW of approximately 350 kg) of British breeding were blocked into pairs based on pre-trial feed intake and used in 2 replicates of similarly designed trials conducted at different times. Within each replicate, 3 steers (one randomly selected from each blocked pair) were fed 60 mg/d of zilpaterol-HCl throughout the trial, whereas the remaining steers received no zilpaterol-HCl. Treatments within each square were corn-based diets: control (CON; 9.6% CP), urea (UREA; 12.4% CP), or DDGS (13.7% CP). Total DMI increased ($P < 0.01$) with zilpaterol but was not affected by diet ($P = 0.76$). Total tract digestion of DM and N were not affected ($P \geq 0.64$) by zilpaterol. Total tract digestion of DM was not different ($P = 0.65$) among diets, but N digestion was less ($P < 0.01$) for CON than for DDGS and UREA. Nitrogen intake increased ($P < 0.01$) with zilpaterol and was greatest ($P < 0.01$) for DDGS, intermediate for UREA, and least for CON. The zilpaterol treatment did not affect urea entry rate ($P = 0.80$) or urea-N recycled to the gastro-intestinal tract (GER; $P = 0.94$), despite increased N intake. Estimated microbial N flow to the duodenum was greater ($P = 0.02$) for zilpaterol than control but did not differ ($P = 0.91$) among diets. When cattle were fed grain-based diets, patterns among treatments were similar for GER and urea entry rate. Because urea entry and GER were not affected by zilpaterol, despite increases in N intake, it appears that the amount of N available for urea entry and GER was reduced by zilpaterol.

Introduction

Zilpaterol-HCl is an orally active β-adrenergic agonist approved as a feed additive for beef cattle in the United States. Orally active β-adrenergic agonists repartition nutrient use from lipid accretion toward skeletal muscle growth (Beerman, 1993). When fed during the final 20 to 40 d on feed, zilpaterol has been shown to increase ADG and feed efficiency of cattle consuming corn-based diets (Vasconcelos et al., 2008; Montgomery et al., 2009) and has been shown to have little effect on DMI (Vasconcelos et al., 2008) or to slightly reduce DMI (Montgomery et al., 2009). Moreover, β-adrenergic agonists can increase the protein composition of carcasses 10 to 26% and increase N retention within 6 h with maximal responses being observed within 24 h (Beerman, 1993). This repartitioning of nutrient use by zilpaterol clearly increases net protein deposition of cattle.

As use of ethanol-fermentation coproducts increases in finishing-cattle diets (Vasconcelos and Galyean, 2007), dietary N available to the ruminal microflora may be more limited when compared to more traditional sources of supplemental N. As finishing cattle consume supplemental N sources with low ruminal degradability, the importance of recycling of N to the rumen may increase. Few studies have measured urea recycling by cattle fed corn-based diets containing β-adrenergic agonists. The goal of our study was to better quantify the amount of urea-N recycled in growing cattle fed corn-based diets supplemented with different sources of N, with or without zilpaterol.

Materials and Methods

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

Two sets of 6 steers (one set had a BW of 372 ± 65 kg, and the other set had a BW of approximately 325 kg) of British breeding were blocked into pairs based on pretrial feed intake (ad libitum) and used in 2 replicates of similarly designed trials conducted at different times. Within each replicate, 3 steers (one randomly selected from each blocked pair) were fed 60 mg/d zilpaterol-HCl (1.25 g/d Zilmax; Intervet Schering-Plough Animal Health, Millsboro, DE) throughout the trial, whereas the remaining 3 steers received no zilpaterol-HCl. Thus, the zilpaterol-HCl treatment was provided in a randomized block design. The level of zilpaterol inclusion was the lowest level of inclusion approved by the Food and Drug Administration at the time the experiment was initiated (Feed Additive Compendium, 2008). Zilpaterol-HCl was mixed manually into the complete diet. The zilpaterol-HCl treatments were initiated on d 4 of the first period and continued throughout the trial. Initiation of the zilpaterol-HCl treatment was delayed to minimize the risk of steers becoming refractory to the β-agonist prior to the end of the trial, yet was initiated soon enough to allow adaptation prior to sample collection.

Within each group of 3 steers receiving the same zilpaterol-HCl treatment, steers were used in a 3 × 3 Latin square concurrent with an identical Latin square involving the group of 3 steers receiving the other zilpaterol-HCl treatment. Treatment sequences within the Latin squares were reversed between replicate groups to balance for carryover effects. Treatments within each square were 3 corn-based diets (Table 7): control (CON; 9.6% CP), urea (UREA; 12.4% CP), or DDGS (13.7% CP).

Diets delivered 3 different levels and sources of CP, with inclusion rates of the supplemental protein sources similar to those commonly used in corn-based diets fed to finishing cattle (Galyean, 1996; Vasconcelos and Galyean, 2007). The supplemental DDGS was from a single source (Dakota Gold; POET Nutrition, Sioux Falls, SD). Dried distiller's grains with solubles was selected as a supplemental protein source because of its elevated undegraded intake protein (UIP) content. Urea was selected as a supplemental N source that is completely available in the rumen.

