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THE EFFECTS OF SLOW RELEASE UREA ON NITROGEN METABOLISM IN CATTLE

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THE EFFECTS OF SLOW RELEASE UREA ON NITROGEN METABOLISM
IN CATTLE

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DISSERTATION
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A dissertation submitted in partial fulfillment of the
Requirements for the degree Doctor of Philosophy in the
College of Agriculture at the University of Kentucky

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2012

ABSTRACT OF DISSERTATION

THE EFFECTS OF SLOW RELEASE UREA ON NITROGEN METABOLISM

IN CATTLE

The objective of this research was to investigate the effects of slow release urea on N metabolism in cattle. The ruminal behavior of Optigen®II and the effect of basal diet on the *in situ* degradability of urea and Optigen®II were evaluated. The effect of slow release urea and its interaction with degradable intake protein (DIP) level in the diet on N retention and excretion was evaluated utilizing 8 Holstein steers in a 4 x 4 Latin square experiment. In addition, the effect of slow release urea and DIP level on ruminal and systemic urea kinetics was evaluated using stable isotope techniques with 8 Holstein steers in a 4 x 4 Latin square experiment. Finally, slow release urea was evaluated under a practical beef production setting. The performance of slow release urea was compared to regular feed grade urea in a 42 day receiving study (288 Angus cross steers) as well as a 70 day growing study (240 Angus cross steers). High forage diets increased the ruminal degradation rate of both urea and slow release urea an increased the extent of degradation of slow release urea when compared to high concentrate diets. Lower DIP concentrations in the diet reduced systemic urea production, ruminal ammonia and plasma urea concentrations and urinary urea excretion under most circumstances but also led to a reduction in N retention, reduced diet digestibility, lower feed intake, lower growth rate and decreased feed efficiency. High DIP intakes increased N retention, growth rate, diet digestibility and improved feed efficiency but also lead to increased excretion on urea N in the urine. Slow release urea improved N retention and efficiency of N retention in high DIP diets when compared to urea and generally reduced plasma urea and ruminal ammonia concentrations. Compared to urea, slow release urea did not significantly improve the production of receiving cattle. However Optigen®II improved the feed efficiency when compared to urea on high concentrate diets but reduced feed efficiency on high forage diets.

Key words: Nutrient Synchrony, Feed Efficiency, Nitrogen Efficiency, Urea Kinetics, Pollution

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FREQUENTLY USED ABBREVIATIONS

BW – Body weight

CP – Crude protein

d – Day

DAPA – Diamino pimelic acid

DIP – Degradable intake protein

DM – Dry matter

DMI – Dry matter intake

FAB – Fluid associated bacteria

G:F – Gain to feed ratio

GER – Gut entry rate of urea

GIT – Gastrointestinal tract

h – Hour

hd – Head, i.e. count of animal number

MCP – Microbial crude protein

min – Minute

N - Nitrogen

NPN – Non protein nitrogen

OM – Organic matter

PAB – Particle associated bacteria

PD – Purine derivatives (Allantoin + uric acid)

RDP – Rumen degradable protein

ROC – Return of urea N to the ornithine cycle

RUP – Rumen undegradable protein

SBM – Soybean meal

TMR – Total mixed ration

UER – Urea entry rate

UFE – Urea N fecal excretion rate

UIP – Undegradable intake protein

UUE – Urinary urea excretion rate

VFA – Volatile fatty acid

CHAPTER 1. INTRODUCTION

Urea is a small organic compound that is very rich in N (44.96% N) that is used to supply degradable intake protein (DIP) to ruminants. Urea is broken down to ammonia in the rumen under the action of bacterial urease (Satter and Slyter, 1974). Microorganisms in the rumen are able to utilize the resulting ammonia to form amino acids which then become available to the host when microbial bodies are digested and absorbed post ruminally (Loosli et al., 1949). The reasons for using urea over other sources of DIP is that urea N is cheaper on a per N basis than any other feedstuff and transportation and storage are cheaper and easier due to the concentrated nature of urea (McPherson and Witt, 1968). However, urea is used rather inefficiently by ruminants compared with other sources that contain true protein (Broderick et al., 2009), and this is due to the fact that the rate at which urea is degraded in the rumen is more rapid than the rate of utilization of the resulting ammonia by rumen bacteria leading to ruminal accumulation and absorption of ammonia and subsequent excretion of urea in the urine (Golombeski et al., 2006; Highstreet et al., 2010). Therefore utilizing urea as a DIP source may lead to excessive excretion of N in the urine. Nitrogen excretion from cattle operations has become a matter of increasing public concern over the last decade (VandeHaar and St-Pierre, 2006) and therefore reducing N excretion from cattle operations has become an increasing priority for ruminant nutritionists. One strategy for improving the utilization of urea by ruminants is to more closely time the fermentation of energy and the availability of ammonia in the rumen (Johnson, 1976). This may be done by increasing the degradability of the carbohydrates in the diet, or alternatively, by reducing the degradation rate of urea. A number of slow release urea products have been

developed for this purpose (Deyoe et al., 1968; Fonnesbeck et al., 1975; Owens et al., 1980). Optigen®II is a lipid coated urea product design to reduce the rate of ruminal degradation of urea. The objectives of this research were to: 1) Characterize the ruminal behavior of Optigen®II; 2) Determine the effects of Optigen®II on N retention, excretion and efficiency; 3) Determine the effect of Optigen®II on systemic urea kinetics; 4) To evaluate the effectiveness of Optigen®II in receiving and growing beef cattle rations.

CHAPTER 2. A REVIEW OF THE LITERATURE

INTRODUCTION

The most important driving factor behind the use of urea as a N source in cattle is the economical advantage of using urea in the place of traditional protein sources. At the most basic level, urea is cheaper on a per N basis than any other N source. For example, if the current market prices for urea and soybean meal (SBM) are compared, SBM costs $347.60 per ton while urea costs $368.60 per ton (U.S. Commodity prices, January, 2012, indexmundi.com). However, when you compare the price of urea and SBM on a per N basis, SBM, which is 48% CP, is only 7.68% N on average, making the price per ton of SBM N about $4526. On the other hand, urea is 281% CP which is 44.96% N, making the price per ton of urea N about $820. This means that urea N is about 5.5 times cheaper than SBM N. In addition to the price per unit N, transportation and handling costs have to be considered as well. Although cost of transportation is affected by multiple factors, the fact that urea is a more concentrated source of N means that it is cheaper to transport per unit N than other less concentrated N sources (McPherson and Witt, 1968).

UREA AS A NITROGEN SOURCE FOR RUMINANTS

Discovery and history of urea as a feedstuff

The utilization of NPN by ruminants as a useful N source has been recognized for over 100 years. Approaching the end of the 19th century, Zuntz (1891) proposed a theory that bacteria in the forestomach of ruminants could utilize simple N components such as amides or ammonia to produce bacterial protein that the animal then had access to by digestion of microbial bodies in the small intestine. By 1936, much work had been done

in the area as the famous biochemist Hans A. Krebs, known for his Nobel award winning work in discovering the citric acid cycle, presented a review (Krebs, 1937) citing over 100 references with conflicting evidence with regard to the theory proposing that ruminants could utilize NPN. At this point, there was still no concrete evidence that ruminants could use non protein nitrogenous compounds for protein synthesis. In 1939, Hart et al. showed rather definitively, in long term growth/slaughter and milk production trials with cattle, that diets with included NPN resulted in improved protein deposition and milk production over low protein controls. Similar results were obtained by other authors over the next few years for both growing ruminants (Harris and Mitchell, 1941; Loosli et al., 1949) and for milk production (Owen et al., 1943). In 1949, Loosli et al. were the first to feed purified diets to ruminants (sheep and goats) in which the only source of N was urea. They became the first authors to demonstrate that the ten essential amino acids are synthesized in large quantities in ruminants fed urea as the only dietary source of N. A few years later, in a study conducted with ruminally fistulated calves, Duncan et al. (1953) demonstrated that all amino acids required by the animals were synthesized in significant quantities by microbial populations in the rumen. Finally, a series of long term studies on dairy cattle were conducted by Nobel prize laureate and animal scientist Artturi Virtanen, in which he demonstrated that dairy cattle were able to live, reproduce and produce moderate amounts of milk on a protein free diet (Virtanen, 1966). Following these findings, a substantial amount of research has focused on the utilization of non protein N and other degradable proteins in the rumen. This research has essentially led to the modern day practice of dividing feed N between that available to ruminal microorganisms (RDP or DIP) and that which escapes ruminal degradation

and may be available for digestion in the small intestine (RUP or UIP)(NRC, 2000, 2001).

General information on how urea is used by the nutritionist

Although the use of urea has potential economic advantages over other N sources, there are limitations and disadvantages to its use. As early as 1953, the limitations of using urea in cattle rations were being realized. Reid (1953) published an extensive review on the use of urea in cattle diets. He recognized that the degradation rate of urea to ammonia in the rumen was likely more rapid than the utilization of the resulting ammonia and that much of the urea N was lost in the urine. The same authors also found that urea was less effective in diets that already contained 12% or more of CP and that urea became unpalatable or resulted in reduced feed intake when dietary inclusion exceeded 1% of DM. Most reports of reduced production in ruminants fed urea are directly attributable to a reduction in DMI (Kertz, 2010; Polan et al., 1976). Since the original observations by Reid, several authors have challenged these recommendations. Kwan et al. (1977) found that dairy cows used urea rather effectively at 1% of DM in the diet up to dietary CP concentrations of 16.6%, but upheld the recommendation of Reid (1953) that when urea is fed at greater than 1% of DM, reductions in DMI and production may result. Broderick et al. (1993) found no difference in DMI when replacing natural protein with 1.33% urea but a reduction in DMI when replacing with 1.63% urea in diets based on alfalfa and corn silage. Utilization of dietary urea is probably more related to degradable protein supply in the diet and the amount of fermentable energy available for the capture of ammonia in the rumen than the absolute CP concentration in the diet (Burroughs et al., 1975). Concerning the quantity of urea in the feed that will cause a

5

depression in DMI, it seems safe to assume that up to 1% of DM as urea should not cause any depression in DMI and this number can probably be increased to 1.5% under certain circumstances (Kertz, 2010). Total mixed ration feeding instead of discreet meal feeding is less likely to result in DMI depression at urea levels above 1% of DM (Kertz, 2010). This is likely related to the amount of fermentable energy available in the rumen. Urea appears less likely to cause a decrease in DMI or toxicity when it is fed along with a readily available source of fermentable energy (Bartley et al., 1976). Fermentation of carbohydrates provides energy to rumen microbial populations. Growing microbial populations utilize ammonia to synthesize microbial proteins and thereby decrease free ammonia in the rumen and consequently ammonia absorbed into the circulation of the animal. Additionally, an increase in fermentable energy will reduce the pH in the rumen resulting in decreased absorption of ammonia across the rumen wall (Bartley et al., 1976). These statements fit well with the theories of Burroughs et al. (1975) on urea fermentation potential that positively relate the potential to use urea in ruminant diets with the amount of TDN in the diet.

Another potential disadvantage to using higher inclusion rates of urea in the diet of cattle is ammonia toxicity. Urea is broken down to ammonia in the rumen under the action of microbial urease activity (Satter and Slyter, 1974). Excessive absorption of ammonia into the blood can overwhelm the ability of the liver to detoxify it back to urea, and ammonia toxicity results. The toxic effects of excessive consumption of urea have been well documented (Antonelli et al., 2004; Bartley et al., 1981; Bartley et al., 1976; Davidovich et al., 1977). The symptoms of urea toxicity, in order of appearance after exposure include: fasciculation, apathy, hyperaesthesia, tremors, rumen stasis,

incoordination, recumbancy, convulsions and death (Antonelli et al., 2004). The required amount of urea to cause toxicity varies widely, however urea fed at as low as 0.35g/kg BW resulted in death in some dairy cattle (Ryley and Gartner, 1968). However, ammonia toxicity from feed urea is somewhat situation dependant. There are wide reports that higher levels of urea are tolerable in the diet when it is fed as part of a TMR, instead of in discrete meals (Kertz, 2010). Animals fed a TMR would be exposed to lower concentrations of urea, with more time for ammonia detoxification across the day. However, Bartley et al. (1976) presented data that indicated that ammonia toxicity was poorly correlated to rumen ammonia concentration. Instead they showed that toxicity related to feed urea was more closely related to rumen pH. When ruminal urea degradation results in a rapid accumulation of ammonia in the rumen, the pH of the rumen may increase sharply, as ionization of ammonia molecules removes free hydrogen ions from solution (Kertz et al., 1983). Increased ruminal pH facilitates a rapid transport of ammonia across the rumen epithelium, resulting in a rapid increase in blood ammonia and the consequent ammonia toxicity (Abdoun et al., 2006). The causal effect of pH on ammonia toxicity was confirmed by Kertz et al. (1983) in a study that was based on adding ammonia equivalent amounts of ammonium chloride (already ionic) and urea, which requires addition of a hydrogen ion to ionize it. The ammonium chloride treatment resulted in increased rumen ammonia concentrations, but no pH elevation and subsequently no toxicity.

Application of urea in feeding systems models

Modern ruminant feeding systems account for the fact that dietary N can be used to feed the rumen microbial population as well as the animal directly (NRC, 2000, 2001).

Depending on the degradation rate of N in the feed and the rate of passage of that feed component, a variable amount of intake N will be available to ruminal microbes, with the remainder available for enzymatic degradation in the post ruminal digestive tract (Zinn and Owens, 1983). Feed N can come from either true protein or NPN. Nitrogen sources that are not degraded in the rumen are available for enzymatic digestion. This source of N, often referred to as rumen bypass, rumen escape or undegraded protein can supply protein to the animal following enzymatic digestion in the post ruminal digestive tract (Armstrong and Hutton, 1975). However, only true protein sources can provide this post ruminal protein source. When urea and other NPN sources are used to provide N in the diet of ruminants, the feeding system has to account for the fact that urea may only provide ruminal N, and that post ruminal availability of urea or NPN cannot improve amino acid supply, unless it is recycled back to the rumen in the urea cycle. In order to avoid using NPN as a post ruminal N source, most feeding systems, including the NRC beef, CNCPS and NRC dairy, assume that the degradation rate of NPN in the rumen to be equal to infinity. Therefore passage rate, by definition is equal to zero and no post ruminal absorption of NPN is recognized.

SLOW RELEASE UREA

In general, the efficiency of utilization of dietary N by cattle is relatively low under normal production conditions (Castillo et al., 2001) with a global average N-efficiency in cattle estimated at 7.7 % (Van der Hoek, 1998). Urea is used rather inefficiently for production of protein products (Broderick et al., 2009) and due to its wide use in ruminant feeds, may be partially responsible for the poor N efficiency in cattle. Low efficiency of utilization of dietary urea has been attributed to the rapid hydrolysis to NH_3

in the rumen by microbial enzymes which occurs at a higher rate than its utilization by rumen bacteria, leading to ruminal accumulation and absorption of ammonia and subsequent excretion of urea in the urine (Golombeski et al., 2006; Highstreet et al., 2010). Therefore, attempts have long been made to produce a form of urea that would degrade more slowly in the rumen, potentially resulting in increased incorporation of ammonia into microbial populations and consequently lower excretion of urea in the urine.

Early slow release urea products include: biuret, an extensively studied compound that is formed by the condensation of two molecules of urea. Biuret has been studied in ruminant diets since the 1970s (Fonnesbeck et al., 1975); Starea, a product produced by cooking grains and urea together to form a product that degraded more slowly (Deyoe et al., 1968) and urea phosphate (Oltjen et al., 1968). More recently, slow release of urea in the rumen has been achieved by binding urea to lignin (Castro et al., 1999) or calcium chloride (Huntington et al., 2006a) or by encapsulating the urea particles with polymers (Galo et al., 2003) or lipids (Garrett et al., 2005; Owens et al., 1980) to reduce the rate of release in the rumen.

The effect of slow release urea is often compared to that of regular feed grade urea to determine its efficacy. Experiments where slow release urea is compared to other true protein sources are somewhat confounded by the presence of amino acids, nucleotides, fermentable energy and other compounds that are present in the feed, that may affect ruminal utilization of N. Therefore, only studies that used isonitrogenous urea as a control treatment are included in this review.

Feed intake and digestibility are known to be intimately related and may be affected by ruminal N availability (Köster et al., 1996). Some authors have reported improvements in feed intake or digestibility for ruminants consuming slow release urea compared to cattle consuming feed grade urea. Cherdthong et al. (2011) investigated the effect of a urea-calcium slow release urea in dairy cattle fed rice straw and found that when compared to urea, urea-calcium mixture increased OM intake as well as OM digestibility. Owens et al. (1980) compared urea to a lipid coated slow release urea with steers fed *ad libitum* cottonseed hulls and found that slow release urea increased cottonseed hull intake when compared to urea but did not see any differences in diet digestibility. In contrast Galo et al. (2003) fed a polymer-coated slow release urea to dairy cattle fed a corn silage based mixed dairy diet and Taylor-Edwards et al. (2009a)Taylor-Edwards et al. (2009b) did not find any effect on DMI or diet digestibility. In addition, Bourg et al. (2012) found no differences between a lipid-coated urea and urea on diet intake or digestibility in a N balance study using Holstein steers fed a diet based on steam flaked corn.