Steers were housed in metabolism crates continuously to allow for total collection of urine and feces. Steers were allowed ad libitum access to water and were fed twice daily in equal amounts at 0700 and 1900 h. The amount of feed offered was near ad libitum intake for each individual steer prior to the experiment.

Experimental periods were 11 d long; each period consisted of 7 d for adaptation to treatment diets and 4 d for sample collection. A urine-collection vessel containing 900 mL of 10% (wt/wt) H_2SO_4 was placed under each steer at 0730 h on d 8 through 11 of each period. At 1100 h, blood (10 mL) was collected by jugular venipuncture into heparinized Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Samples were placed in ice water immediately after collection and centrifuged at 1,200 × g for 15 min within 1 h of collection. Plasma was isolated and frozen for analysis of urea-N and amino acid concentrations.

A temporary indwelling jugular catheter (MILACATH - Long Term 1410-2P, MILA International, Erlanger, KY) was placed for infusion of $^{15}N^{15}N$-urea, a marker used to measure urea kinetics. The $^{15}N^{15}N$-urea solution was prepared using sterile techniques in a laminar-flow hood by combining 3.6 g of $^{15}N^{15}N$-urea (99%, Medical Isotopes, Inc., Pelham, NH) with 1 L of sterile saline solution (0.9% NaCl). The solution was passed through a 0.22-μm filter (Sterivex,

Millipore Corporation, Billerica, MA) into a sterilized glass container. A sterilized rubber septum was crimped onto the container after filtration, and the solution was stored at 4°C until use. The $^{15}N^{15}N$-urea solution was prepared immediately prior to the initial infusion for each period.

Sterile saline solution was infused continuously after catheters were placed on d 8 until 0730 h on d 9 of each period. Continuous infusion of the $^{15}N^{15}N$-urea solution (4.16 mL/h) began at that time and continued through the end of each period. The infusion of the $^{15}N^{15}N$-urea solution delivered 0.48 mmol of urea-N/h via a programmable syringe pump (BS-9000 Multi-Phaser, Braintree Scientific, Inc., Braintree, MA).

A basal mix that constituted 80% of the DM of each treatment diet was manually mixed each day with dry-rolled corn, DDGS, or a combination of dry-rolled corn and urea to constitute each treatment diet. These dietary components were sampled (100 g/d) as diets were mixed and samples were frozen (-20°C). If any orts were present, they were removed at 0655 h, weighed, and frozen (-20°C). Daily fecal and urinary output was collected at 0730 h daily and weighed. Urine samples from d 8 through 11 were mixed thoroughly and then 1% was sampled and frozen. Concurrently, a representative portion of urine was mixed with 0.05*M* H_2SO_4 (1 part urine with 4 parts 0.05*M* H_2SO_4), such that the final solution weight was equal to 1% of the daily urinary output, and frozen for analysis of urinary purine derivatives and creatinine. Fecal samples from d 8 through 11 were mixed thoroughly by hand and 5% of the daily output was sampled and frozen.

Intake, digestion, and N balance were calculated using feed and ort samples collected from d 7 to 10 which corresponded with urine and fecal samples collected from d 8 to 11. Total feces and urine collected on d 8 of each period were used to estimate background enrichments of

^{15}N, whereas total feces and urine collected on d 11 of each period were used to measure plateau enrichments of ^{15}N for each animal; all samples for analysis of ^{15}N enrichments were frozen (-20°C). Concurrently, 20 g of urine was diluted with 80 g of 0.05 *M* H_2SO_4 and frozen (-20°C) for later analysis of ^{15}N enrichments in urinary purine derivatives.

Laboratory Analyses

Within period, feed samples were pooled across day on an equal weight basis. Ort and fecal samples were composited by steer within period. Feed, ort samples, and subsamples of feces were dried at 55°C in a forced-air oven for 72 h, air-equilibrated for 24 h, and weighed to determine partial DM. Once dried, all samples were ground to pass a 1-mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific USA, Swedesboro, NJ). The DM of feed, ort, and fecal samples was determined by drying for 24 h at 105°C in a forced-air oven. The OM was determined by ashing for 8 h in a muffle oven at 450°C. The N contents of feed, wet feces, ort, and urine samples were determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation St. Joseph, MI), and CP was calculated as N × 6.25. Dried fecal samples were analyzed for ^{15}N enrichments using a stable isotope elemental analyzer (ThermoFinnigan Delta Plus, Thermo Electron Corporation, Waltham, MA). Starch concentrations of feed were determined using the procedures of Herrera-Saldana and Huber (1989) with glucose measurement conducted according to Gochmann and Schmitz (1972).