Slow release urea has been shown to affect ruminal fermentation characteristics. Most notably, slow release urea is intended to reduce the release rate of NH_3 in the rumen. Most reports on slow release urea have shown a reduction in ruminal NH_3 concentration when measured (Cherdthong et al., 2011; Huntington et al., 2006b; Taylor-Edwards et al., 2009d). This is the reason why slow release urea presents a lower risk for ammonia toxicity than feed grade urea as was demonstrated by Owens et al. (1980). Ruminal NH_3 concentration is often related to ruminal pH, as the protonation of NH_3 to NH_4^+ when ammonia from urea ionizes, can result in an increase in ruminal pH (Kertz et

al., 1983). Consequently, there are reports of higher ruminal pH for animals fed urea than those fed slow release urea (Cherdthong et al., 2011; Taylor-Edwards et al., 2009b).

The analysis of ruminal VFA data on slow release urea studies is less common. Cherdthong et al. (2011) reported that slow release urea did not change the total VFA produced but led to an increase in the proportion of propionate produced. Other authors have reported no change in the total VFA or proportions of VFA produced when urea was compared to slow release urea (Taylor-Edwards et al., 2009c; Xin et al., 2010).

The proposed mechanism of action of slow release urea is that it will lead to a more closely timed synchrony of energy and N availability in the rumen, leading to increased capture of ruminal N in microbial protein (Johnson, 1976). Cherdthong et al. (2011) reported that a urea-calcium based slow release urea resulted in increased counts of total and cellulolytic bacteria estimated both by direct count (CFU/mL) and as estimated by real-time PCR in diets based on cassava chips fed to lactating dairy cattle. Galo et al. (2003) reported no change in microbial protein production estimated by the urinary excretion of purine derivatives on mixed diets fed to lactating dairy cattle.

The purpose of any nutritional strategy designed to improve microbial protein production is to improve productive output of the animals, or alternatively, to increase the efficiency of production of the animals. Cherdthong et al. (2011) reported an increase in total and 3.5% fat corrected milk yield in dairy cows fed a urea-calcium based slow release urea when compared to urea. Xin et al. (2010) reported that although slow release urea did not increase milk production, it did lead to an increase in the percentage of milk protein and a reduction in milk urea N when compared to urea. In finishing Angus steers

fed a steam flaked corn diet, Bourg et al. (2012) did not find any difference in performance or carcass composition when feeding a lipid coated urea but it resulted in a tendency for higher gain to feed ratio than an isonitrogenous amount of urea. In contrast, Taylor-Edwards et al. (2009) reported that urea tended to result in higher gains in beef cattle than slow release urea, but also noted an interaction with the quantity of urea in the diet, where urea resulted in better growth at low levels of supplementation whereas slow release urea was superior at higher levels of supplementation with the exception of the highest level. A similar trend was evident for feed efficiency.

Therefore the response to supplementation of slow release urea in the place of feed grade urea is somewhat situation dependant and probably varies significantly between, and within the various slow release urea products available, as well as the wide variety of diets and production situations investigated.

STRATEGIES FOR MINIMIZING N WASTE IN CATTLE OPERATIONS

Due to increased population and income levels, particularly in developing countries, worldwide production of meat and milk will have to double within 50 years (Dijkstra et al., 2011). This will require a massive increase in the productive output from animals without any appreciable increase in land availability. However, maximizing production from ruminants is often associated with an increase in excretion of waste products that may be harmful to the environment (VandeHaar and St-Pierre, 2006). Nitrogen is one of the major sources of pollution from ruminant operations, along with phosphorus and methane. Nitrogen is of particular concern in dairy cattle production (Arriaga et al., 2009) and N pollution results in eutrophication of natural water sources, pollution of groundwater with nitrates and atmospheric pollution by de-nitrification and

ammonia volatilization (Dijkstra et al., 2011). The goal for nutrient management on farms, including beef and dairy farms, is the efficient utilization of nutrients, as this affects both environmental pollution as well as the farmers' bottom line (Oenema and Pietrzak, 2002). Minimizing N pollution is necessary at all stages of production, from crop production to feeding and management practices and manure management (Rotz, 2004). This section will focus on nutritional strategies to limit N excretion.

The N efficiency in US dairy herds is estimated to be between 20 and 30% (Kohn et al., 1997; Oenema and Pietrzak, 2002) whereas pasture produced (Hutchings et al., 1996) and feedlot finished beef (Bierman et al., 1999) have a N efficiency of 10% or less. Efficiency of N use by animals can be improved by more closely matching dietary supply of degradable and undegraded N with the requirements of the animal, and by increasing animal production, resulting in a dilution of the maintenance protein requirement per unit of protein product (Rotz, 2004). This is difficult to achieve whilst still attempting to minimize feed cost as is the goal of most farmers. A major step forward in more closely matching the feed and requirement N took place when feeding system moved away from total or CP content of diets to one where the ruminal available N and post ruminal protein availability are considered separately (NRC, 2000, 2001). Microbial protein digested in the small intestine, and produced from ruminally available protein is typically the primary source of amino acids in cattle (NRC, 2000; Spicer et al., 1986) followed by proteins that escape ruminal degradation and are digested post ruminally. Overfeeding degradable N may result in excessive losses of N in the urine (Marini and Van Amburgh, 2005) while underfeeding it may reduce digestion and microbial protein production (Köster et al., 1996; Satter and Slyter, 1974). Overfeeding DIP or RUP or an imbalance

between DIP and RUP in excess of requirements will increase the total CP required for a particular level of production, leading to increased N excretion per unit N intake (Marini and Van Amburgh, 2005), thus increasing feed costs and N excretion rate at a particular level of production. High CP diets result in higher rate of excretion of N than low CP diets, even if the diets are balanced for degradable and undegradable protein. Tomlinson et al. (1996) reported that increasing CP concentration in the diet from 12 % to 18 % resulted in 2.3 times greater urinary N excretion and 0.25 times greater fecal N excretion. Similarly, Frank et al. (2002) reported that increasing dietary CP concentration from 14 % to 19 % increased ammonia emissions by 300% and also increased nitrous oxide emission from manure (Külling et al., 2001). Rotz (2004) predicted that overfeeding of RDP is responsible for much of the excessive N excretion and poor N economy of dairy cattle.

A management strategy for improving the accuracy of supply of DIP and RUP to the animals' requirements is to feed animals in smaller groups so that the formulated diet more closely resembles the requirements of each individual animal (St-Pierre and Thraen, 1998). For example, if dairy cattle are divided by stage of lactation, the farm manager can more closely meet the high N requirements of the early lactation cow without over feeding the mid and late lactation cows, resulting in improved N economy throughout the herd. Some traditional forage sources such as alfalfa silage contain relatively large amounts of rapidly rumen degradable protein, which may result in increased excretion of N in the urine. Using a forage source with lower degradable N, such as corn silage can result in a reduction in DIP supply, decreased CP concentration in the diet and decreased N pollution (Dhiman and Satter, 1997) and any deficit in rumen available N that may

14

result from the use of corn silage may be easily overcome by utilizing an inexpensive NPN source. Finally, when supplementing diets with DIP, there is evidence that utilizing a DIP source that is degraded more slowly will lead to a reduction in urinary excretion of urea associated with lower extremes in ruminal ammonia concentration (Galo et al., 2003).

METHODOLOGIES FOR STUDYING N METABOLISM

Following is a brief overview of some of the methods used to study N and urea metabolism in ruminants. This list is by no means comprehensive, but contains many of the currently used and classical techniques for studying N metabolism. Included are methodologies used to study urea metabolism, nutrient absorption and microbial protein production in ruminants.