Concentrations of allantoin, uric acid, and creatinine were determined in pooled (d 8 to 11) urine samples using reverse-phase HPLC, adapted from Shingfield and Offer (1999). Samples were analyzed on a Hewlett-Packard 1050 Ti-Series liquid chromatography system (Hewlett-Packard, Palo Alto, CA) equipped with an Acutect 500 UV/VIS detector (Thermo Fisher Scientific Inc, Waltham, MA) set at 218 nm and an autosampler (AS 1000

SpectraSYSTEM, Thermo Fisher Scientific Inc, Waltham, MA). Separation of the sample components was achieved using a 5-μm Discovery BIO Wide Pore C_{18} column (250 × 4.6 mm i.d.; Sigma-Aldrich, St. Louis, MO) with a 5-μm Discovery BIO Wide Pore C_{18} guard column (20 × 4.6 mm i.d.; Sigma-Aldrich, St. Louis, MO). The mobile phase was prepared by dissolving 1.01 g of sodium 1-heptane sulfonic acid and 0.86 g of ammonium phosphate into 1 L of deionized H_2O with 35 mL of methanol and 70 μL of triethylamine added. The pH was adjusted to 3.2 with HCl and the entire solution was filtered (0.45 μm MAGNA-R, MSI, Westboro, MA) and degassed with He. Urine samples were diluted to be within the linear range of the standards (20/1) with a diluent that was prepared by dissolving 0.86 g ammonium phosphate and 1.01 g sodium 1-heptane sulfonic acid into 1 L of H_2O (pH was adjusted to 2.1 with HCl). Diluted samples were filtered (0.45 μm Syringe Filter Fisherbrand, Fisher Scientific, Pittsburgh, PA) and stored at 4^{o}C until analysis. Sample injection volume was 5 μL. Chromatography at room temperature (approximately 24^{o}C) was achieved at an initial flow-rate of 0.5 mL/min (10 min), followed by 1.5 mL/min (29 min), and then reduced to 0.5 mL/min (1 min), with a total run time of 40 min.

Urinary urea and ammonia concentrations were determined colorimetrically using an AutoAnalyzer (Technicon Analyzer II) according to the methods of Marsh et al. (1965) and Broderick and Kang (1980). Measurement of ^{15}N enrichment of urinary urea was accomplished using an adaptation of the techniques of Wickersham et al. (2009b). Ammonia was removed from the samples by pipetting urine containing 30 μmol of urea onto a column (Poly-Prep Chromatograph Columns 0.8×4 cm, Bio-Rad Laboratories, Hercules, CA) containing 2 mL of a strong cation exchange resin (Dowex 50W-X8, 100 to 200 mesh, H^+ form, Sigma Chemical, St. Louis, MO). The subsequent effluent was discarded. The column was rinsed twice with double-

deionized water (10 mL/rinse) and the effluent discarded. A final rinse (10 mL) of double-deionized water was then applied to the column and the effluent was collected and analyzed for urea (Marsh et al., 1965) and ammonia (Broderick and Kang, 1980). After it was determined that no ammonia was present, a volume containing 3 μmol of urea was pipetted into an Exetainer tube (Labco International, Houston, TX), and the total volume was brought to 4 mL with double-deionized water and frozen (-20^{o}C).

Sodium hypobromite was prepared according to the procedures of Sprinson and Rittenberg (1949). Bromine (50 g, 99.5%, Fisher Scientific, Pittsburgh, PA) was dissolved into 100 mL of NaOH (40% wt/wt) that had been previously cooled to 0^{o}C in an ice bath. The solution was brought to a final volume of 156 mL with the addition of cooled sodium hydroxide (40% wt/wt).

Samples were allowed to thaw at room temperature (approximately 24^{o}C) prior to performing the Hoffman degradation. Ultra-high purity He was bubbled through the samples for approximately 5 min and then samples were immediately frozen in liquid N_2. Sodium hypobromite (0.3 mL; previously bubbled with ultra-high purity He) was pipetted into the Exetainer tube, and the tube was immediately capped with a Hungate stopper (13 mm, Bellco Glass Inc, Vineland, NJ). A vacuum pump (pressure of less than 50 mtorr) was used to remove gas from the tube, and ultra-high purity He was added; this process was repeated 5 times. After the final addition of He, the sample was removed from the liquid N_2 and allowed to thaw at room temperature. Once thawed, the sample was placed in a water bath (60^{o}C) for 5 min, vortexed, and then placed back in the water bath for an additional 10 min to speed the Hoffman degradation. Samples were analyzed for $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ using a stable isotope gas bench (ThermoFinnigan Delta Plus).