Urea recycling methodologies

The method used to evaluate urea kinetics was developed by Sarraseca et al. (1998) for use in sheep and was later refined by Lobley et al. (2000). The method was later validated for use in cattle (Archibeque et al., 2002; Marini and Van Amburgh, 2003). The premise of the method is that by continuously infusing animals with $^{15}N^{15}N$ urea, and allowing sufficient time for the concentration of $^{15}N^{15}N$ urea and its metabolite $^{15}N^{14}N$ to reach steady state, and by measuring the enrichment of the two urea species in the urine, systemic urea metabolism may be determined. If animals are not fed under steady state conditions (i.e. multiple small meals per day) urine should be collected for a full 24 h period in order to account for the potential effect that meal feeding (non-steady state) might have on the enrichment of N species in the urine (Wickersham et al., 2009). The parameters estimated by the urea kinetics method include the following: Urea entry

15

rate (UER), which is the rate of appearance of urea N in the blood. It represents the urea synthesis rate occurring in the liver (Lobley et al., 2000). This urea (UER) can suffer only one of two fates: It can be excreted in the urine or it can enter the gastrointestinal tract. Urinary urea excretion (UUE) is the rate of excretion of urea in the urine. Entry into GIT (GER) is the rate of entry of urea N into the gut (Sarraseca et al., 1998) and is commonly referred to as urea recycling. Return to the ornithine cycle (Gorocica-Buenfil and Loerch, 2005) represents urea N that is returning from the gut (NH_3 absorbed across the rumen epithelium into the blood) and is re-synthesized to urea in the liver. Urea N excreted in the feces (UFE) is N that originates from urea, which is excreted in the feces. Urea N utilized for anabolism (UUA) is N that originates from urea, which is used for anabolic purposes, mainly via microbial protein. The model also allows the estimation of fractional transfers of urea between body pools (Lobley et al., 2000). The UER to urine (u) is the fraction of UER that ends up in the urine. The fraction of UER that enters the GIT is then denoted by (1-u). The GER to ROC (r) is the fraction of GER that returns to the urea cycle for re-synthesis to urea. The GER to feces (f) is the fraction of GER that ends up in the feces. The GER to UUA is the fraction of GER that is used for anabolic purposes.

In order to determine urea kinetics experimentally, animals should be set up as for a N balance experiment where total and separate collection and quantification of feces and urine can be accomplished. Prior to infusions, samples of urine and feces should be taken in order to determine the natural abundance of ^{15}N (Lobley et al., 2000). Animals are fitted with jugular catheters which can be connected to a pump to perform the infusion. Rate of infusion is calculated buy determining how much $^{15}N^{15}N$ urea is needed

per day to result in enrichment of $^{15}N^{15}N$ urea in the body urea pool to approximately 0.15-0.25 atom percent excess at plateau. Continuous infusion requires at least 48 hrs before both $N^{15}N^{15}$ and $N^{15}N^{14}$ are at plateau levels (Marini and Van Amburgh, 2003). Total daily fecal and urine output is recorded and subsamples of acidified urine and feces are taken daily. Daily urine samples are then analyzed for total urea content and proportions of $^{15}N^{15}N$, $^{15}N^{14}N$ and $^{14}N^{14}N$ urea isotopes by isotope ratio mass spectrometry (Marini and Attene-Ramos, 2006). Fecal ^{15}N is also estimated. Required variables to measure: $^{15}N^{15}N$ urea in the dose, fecal N and ^{15}N enrichment (APE), total urine urea N, proportions of $^{15}N^{15}N$ and $^{15}N^{14}N$ urea in the urine. These inputs can be used with the equations described by Lobley et al. (2000) to determine UER, UUE, UFE, GER, ROC, UUA. Corrections have to be made to allow for the $^{14}N^{15}N$ urea present in the infusate and that which is produced by so-called non-monomolecular reactions during the hypobromite release of N_2 gas which are required to accurately determine and differentiate between $^{15}N^{15}N$ and $^{15}N^{14}N$ in the model (Lobley et al., 2000).

Methodologies for estimating microbial protein production

Yield of microbial protein from the rumen remains one of the most studied fields of ruminant nutrition and yet still one of the most difficult to predict and measure (Perez et al., 1996). Markers are traditionally used to determine microbial protein production as the separation of protein fractions from feed and bacterial origin is difficult. Samples can be collected from the omasum, abomasum or duodenum, if surgically prepared animals are available, or microbial markers in the urine can be used to estimate microbial protein production (Shingfield and Offer, 1999). In order to determine microbial protein production by sampling the GIT, animals have to be surgically fitted with ruminal,

17

omasal, abomasal or duodenal fistula. Digesta may then be sampled and an indicator of microbial protein can be used to calculate MCP production. However, a problem arises in that the concentration of various markers tends to vary between different fractions of bacteria and digesta (France and Siddons, 1986). There also has to be a measure of digesta flow to get to total microbial protein when samples are taken directly from the intestine, and multiple markers are required to determine the flow of the various digesta fractions. Digesta are collected at the omasum, abomasum or proximal duodenum. Collected digesta have to be fractionated and a measure of the flow rate of each component is needed in order to calculate total microbial protein production. Digesta fractionation and flow markers are discussed first, followed by a discussion on the various markers of microbial protein that are used.

When collecting digesta directly from the gut, the digesta flow rates may be estimated by a triple marker method described by France and Siddons (1986). Other similar methods are available and are generally based on the same principles. Indigestible NDF, $YbCl_3$ and CoEDTA are used as markers of the large particle, small particle and fluid digesta respectively. Known quantities of CoEDTA and $YbCl_3$ are infused continuously into the rumen via a rumen cannula. Digesta are collected from the omasum, abomasum or duodenum via a surgically fitted cannula at various intervals after feeding. Pooled samples of digesta are then fractionated by centrifugation, blending and separation techniques to yield large particle solid (LP), fluid phase (FP) and small particle phase (SP). These are freeze dried and ground through a 1mm screen. Composite samples are also processed for the fractionation and isolation of fluid associated bacteria (Faber et al.) and particle associated bacteria (Udén et al.). Protozoa can also be

18

determined in the composite sample by sedimentation analysis described by Hristov et al. (2001). Total protein flow in each fraction can be calculated using the flow marker concentration to estimate flow of that fraction and the microbial marker to estimate microbial protein content of that fraction. The sum of protein flows of all 3 fractions gives the estimate of microbial protein production.

Microbial protein production may also be estimated less invasively my measuring the excretion of metabolites of microbial protein excreted in the urine. Urinary excretion of purine derivatives, determined by the methods revised by Shingfield and Offer (1999) has been used to estimate microbial protein production in ruminants (Chen et al., 1995). Total collection of urine allows the estimation of total MCP production by measuring metabolites in the urine that are exclusively of microbial origin. Utilizing urinary purine derivative excretion to estimate microbial protein production is based on the assumption that the nucleic acids digested in the small intestine are entirely microbial in origin. Dietary purines are considered to form a negligible part of intestinally absorbed nucleic acids as most ruminant feeds contain relatively low concentrations of nucleic acids, most of which are degraded by the microbes in the rumen (Chen and Ørskov, 2004). Microbial purines are completely converted to uric acid during absorption through the intestinal mucosa and a variable amount of uric acid is converted to allantoin in the liver (Chen and Ørskov, 2004). Purine derivatives excreted in the urine originate from microbial purines absorbed in the small intestine as well as those from the animals tissues as a result of regular tissue turnover (Chen et al., 1992). However, the endogenous contribution to urinary purine derivative excretion has been determined to be negligible compared to the amount of purines from microbial origin in ruminants (Chen et al., 1990d). An estimate

of the purine content in microbial protein, which can be measured from collected rumen fluid or estimated, allows prediction of microbial protein production.

There are a number of internal markers that can be used to estimate microbial CP in digesta. In addition external markers, infused into the rumen, may also be used. Following is a brief description on some of the most commonly used microbial markers. As mentioned above, microbial purines may be used as an internal microbial marker to estimate microbial protein in digesta. The method directly associates the amount of adenine and guanine in a digesta sample to MCP production by knowing the purine: protein ratio in the bacterial population. Assumptions of the method are that all purines in digesta samples are microbial in origin, which may not be true especially if large amounts of low degradable animal proteins are fed, such as fishmeal. The method also assumes that the purine content of microbes is constant. This assumption may not always be valid as the composition of microbes may vary between FAB and PAB and protozoa (Merry and McAllan, 1983). Therefore digesta fractionation is important to improve estimate of MCP production with this method. In addition, the purine to microbial protein ratio can be determined for each individual experiment by isolating rumen bacteria and determining the microbial marker to microbial protein ratio. Total purines in digesta samples can be determined by HPLC with the refined method described by Reynal et al. (2003).

Another microbial marker that has been used historically is diamino pimelic acid (DAPA). Diamino pimelic acid is an amino acid of bacterial origin and is present in the cell walls of some bacteria (Seltmann and Holst, 2002). However, DAPA values of feed have been found to range between 18 and 40% of bacteria DAPA levels (Rahnema and

Theurer, 1986), making them inappropriate as a reliable indicator of microbial protein. In addition, DAPA to protein levels are not constant and concentrations vary among different bacteria with more than 4 times the mean concentration (Broderick and Merchen, 1992). Also, due to the fact that DAPA is part of the cell wall of bacteria, the size and therefore the fed state of the bacteria will affect DAPA:Protein ratios (Broderick and Merchen, 1992). D-alanine is an amino acid that is present in the cell wall of bacteria and has been proposed as an internal marker for microbial protein production. (Garrett et al., 1987). However, it has been shown to greatly overestimate MCP flow with large variation in estimates of MCP production at the small intestine in *in vivo* experiments (Quigley and Schwab, 1988).

In addition to internal markers of microbial protein synthesis, a number of external markers have been used. Typically these have been radio labeled (^{35}S, ^{32}P) or stable isotope (^{15}N) labeled compounds that are infused in the rumen and incorporated into the microbial protein and so provide an estimate of microbial protein synthesis (Broderick and Merchen, 1992). Inorganic forms of ^{15}N such as (^{15}NH$_4$)$_2$SO$_4$ have been extensively used to study microbial protein production. ^{15}NH$_3$ infused in the rumen will label bacteria directly when used by ruminal bacteria for producing amino acids. Enrichment of ^{15}N in digesta determined by mass spectrometry (Hardarson, 1990) can then be used to estimate microbial protein production by the same methods used for internal markers. Radioisotopes are used in an analogous fashion to ^{15}N. In the case of ^{35}S, it is incorporated into the sulfur containing amino acids methionine, cysteine, homocysteine and taurine (Brosnan and Brosnan, 2006) during protein synthesis (Beever et al., 1974). However, the use of stable isotopes is generally preferred to the use of

radioisotopes due to the health hazards of working with radioactive compounds. Therefore, since the technology has been developed to detect stable isotopes at far lower concentrations (Isotope ratio mass spectrometry), the use of radioisotopes to study microbial protein production has declined.

Poor N economy in ruminants and the resulting excessive excretion of N in to the environment surrounding cattle operations is exacerbated by the inclusion of urea in most diets for ruminants. The rapid rate of degradation of urea in the rumen and the subsequent absorption and excretion of any excess urea N means that there is potential for improving N economy and production while simultaneously reducing N pollution if the efficiency with which urea is utilized can be improved. Therefore the objectives of this dissertation were to compare the effects of feed grade urea vs. slow release urea (Optigen®II) on N metabolism in cattle. Specific objectives were to:

1. Characterize the ruminal behavior of urea and Optigen®II under varying dietary conditions.

2. Determine the effects of Optigen®II on N retention, excretion, N economy and systemic urea kinetics.

3. Determine the efficacy of Optigen®II in receiving and growing beef cattle rations.

CHAPTER 3. EVALUATION OF THE RUMINAL DISAPPEARANCE OF

OPTIGEN®II AND UREA IN SITU

INTRODUCTION

In order to study the effects of slow release urea on N metabolism and production in cattle, it is important to characterize the ruminal behavior of Optigen®II under varied circumstances. Therefore *in situ* methodologies were developed in order to study the behavior of Optigen®II and urea in the rumen. These methodologies focus on determining the actual urea disappearance from polyester bags instead of utilizing traditional techniques based on DM or N disappearance (Vanzant et al., 1998). Traditional *in situ* assays cannot be used for urea and other soluble compounds as the required rinsing procedures would result in solubilization of the *in situ* residues, leading to over estimation of ruminal disappearance. In addition, measuring urea directly negates the need to correct for microbial contamination in the residue. Three experiments were conducted in order to study the ruminal behavior of Optigen®II and urea. In Experiment 1, the ruminal urea disappearance of 3 Optigen®II batches were compared to that of urea in steers fed a diet with a 70:30 concentrate to forage ratio. In Experiment 2, the ruminal urea disappearance characteristics of 5 different Optigen®II batches were determined in a 100% alfalfa hay cube diet to characterize the ruminal behavior of all available Optigen®II batches. In Experiment 3, the effects of basal diet on the ruminal urea disappearance from urea and Optigen®II were determined. The hypotheses were that urea degradation would be extremely rapid and that Optigen®II would degrade more slowly over time. Additionally, we hypothesized that Optigen®II and urea would have

similar ruminal urea disappearance between animals fed a high forage diet and those fed a high concentrate diet.

MATERIALS AND METHODS

The method for all in situ experiments focused on determining the ruminal disappearance of urea from Optigen®II or urea samples at fixed time points in order to characterize the disappearance of urea over time. Instead of determining DM or N disappearance over time as in traditional in situ assays, we determined actual urea disappearance by dissolving in situ residues in an acidic solution after incubation in the rumen. The acidic solution was used to eliminate any residual urease activity in the residues after removal from the rumen (Muck, 1982). Subsequent determination of urea concentration of solutions allowed estimation of urea remaining in the in situ residues. This eliminated variation in estimated recovery rates due to variation in rinsing technique and microbial contamination.

Experimental design

Experiment 1

To characterize the ruminal behavior of urea and Optigen®II, a complete set of all samples (urea and 3 Optigen®II samples) were incubated in each of two ruminally cannulated Holstein steers consuming a 70:30 concentrate:forage ratio diet consisting of corn silage, cracked corn and a soybean/vitamin/mineral supplement. Animals were adapted to the diet for 2 weeks prior to performing the *in situ* analysis. The time points for duration of the incubation were 0, 1, 2, 4, 6, 8, 10 & 24 h.

Experiment 2

To compare the rate and extent of ruminal degradation of Optigen®II from different batches, a complete set of each of 5 Optigen®II batch samples were incubated in triplicate in a single Holstein steer fed a diet consisting of alfalfa cubes plus a vitamin/mineral supplement. The steer had been consuming the diet for several weeks and was thus adapted to the diet. The time points for this incubation were 0, 3 and 24 h. The 0 h time point was not incubated in the rumen and allowed the determination of the urea content of each of the Optigen®II samples. The 3 h time point allowed estimation of the rate of degradation of Optigen®II samples while the 24 h time point allowed the estimation of the extent of ruminal degradation.

Experiment 3

The objective of this experiment was to determine the effects of basal diet on the degradation of urea and Optigen®II in the rumen. The degradation of urea and Optigen®II were determined on a 70% concentrate diet and a 100% forage diet respectively. The 100% forage diet consisted of *ad libitum* fescue hay plus a vitamin mineral supplement. The 70% concentrate diet consisted of 30% of fescue hay / cottonseed hulls (CSH) blend and 70% of a cracked corn and soybean meal blend (70% concentrate diet, Table 3). Additionally, diets were top dressed with 0.1% urea in order to adapt animals to having urea in the diet. Animals were adapted to diets for three weeks before each in situ procedure was performed. The Optigen®II product batch used in this experiment was #311622-2. To limit the total amount of urea fed to each animal, separate experiments using different animals were conducted for determination of urea

and Optigen®II disappearance respectively. For determination of urea disappearance, 4 Angus steers (Average BW = 310 kg) were randomly assigned to either the 100% forage or 70% concentrate diets (n = 2). For determination of Optigen®II disappearance, 4 Angus steers (Average BW = 282 kg) were randomly assigned to either the 100% forage or 70% concentrate diets. For the Optigen®II experiment, treatments were then crossed over and the experiment was repeated (n = 4) after a further 3 weeks of diet adaptation. Urea samples were incubated in the rumen for 0, 5, 10, 15, 20, 25, 30, 40, 50 & 60 min. Optigen®II samples were incubated in the rumen for 0, 1, 2, 4, 6, 8, 10 & 24 h.

Sample preparation and ruminal incubation

For each time point, triplicate 10.0 g samples of Optigen®II or urea were weighed out into polyester bags (R510, 5x10 cm, 50 μm pore, Ankom Technology, Macedon, NY), and sealed with a heat sealer. For all three experiments, triplicate Optigen®II and urea samples were placed into a single weighted mesh bag at each incubation time. The mesh bag had a string (60cm) attached, the end of which was left outside of the rumen for easy removal. Bags were placed in the ventral rumen sequentially and then all bags were removed at the same time at the end of the incubation to achieve the appropriate incubation times for the particular experiment. Upon removal, individual polyester bags were immediately flash frozen in liquid N and stored at -80°C until they were processed.

Processing of In situ residues

For each polyester bag, a clean plastic funnel was placed in a 500 mL medicine bottle and the frozen polyester bag was cut into 4-5 pieces above the funnel. Approximately 350 mL of 1M HCl was used to rinse all residues including the bag into the medicine bottle. The exact amount of HCl added was monitored and recorded with a

countertop digital scale. The bottles were then capped and placed in a 100ºC water bath

for 25 minutes in order to dissolve Optigen®II granules and urea into the solution. After

incubation, bottles were vigorously agitated and a 10 mL sample of the liquid portion was

collected and frozen (-20ºC) until urea analysis. Urea was analyzed by colorimetric assay

using a Technicon AAII Autoanalyzer (Marsh et al., 1965).

Calculations

In order to characterize the urea disappearance from Optigen®II, it was necessary

to determine the initial amount of urea in the polyester bag and the final amount of urea

in the residue after ruminal incubation. In order to estimate initial urea content of

Optigen®II samples, the percentage urea in the Optigen®II granules had to be calculated.