Purine derivatives in urine were analyzed for total ^{15}N enrichment using a stable isotope elemental analyzer (ThermoFinnigan Delta Plus). Purine derivatives were isolated from the diluted urine samples using a modification of the methods of Chen et al. (1998). Urine (6 mL) was combined with 3 mL of 6 *M* ammonia hydroxide and vortexed. This solution was then pipetted over a column (Poly-Prep Chromatograph Columns 0.8×4 cm, Bio-Rad Laboratories, Hercules, CA) containing 2 mL of an anion exchange resin (Dowex 1×8 chloride form, 100 to 200 mesh, Sigma Chemical, St. Louis, MO), rinsed with 12 mL of double-deionized water, and the effluent was discarded. A final rinse of the columns was performed with 4 mL of 0.1 *M* HCl, and the effluent was collected for analysis. Then, 20 μL of 40% (wt/wt) NaOH was added to the final effluent (to ensure evaporation during drying of any residual ammonia remaining from the ammonia hydroxide) and the samples were vortexed for 10 s. Samples were then pipetted to deliver 0.1 mg of N into microcentrifuge tubes (Fisherbrand, Premium Flat Top Microcentrifuge Tubes, Fisher Scientific, Pittsburgh, PA) and dried at 90°C within a dry block heater for 6 h (Pierce Reacti-Therm III Heating Module, Thermo Fisher Scientific, Rockford, IL). The dried purine derivatives were resolubilized in 150 μL of double-deionized water and vortexed and the solution was transferred into pressed tin capsules (5×9 mm). The pressed tin capsules were then placed into a 96-well microtiter plate and dried at 63°C for 4 h in a dehydrator (American Harvest, Snackmaster Dehydrator Model 2200/FD-30, Chaska, MN).

Colorimetric determinations of plasma urea (Marsh et al., 1965), plasma creatinine (Chasson et al., 1961), and plasma glucose (Gochman and Schmitz, 1972) were completed with an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL). Plasma amino acids were analyzed using a Hewlett-Packard 5890 gas chromatograph (Hewlett-

Packard, Palo Alto, CA) with a flame ionization detector in combination with a GC-FID free amino acid analysis kit (EZ:faast Kit, Phenomenex, Torrance, CA).

Calculations

Urea kinetics were calculated according to the methods of Lobley et al. (2000). Microbial N supply was calculated from urinary excretion of purine derivatives using the procedures of Chen and Gomes (1992) and capture of recycled N by ruminal microbes was calculated as microbial N flow × ratio of ^{15}N enrichment of urine purine derivatives divided by ^{15}N enrichment of urinary urea (Wickersham et al., 2009a). This assumes that microbial enrichments were the same as for urinary purine derivatives (Hristov et al., 2005). Corrected microbial N supply and corrected capture of recycled N by ruminal microbes were calculated with the modifications suggested in Chapter II of this thesis.

Statistical Analysis

Data from one steer was not obtained because it was injured and removed from the study. Another steer injured its leg at the onset of the final period and no observations were obtained from it. All data from one steer for 2 periods were excluded because its DMI that was less than 30% of the average intake of all other cattle. Observations related to urea kinetics from 2 animals during one period were excluded as outliers because studentized residuals for urea entry rate were greater than 3. Data for plasma AA from all steers in one period within one Latin square and from one steer in a single period were missing because insufficient amounts of plasma were available for analysis.

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Terms in the model were zilpaterol, replicate, period within replicate, diet, and diet×zilpaterol. Animal within square was included as a random effect. The LSMEANS option was used to

calculate treatment means. Means were separated using pair-wise *t*-tests when the *F*-test was significant. Statistical significance was declared at $P \leq 0.10$, and tendencies were considered at $0.10 < P \leq 0.20$.

Results and Discussion

Dry matter intakes of steers fed zilpaterol were greater ($P < 0.01$) than of steers that were not fed zilpaterol (8.5 kg/d and 6.6 kg/d, respectively, Table 8). Differences in DMI were due to unexpectedly greater refusals by steers not receiving zilpaterol than by those receiving zilpaterol (27% vs. 7%), rather than differences in amounts of feed offered. Differences in intake due to zilpaterol were not expected and are contrary to the observations of others (Vasconcelos et al., 2008; Montgomery et al., 2008). Vasconcelos et al. (2008) fed finishing cattle corn-based diets similar to ours with greater levels of zilpaterol (8.33 mg/kg diet DM) and reported no differences in intakes. Montgomery et al. (2008) reported that zilpaterol slightly decreased DMI. The reasons for theses differences are unclear.

In response to zilpaterol, OM and starch intakes followed patterns similar to that for DMI. Additionally, starch intake by steers fed DDGS was less ($P < 0.01$; 3.9 kg/d) than for those fed CON and UREA (4.9 kg/d each). These differences in starch intake were expected because we replaced dry-rolled corn with the DDGS; however, there were no differences among dietary treatments when ME concentrations were calculated using tabular values from NRC (1996).

Nitrogen intake (Table 8) was greatest with DDGS ($P < 0.01$; 176 g/d), intermediate with UREA (161 g/d), and least with CON (120 g/d). These differences were expected. Moreover, zilpaterol increased ($P < 0.01$) N intake (171 g/d vs. 134 g/d). Increases in N intake in response to zilpaterol were not expected but were proportional to the increase in DMI. Fecal N (g/d) for steers receiving zilpaterol was greater ($P = 0.09$; 41.4 g/d) than their counterparts that received no zilpaterol (32.7 g/d), but total tract digestibility of N was not affected by zilpaterol. Therefore, differences in fecal N output were related to differences in intake of feed N. Fecal N was

greatest ($P = 0.03$; 41.7 g/d) for DDGS, UREA was intermediate (36.0 g/d), and CON was least (33.5 g/d). Apparent total tract digestion of N was greater ($P < 0.01$) for UREA (77.5%) and DDGS (76.6%) than for CON (72.0%): UREA and DDGS did not differ from each other. Although urinary N losses were not affected by either zilpaterol or supplemental N treatments, the amount of urinary N lost as purine derivatives (allantoin and uric acid) was greater for zilpaterol than control ($P = 0.02$). Urinary creatinine losses tended ($P = 0.20$) to be greater for cattle receiving zilpaterol (5.7 g/d) compared to the cattle not receiving zilpaterol (5.1 g/d), suggesting a greater lean tissue mass in these animals. Nitrogen retention was greater ($P = 0.05$) when cattle consumed zilpaterol (70 v. 43 g/d). Additions of supplemental N as DDGS increased ($P = 0.10$; 72 g/d) N retention when compared to CON (37 g/d), whereas UREA was intermediate (61 g/d).

Dietary treatment effects on N retention may have been a response to increases in metabolizable protein (MP) supply. When Wessels and Titgemeyer (1997) limit fed steers (254 kg) to gain 1 kg/d with corn-based diets, increasing levels of dietary protein led to linear increases ($P < 0.05$) in N retention. Gleghorn et al. (2004) fed steam-flaked corn-based diets to cattle (300 steers at 357 kg and 236 steers at 305 kg) over the course of 2 experiments. They observed an increase in average daily gain (ADG) during the initial 56 d on feed as dietary N (and presumably MP) increased, regardless of the N source. Cole et al. (2006) reported N retention by steers (315 kg) were not improved by feeding levels of CP as high as our DDGS diets during the first 112 d on feed or during the final 56 d on feed. Perhaps N retention by our steers was improved by increases in MP because of their relatively young age and light BW. Similar increases in response to DDGS were observed in Chapter II of this thesis.

No measureable differences were observed due to zilpaterol for urea kinetics, when expressed either as gross amounts or as proportions (Table 9). Zilpaterol unexpectedly increased intakes in our experiment, and it is difficult to separate the effects of zilpaterol per se from those of increased intakes. Increases in N intake lead to increases in urea entry rate and GER (Reynolds et al., 1991); however, zilpaterol may repartition N such that more N is directed to lean tissue accretion. Our initial hypothesis was that increases in N retention in response to zilpaterol would lead to less catabolism of AA, less urea production, and less urea recycling to the GIT. In light of the greater N intake of zilpaterol-fed cattle and no change in either urea produced or recycled to the GIT, it is possible that the effects of N intake and zilpaterol counteracted one another in our measurements.

Urea-N entry rate was numerically greatest for DDGS (193 g/d) when compared to CON (141 g/d) or UREA (138 g/d). Gut entry of urea-N was also was numerically greater for DDGS (151 g/d) than for UREA (101 g/d) or CON (111 g/d). The amount of urea-N which entered the GIT and was subsequently returned to the ornithine cycle was numerically greater for DDGS (78 g/d) than either CON (49 g/d) or UREA (50 g/d). Urea-N lost to the feces decreased for CON ($P = 0.09$; 4.4 g/d) compared to DDGS (6.7 g/d) or UREA (5.6 g/d).

Reynolds et al. (1991) observed that concentrate-fed heifers with BW and DMI similar to our steers had a net flux of urea-N across the liver 166 g/d. Moreover, Reynolds et al. (1991) found flux of urea-N across the liver to be approximately 2 times greater when cattle had high vs. low intakes of N. These hepatic urea-N fluxes were similar to our measures of urea entry rate, although flux of urea-N across the liver does not account for synthesis of urea by non-hepatic tissues.

Wickersham et al. (2008b) observed that when cattle were fed poor-quality forage, increases in DIP supplementation and total N intake led to greater urea entry rates. Huntington (1989) observed nearly a 2-fold increase in flux of urea-N across the liver when cattle were provided 42% more dietary N. Additionally, when we (Chapter II) fed identical diets, urea entry rate was related to N intake.

In Chapter II, we observed increases in the recycling of endogenously synthesized urea to the GIT (GER) when N intake increased. Similarly, Wickersham et al. (2008b) observed that GER followed N intake and urea entry and Reynolds et al. (1991) reported that increased flux of urea-N across the portal-drained viscera as DMI and N intake increased. Interestingly, zilpaterol had no effects on GER despite greater ($P < 0.01$) N intakes.