The 0 h Optigen®II samples were used for this purpose. Urea recovered per mass of

Optigen®II in each replicate of the assay provided an estimate of the urea content of each

Optigen®II sample. The initial urea in each polyester bag was estimated by multiplying

the weight of the Optigen®II sample by the percentage urea in that sample. The final

amount of urea in the *in situ* residues was determined by multiplying the concentration of

the resulting solution by the volume of the solution (mmol/L x L = mmol urea). Percent

urea disappearance was then calculated by expressing the weight (g) of urea remaining in

the residue as a percentage of initial urea and subtracting from 100 to get disappearance

(Equation 3.1)

$$\% \ Urea \ disappearance = 100 - (\frac{initial \ urea, g - residual \ urea, g}{initial \ urea, g}) \ x \ 100 \qquad (3.1.)$$

The data were then plotted as percentage urea disappearance over time and fit to a one phase association model adapted from Ørskov and McDonald (1979) (Equation 3.2) using GraphPad Prizm Software (GraphPad Software, San Diego, CA).

$$p = b \, (1 - e^{-ct}) \tag{3.2}$$

Where:

p= urea disappearance (%),

b= urea fraction available over time

c= fractional rate of disappearance of fraction

Statistical analysis

For Experiments 1 and 3 data were analyzed with proc mixed of SAS (SAS Inst. Inc., Cary, NC) as a split plot in time with steer as the main plot and time (h) as the subplot. The following model was used for dependant variables:

$$Y_{ijk} = \mu + C_i + R_j + A_k + T_l + AT_{kl} + E_{ijkl}$$

where:

μ = Overall mean

Y_{ijk} = Observation

C_i = Fixed effect of period

R_j = Random effect of animal

A_k = Fixed effect of basal diet

T_l = Fixed effect of time

AT_{kl} = Treatment by time interaction

E_{ijkl} = Residual Error

In addition, where relevant, rate and extent parameters from model fitting were analyzed as a completely randomized design using proc mixed of SAS. Differences among treatments were considered to be significant when $P < 0.05$, whereas when $P > 0.05$ but < 0.10 differences were considered to indicate a trend.

RESULTS

Experiment 1

As expected, ruminal urea disappearance was complete by the first sampling time (1h, Figure 3.1). This rapid disappearance is reflected in the variables in Ørskovs' equation representing rate (*c*) and extent (*b*) of degradation (Table 3.1, Figure 3.2). Optigen®II batch #280752-2 had the highest extent of urea disappearance of the Optigen®II samples and a rate similar to #311622-2. However #311622-2 had a lower extent of disappearance than #280752-2. Optigen®II batch #299702 had a higher rate but a lower extent of urea disappearance than the other two Optigen®II products tested.

Experiment 2

Optigen®II batches: 280752-2, 321245-3 and 400569-4 had similar extent of degradation with 280752-2 having the highest and 400569-4 having the lowest rate of degradation respectively (Table 3.2). Disappearance curves generated by Ørskovs' one

phase association model are presented in Figure 3.3. Optigen®II products 311622-2 and especially 299702 had lower extent of degradation than the other three products.

Experiment 3

Urea disappearance from both feed grade urea and Optigen®II was affected by basal diet. For feed grade urea, the extent of disappearance had already reached a maximum (~99%) by ten minutes of incubation in the rumen. However, there was a significant difference between forage and concentrate diets on the percentage urea disappearance at 5 minutes of ruminal incubation. Forage diets had a higher percentage of urea degraded (95.3 vs. 77.5%, $P < 0.05$, Figure 4) at 5 minutes. For Optigen®II, basal diet had an effect on ruminal urea disappearance with average disappearance being higher for forage than for concentrate diets (65.8 vs. 58.6%, $P < 0.0001$, Table 3). All time points for Optigen®II from 2 to 24 h had significantly higher urea disappearance for forage vs. concentrate diets ($P = 0.0005$, Figure 5). After fitting the data to the Ørskov model, it was not possible to detect the difference between forage and concentrate diets on the rate or extent of urea disappearance from feed grade urea samples or on the rate of Optigen®II degradation. However, there was a tendency for the extent of urea disappearance from Optigen®II to be higher for forage vs. concentrate diets (86.6 vs. 78.2, $P = 0.07$, Table 4).

DISCUSSION

Experiment 1

Urea degradation was rapid and completed by 1 h of incubation in the rumen. However, due to the fact that no other data points between 0 and 1 h were sampled, the

estimate of rate of digestion is probably not accurate. The fractional rate of degradation estimate of 525.1 h^{-1} implies that 52,510 % of the urea is degraded each hour, or that 100% of urea is degraded within 6.9 s. We feel that this is probably an overestimate of the degradation rate, as Experiment 3 revealed a much slower degradation rate. However, the theoretical ability of rumen fluid to degrade urea to ammonia (urease activity) is immense. Cook (1976) reported that in animals fed a pelleted barley diet, maximal urease activity was 0.69 g urea / 100 mL rumen fluid / h. Therefore, if you assume a steer has an 80 L rumen liquid volume (Moloney et al., 1993), capacity to convert urea to ammonia is approximately 55.2 kg / h, or 15 g / s. Optigen®II #280752-2 had the most favorable degradation pattern in the rumen under the conditions that were tested. It demonstrated delayed release characteristics and reached a plateau at approximately 80% degradation at around 20 h of ruminal incubation, indicating that 20% of the urea was still not available by 20 h. From this study, it appears that the Optigen®II batches #299702 and #311622-2 may be overprotected, or that a substantial fraction of the urea remains unavailable after 24 h of incubation, under these conditions. These batches were not used in any of the research in the following chapters. More experiments needs to be conducted under a wider variety of dietary conditions in order to determine the validity of these results.

Experiment 2

In terms of extent of urea disappearance from Optigen®II products, batches #280752-2, #321245-3 and #400569-4 are relatively similar, reaching a plateau of around 80%. Therefore, a maximum of 80% of the urea present in these samples is available for ruminal metabolism. The remaining 20% would not be available for ruminal metabolism,

unless it was digested post ruminally, and recycled back to the rumen. Optigen®II batch #311622-2 and especially batch # 299702 have a significant proportion of urea that is not available in the rumen. With only 60-80% of urea available in the rumen with varying products, care should be taken when interpreting experimental responses between Optigen®II and isonitrogenous amounts of urea, as ruminally available urea will differ. When comparing rate of ruminal degradation, all Optigen®II sources demonstrated slow release of urea in the rumen. Of the three Optigen®II sources with approximately 80% extent of ruminal degradation, #400569-4 had the slowest and #280752-2 had the fastest degradation rate. When comparing the rates and extents of degradation of Optigen®II products used in both Experiments 1 and 2 (Table 3.2), it appears as if the rate of degradation of all 3 products tested in both experiments was higher in Experiment 2 than Experiment 1. It was hypothesized that this may be due to the nature of the diets fed to the animals in each of these experiments with animals in Experiment 1 being on a relatively high concentrate diet (70% concentrate) and animals on experiment to being on an all forage diet (100% alfalfa cubes).

Experiment 3

The 100% forage diets led to a higher urea disappearance rate and extent from Optigen®II samples, as well as a higher rate of disappearance from feed grade urea samples. Urea is rapidly degraded in the rumen and the percentage disappearance had reached a maximum by 10 minutes of ruminal incubation. Therefore a difference in the extent of degradation was not expected. However, differences in ruminal urea disappearance at 5 minutes for feed grade urea and for all time points from 2 to 24 h for Optigen®II samples indicates a marked effect of basal diet on urea and Optigen®II

degradation in the rumen. It is possible that the microbial populations that were prevalent in high forage diets may have higher urease production than populations prevalent in the high concentrate diets. Cook (1976) fed diets varying in concentrate level and reported numerically higher ruminal urease activity in sheep fed hay and grass cubes compared to those fed hay and grass cubes plus concentrates. Additionally, it is known that pH affects urease activity, with urease activity at its highest at a pH of between 6.8 and 8.5, and that urease activity is completely eliminated at a pH below 3 (Mahadevan et al., 1977; Muck, 1982). High concentrate diets may have lead to a depression in rumen pH and depressed ruminal pH may have resulted in a depression of urease activity in the rumen and the subsequent depression of degradation of urea from both Optigen®II and urea. Additionally, pH is known to affect ruminal lypolytic activity, with rumen lypolysis being significantly depressed at pH below 6 (Van Nevel and Demeyer, 1996). Enzymatic degradation of the lipid coating of Optigen®II granules is the proposed mechanism by which urea is released. Therefore, reduction in lypolysis in the rumen of animals fed a high concentrate diet may have lead to slower release of urea from Optigen®II granules. Alternatively, passage rate, or anything that might affect urea solubility in the rumen, such as digesta viscosity, content of dissolved compounds and pH may have resulted in differences between the basal diets, however, these variable were not measured.