Others (Cocimano and Leng, 1967; Reynolds et al., 1991; Wickersham et al., 2008a, 2009b; Chapter II of this thesis) have observed that the amount of urinary urea excreted in the urine increased as N intake increases and that plasma urea-N (PUN) is closely related to N intake (Somers, 1961; Preston et al., 1965). Additionally, Cocimano and Leng (1967) observed that urea-N excreted in the urine of sheep increased as PUN increased, as long as the renal capacity to eliminate urea was not exceeded or PUN was not low. Zilpaterol did not affect ($P = 0.49$) PUN, but UREA tended ($P = 0.12$; 4.6 m*M*) to increase PUN compared to DDGS (3.8 m*M*) or CON (3.4 m*M*).

Urea excreted in urine (Table 9) was not affected by the zilpaterol treatment ($P = 0.47$) or by diet ($P = 0.51$). Urea excreted in the urine numerically followed differences in N intake due to diet, but it did not follow diet effects on PUN. In our study, PUN was low when compared to the values reported by Cocimano and Leng (1967). Based on relationships between urinary urea excretion and PUN presented by Cocimano and Leng (1967), our differences in PUN should

have yielded only small impacts on urinary urea excretion. Similarly, Wickersham et al. (2008b) observed that PUN concentrations as high as 5.0 m*M* led to less urinary urea-N excretion (18.5 g/d) than we observed (30 g/d) with PUN as low as 3.0 m*M* (CON/+ Zilpaterol treatment). Clearly, factors beyond PUN impact urinary urea excretion.

Amounts of recycled N captured by ruminal microbes were not affected by zilpaterol. The lack of effect of zilpaterol was not surprising given that urea production and GER were not impacted by zilpaterol. Cattle fed DDGS tended ($P = 0.16$) to capture more recycled urea-N in ruminal microbes than did cattle fed the other diets. We (Chapter II of this thesis) observed a similar treatment response previously, and this likely reflects the numerically greatest GER for the DDGS treatment as well as the relatively low supply of RAN from the DDGS diet. Wickersham et al. (2009b) reported increased incorporation of recycled urea-N into total microbial N with increasing levels of UIP supplementation, which corresponded to increasing amounts of urea production and GER. Moreover, Wickersham et al. (2008b) observed that microbial capture of recycled N as a proportion of GER was decreased when RAN was increased by DIP supplementation.

Total bacterial N flow to the duodenum, when estimated from purine derivatives by either the equation of Chen and Gomes (1992) or by the adaptation to that equation proposed in Chapter II, did not differ among diets but was greater when steers received zilpaterol ($P = 0.02$). Cattle receiving zilpaterol had nearly 34% greater flows of microbial N to the duodenum, which reflected the greater feed intakes by steers on this treatment.

Conclusions

Urea kinetics of cattle were not impacted by the zilpaterol treatment, in spite of the fact that steers fed zilpaterol had unexpectedly greater intakes of DM and N than steers not receiving

zilpaterol. This lack of response to zilpaterol may have been due to repartitioning actions of zilpaterol suppressing urea production.

Understanding the effects of β-adrenergic agonists, such as zilpaterol-HCl, on N recycling will allow nutritionists to provide diets that more closely match the nutrient needs of finishing cattle consuming corn-based diets. Additionally, cattle may rely heavily upon their innate ability to recycle N to the rumen to optimize ruminal function as DDGS remain prevalent in finishing cattle diets. As the dietary nutrients provided to cattle are more appropriately matched with cattle's requirements, costly overfeeding of N and wasteful nitrogenous excretions may be prevented.

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Table 7. Composition of diets fed to steers (% of DM)

	Dietary treatment		
Item	Control	Urea	DDGS
Ingredient			
Dry-rolled corn	82.5	81.5	62.5
Dried distiller's grains with solubles	---	---	20.0
Alfalfa hay, late bloom	10.0	10.0	10.0
Cane molasses	6.0	6.0	6.0
Urea	---	1.0	---
Limestone	1.0	1.0	1.0
Salt	0.3	0.3	0.3
Mineral and vitamin premix[1]	0.2	0.2	0.2
Chemical Composition			
DM[2]	84.1	84.2	84.8
OM[2]	95.3	95.3	94.6
CP[2]	9.6	12.4	13.7
Starch[2]	65.5	64.7	52.3
Degradable intake protein[3]	5.1	7.8	5.9
ME,[3] Mcal/kg	2.97	2.94	2.97
Neutral detergent fiber[3]	12.7	12.6	20.1

[1]Provided to diets (DM basis) 50 ppm Mn, 50 ppm Zn, 10 ppm Cu, 0.5 ppm I, 0.2 ppm Se, 11.8 ppm thiamin, 1,860 IU of vitamin A/kg, 233 IU of vitamin D/kg, 25 IU of vitamin E/kg.
[2]Based on laboratory analyses.
[3]Calculated using the tabular values of NRC (1996).

Table 8. Effects of nitrogen supplementation and zilpaterol inclusion on intake, digestion, and nitrogen retention in steers consuming corn-based diets supplemented with no protein (CON) with dried distiller's grains with solubles (DDGS), or with urea

	+ Zilpaterol-HCl			- Zilpaterol-HCl				P^2		
Item	CON	DDGS	UREA	CON	DDGS	UREA	SEM[1]	Zilpaterol	Diet	Interaction
No. of observations	5	5	4	6	5	5				
Intake, kg/d										
DM	8.51	8.52	8.45	6.46	6.49	6.89	0.50	<0.01	0.76	0.61
OM	8.13	8.10	8.09	6.29	6.27	6.70	0.45	<0.01	0.66	0.59
Starch	5.58	4.47	5.43	4.24	3.41	4.47	0.30	<0.01	<0.01	0.45
Total tract digestion, %										
DM	77.4	77.4	76.4	76.2	79.1	77.8	2.4	0.80	0.65	0.62
OM	78.4	78.7	77.6	77.7	80.4	79.3	2.5	0.71	0.66	0.70
N	72.5	75.4	79.3	71.5	77.9	75.7	1.9	0.64	<0.01	0.19
Nitrogen, g/d										
Intake	138.0	197.5	177.0	101.9	154.5	145.1	11.2	<0.01	<0.01	0.79
Fecal	37.9	49.2	37.2	29.1	34.1	34.9	4.4	0.09	0.03	0.11
Urinary	51.2	60.6	63.1	47.1	63.9	64.4	12.3	0.98	0.36	0.93
Urea	29.6	37.9	42.6	29.3	47.0	47.5	12.5	0.62	0.35	0.90
NH_3	1.5	2.1	1.2	1.2	1.5	0.7	0.78	0.44	0.52	0.97
Purine derivatives[3]	14.1	14.6	14.3	11.1	11.9	11.8	1.1	0.02	0.76	0.95
Allantoin[3]	12.9	13.4	13.1	10.2	11.0	10.9	1.0	0.02	0.74	0.93
Uric acid[3]	1.20	1.23	1.24	0.86	0.86	0.88	0.12	0.02	0.94	0.99
Creatinine[3]	5.71	5.73	5.51	4.87	5.05	5.12	0.40	0.20	0.88	0.52
Retained	48.9	87.4	74.6	25.8	57.3	47.0	17.1	0.05	0.10	0.97

[1]When SEM differ among treatments, the largest value is reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]One observation for CON/+ Zilpaterol was removed because the analysis was not considered reliable.

Table 9. Interactions of nitrogen supplementation and zilpaterol inclusion on urea kinetics of steers consuming corn-based diets supplemented with no protein (CON), with dried distiller's grains with solubles (DDGS), or with urea.

	+ Zilpaterol-HCl			- Zilpaterol-HCl				P^2		
Item	CON	DDGS	UREA	CON	DDGS	UREA	SEM[1]	Zilpaterol	Diet	Interaction
No. of observations[3]	5	5	3	6	5	4				
Urea kinetics, g/d										
Urea-N entry rate (UER)	156	169	134	126	217	142	52	0.80	0.36	060
Urinary urea-N eliminated (UUE)	30	38	31	29	47	44	15	0.47	0.51	0.81
Gastro-intestinal entry rate (GER)	126	132	109	97	169	93	47	0.94	0.39	0.58
Returned to ornithine cycle (ROC)	55	72	50	44	84	48	22	0.96	0.22	0.77
Urea-N utilized for anabolism (UUA)	65	54	54	49	79	42	26	0.97	0.67	0.46
Urea-N excreted in feces (UFE)	5.3	6.9	5.5	3.5	6.3	3.5	2.0	0.50	0.09	0.73
Fractional urea kinetics										
UUE/UER (u)	0.22	0.27	0.18	0.25	0.24	0.30	0.089	0.57	0.96	0.58
GER/UER	0.78	0.73	0.82	0.75	0.76	0.70	0.089	0.57	0.96	0.58
ROC/UER (ρ)	0.34	0.40	0.36	0.32	0.38	0.30	0.042	0.22	0.18	0.84
ROC/GER (r)	0.47	0.55	0.46	0.43	0.52	0.44	0.086	0.64	0.36	1.00
UUA/GER (a)	0.50	0.39	0.49	0.52	0.45	0.51	0.080	0.59	0.30	0.95
UFE/GER (f)	0.036	0.056	0.046	0.047	0.035	0.047	0.014	0.76	0.87	0.25

[1]When SEM differ among treatments, the largest value is reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]Two observations were removed as outliers (for urea-N entry rate, the absolute value of the studentized residual was greater than 3).