CONCLUSIONS

There is a relatively wide range in both rate and extent of ruminal disappearance of urea from Optigen®II from different batches. It is important, when interpreting results from experiments with Optigen®II, that rate and extent of degradation of the product under evaluation are considered. It may also be important to consider that basal diet also

has an effect on the rate and extent of degradation of Optigen®II when interpreting results. The effect of basal diet on Optigen®II urea disappearance may have some practical application for utilizing Optigen®II products of varying degradability. It is possible that Optigen®II with higher tested degradation rates can be recommended for higher concentrate diets and that less degradable batches may be indicated for forage dominated diets. It is well know that urea is more toxic when fed to animals consuming high forage diets than high concentrate diets. Decreased toxicity is generally assumed to be due to the ability of the microbial population utilizing concentrates to assimilate available ammonia, and because lower rumen pH reduces the ruminal epithelial permeability to ammonia. However, it appears that lowered ruminal degradation rate may play a part in the reduced toxicity of urea in high concentrate diets.

Table 3.1. Ingredient composition of the 70% concentrate diet, Experiment 3

Ingredient	% of DM
Fescue hay	19.0
Shelled corn	55.9
Cottonseed hulls	11.0
Soybean meal blend[1]	14.0
Urea	0.1
Totals	**100.0**

1 Soybean meal plus vitamin and mineral premix

Table 3.2. Rate (c) and maximum extent (b) of digestion of urea and Optigen®II samples in the rumen according to the model, $p = a + b(1-e^{-ct})$, Experiment 1 and 2

Variable	Urea	Optigen®II Product				
		280752-2	299702	311622-2	321245-3	400569-4
Experiment 1						
b (%)	100.5	80.8	55.6	57.9	*	*
c (rate, %/h)	525.1	13.3	20.4	13.0	*	*
Experiment 2						
b (%)	*	78.55	62.36	72.7	79.99	77.68
c (rate, %/h)	*	31.92	29.74	17.73	22.98	18.08

* Not measured

Table 3.3. Effects of basal diet on the disappearance of urea from feed grade urea and Optigen®II in the rumen, Experiment 3

Urea disappearance, %	LSM for Diet			P-value		
	Forage	Concentrate	SEM	Diet	Time	Diet*Time
Urea	98.5	96.2	0.9	0.224	<0.0001	<0.0001
Optigen®II	65.8	58.6	2.5	<0.0001	<0.0001	0.0005

Table 3.4. Effects of basal diet on rate (c) and maximum extent (b) of disappearance of urea from urea and Optigen®II samples in the rumen according to the model, $p = a + b (1-e^{-ct})$, Experiment 3

Item	Forage	Concentrate	SEM	P-value
Maximum extent, % (b)				
Feed grade urea	99	99.5	0.8	0.66
Optigen®II	86.58	78.15	2.3	0.07
Rate,h^{-1} (c)				
Feed grade urea	44.1	22.4	9.9	0.26
Optigen®II	0.42	0.38	0.05	0.46