Table 10. Effects of nitrogen supplementation and zilpaterol inclusion on ruminal microbial capture of recycled N (MNU) in steers consuming corn-based diets supplemented with no protein (CON), with dried distiller's grains with solubles (DDGS), or with urea

	+ Zilpaterol			- Zilpaterol				*P*[2]		
Item	CON	DDGS	UREA	CON	DDGS	UREA	SEM[1]	Zilpaterol	Diet	Interaction
No. of observations[3]	4	5	4	6	5	5				
Microbial N[4] g/d	190	196	192	143	154	153	17	0.02	0.78	0.95
Microbial N[5] g/d	146	151	147	110	118	118	13	0.02	0.78	0.95
Microbial N from Urea (MNU),[4,6,7] g/d	47	52	38	24	49	24	16	0.41	0.16	0.49
% of urea entry (UER)[4,6,7]	24	30	18	24	22	21	6	0.67	0.53	0.50
% of gastro-intestinal entry (GER)[4,6,7]	34	44	22	33	28	29	9	0.64	0.44	0.31
MNU, corrected,[5,6,8] g/d	58	63	47	29	59	30	19	0.41	0.16	0.49
% of UER, corrected[5,6,8]	30	37	22	29	26	26	8	0.67	0.53	0.50
% of GER, corrected[5,6,8]	41	53	26	40	34	35	12	0.64	0.44	0.31

[1]When SEM differed among treatments the largest value was reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]One observation was removed from analysis of all responses in this table (from CON/+ Zilpaterol) because the analysis of urinary purine derivatives was not considered reliable.
[4]Microbial N flow to the duodenum calculated using the equations of Chen and Gomes (1992).
[5]Microbial N flow to the duodenum calculated as 0.558 × the absorption of microbial purines (Chapter II).
[6]Two observations were removed from all analyses of MNU (from Urea/+ Zilpaterol, n=3 following removal; from Urea/ - Zilpaterol, n=4 following removal) because values for UER were outliers (absolute value of the studentized residual was greater than 3).
[7]Calculated using enrichment of urinary purine derivatives.
[8]Calculated using enrichment of urinary purine derivatives multiplied by 1.58 to account for lower enrichment in purine derivatives than in microbial N (Chapter II).

Table 11. Effects of nitrogen supplementation and zilpaterol inclusion on plasma metabolites in steers consuming corn-based diets supplemented with no protein (CON), with dried distiller's grains with solubles (DDGS), or with urea

	+ Zilpaterol-HCl			- Zilpaterol-HCl				*P*[2]		
Item	CON	DDGS	UREA	CON	DDGS	UREA	SEM[1]	Zilpaterol-HCl	Diet	Interaction
No. of observations	5	5	4	6	5	5				
Urea-N, m*M*	2.9	3.5	4.5	3.8	4.0	4.7	1.0	0.48	0.42	0.93
Glucose, m*M*	5.53	5.87	5.97	5.84	6.06	5.86	0.28	0.69	0.14	0.32
Creatinine, m*M*	85.7	86.7	88.0	89.5	87.3	91.7	6.0	0.65	0.80	0.90
Plasma amino acids[3], μ*M*										
No. of observations[3]	4	3	3	5	4	4				
Ala	256	257	212	240	194	267	34	0.78	0.67	0.15
Gly	270	290	211	315	242	235	60	0.87	0.41	0.64
Val	243	240	238	200	207	229	28	0.19	0.87	0.76
Leu	216	201	170	154	142	178	26	0.08	0.80	0.25
Ile	87	79	82	71	72	78	10	0.24	0.83	0.69
Thr	53	47	70	50	46	61	7	0.41	0.05	0.84
Ser	57	67	79	59	70	75	11	0.95	0.12	0.90
Pro	103	97	73	79	65	83	13	0.17	0.41	0.16
Asn	25	23	26	23	24	27	4	0.93	0.50	0.86
Asp	12	10	8	9[4]	7	7	2	0.21	0.27	0.84
Met	27	25	22	23	20	24	3	0.24	0.40	0.35
Glu	171	166	114	145	135	141	23	0.54	0.31	0.31
Phe	73	63	58	56	52	68	5	0.13	0.30	0.03
Gln	95	92	116	78	118	102	26	0.94	0.48	0.51
Orn	121	95	87	94	80	100	26	0.60	0.64	0.65
Lys	92	73	82	88	83	107	17	0.39	0.54	0.55
Tyr	73	58	52	51	49	59	8	0.18	0.40	0.13
Trp	40	41	38	31	39	42	7	0.71	0.54	0.48

[1]When SEM differed among treatments the largest value was reported.
[2]Significance was declared at $P \leq 0.10$ and tendencies at $0.10 > P \leq 0.20$.
[3]Plasma was not available for analysis of 7 observations.
[4]Outlier removed from this treatment for Asp.