Figure 3.1. Ruminal urea disappearance from polyester bags over time of urea and three different Optigen®II products, Experiment 1. Each data point represents the average disappearance from triplicate polyester bags for each animal at each time point
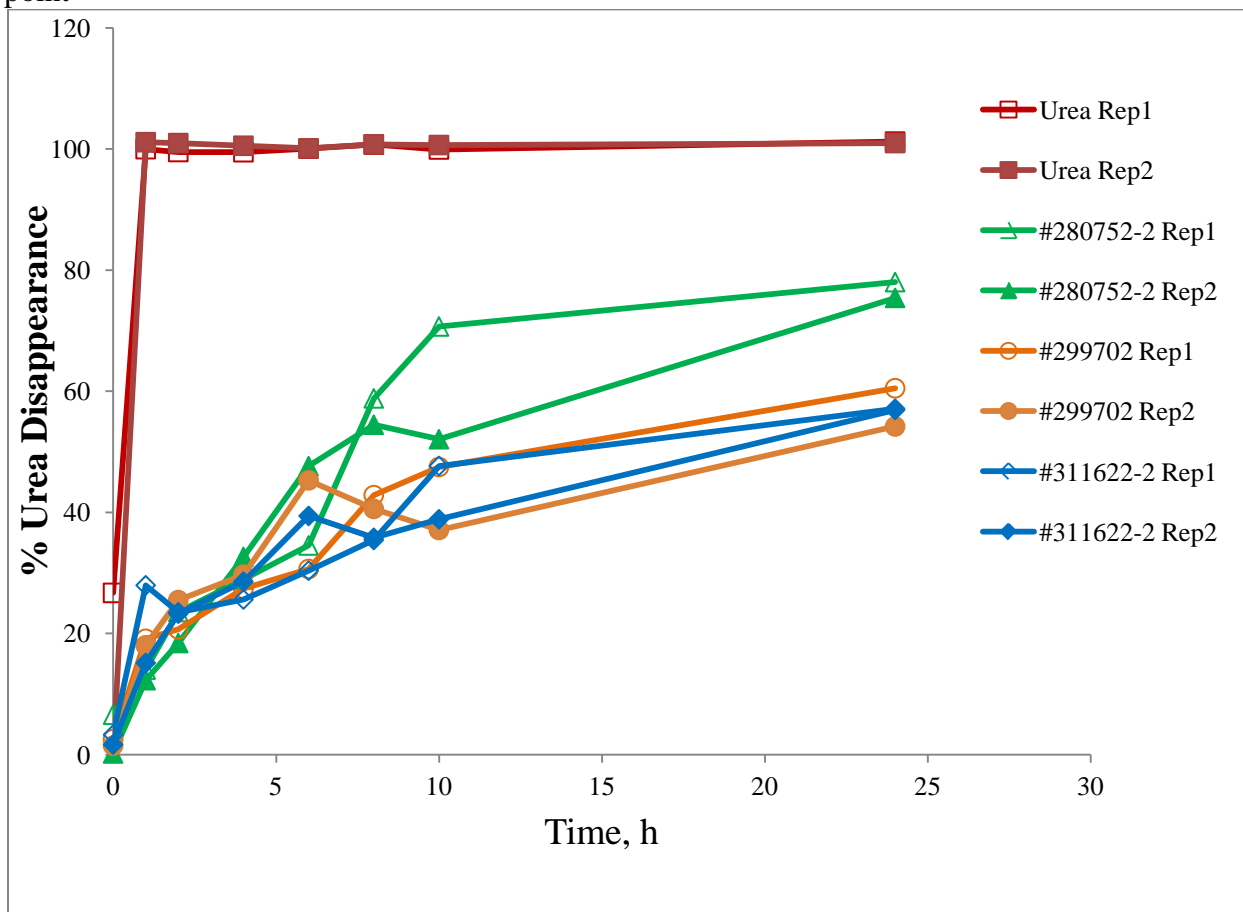
Figure 3.2. Ruminal urea disappearance from polyester bags over time of urea and three different Optigen®II products (model generated), Experiment 1
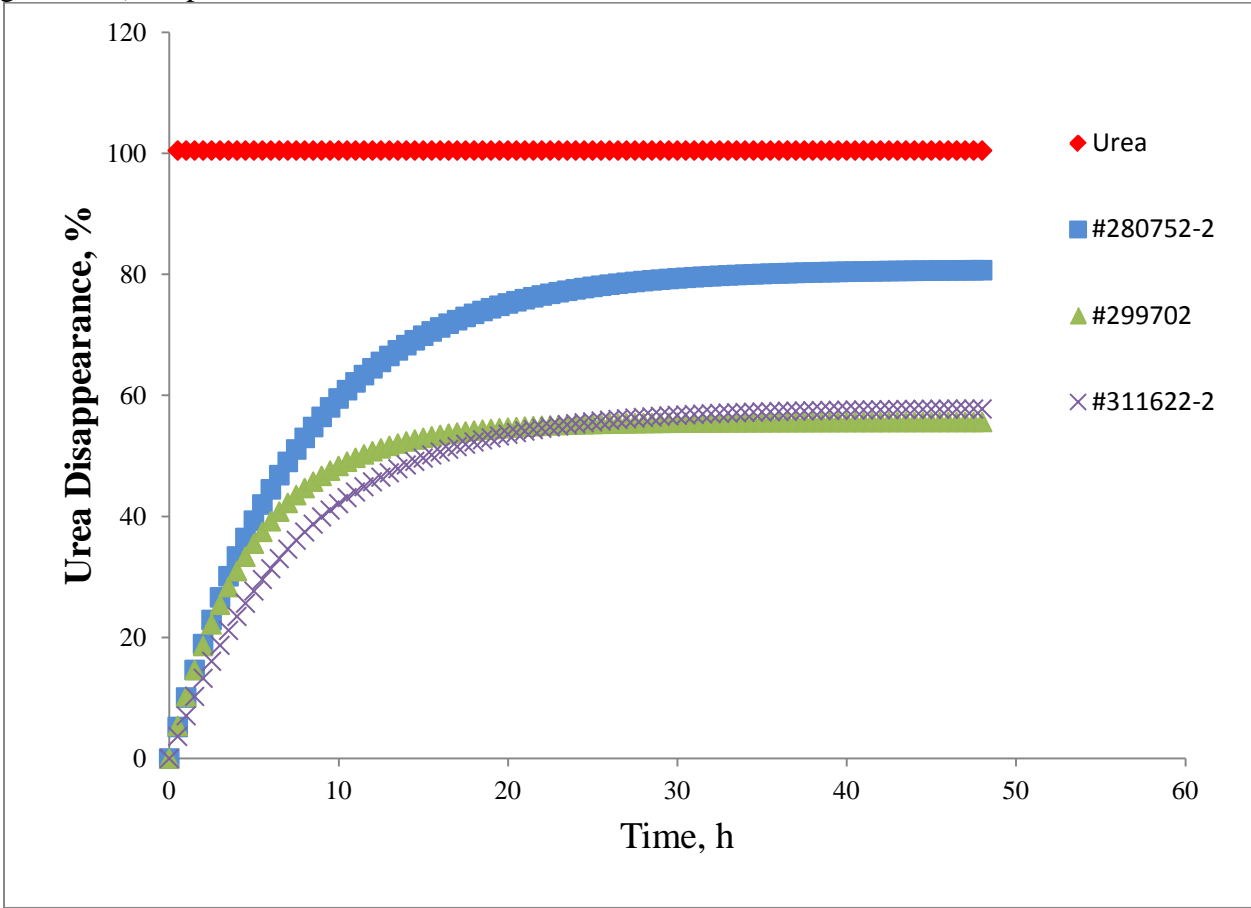
Figure 3.3. Ruminal urea disappearance from polyester bags over time of 5 Optigen®II product batches (model generated), Experiment 2
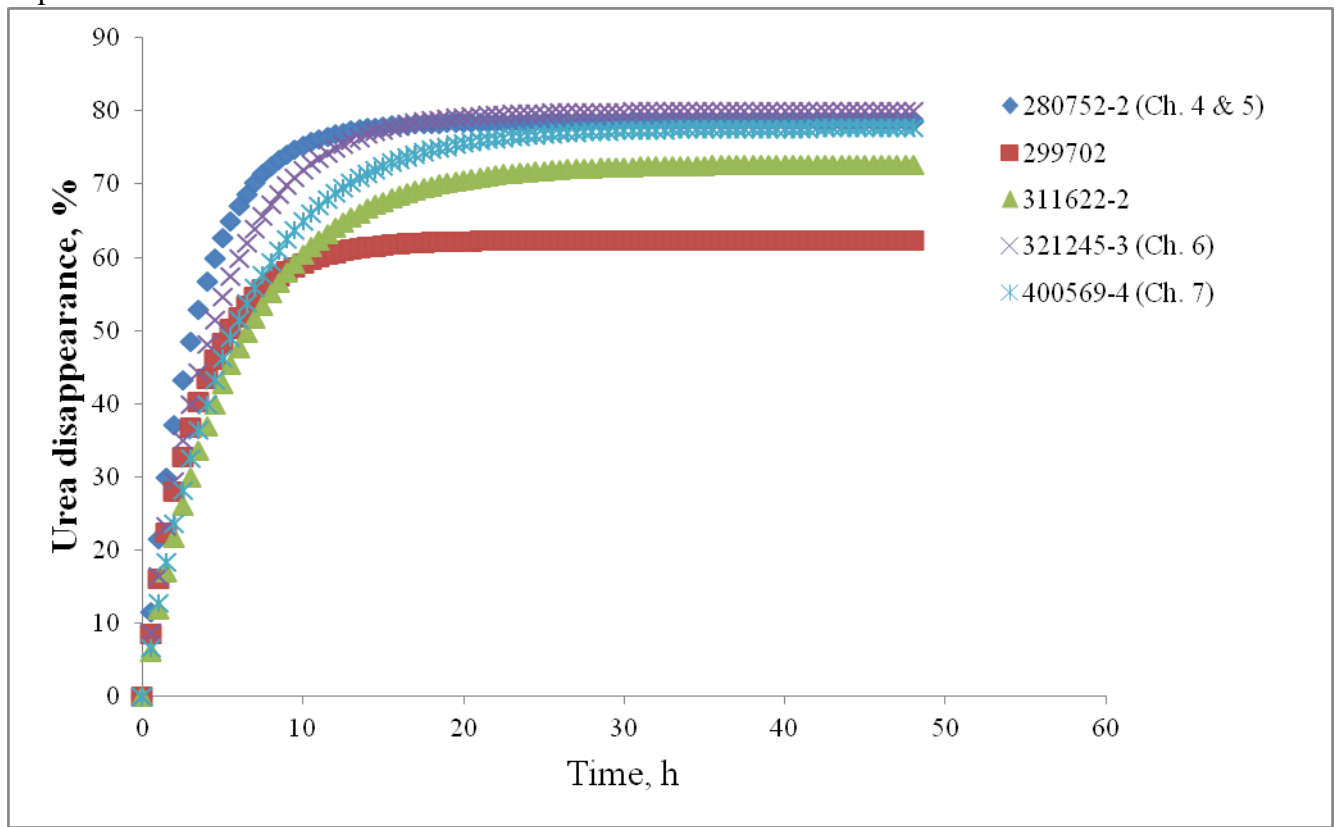
Figure 3.4. Ruminal urea disappearance of feed grade urea in animals fed 100% forage and 70% concentrate diets, Experiment 3
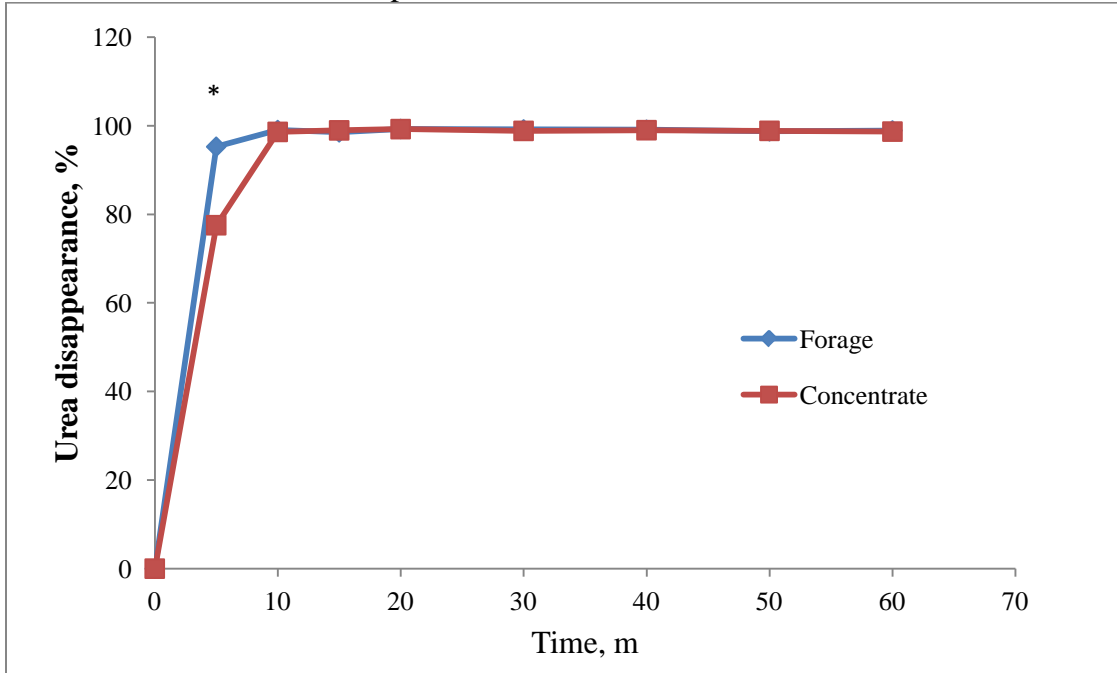
* Treatments differ at indicated time point (P < 0.05)

Figure 3.5. Ruminal urea disappearance from Optigen®II (#311622-2) in animals fed 100% forage and 70% concentrate diets, Experiment 3
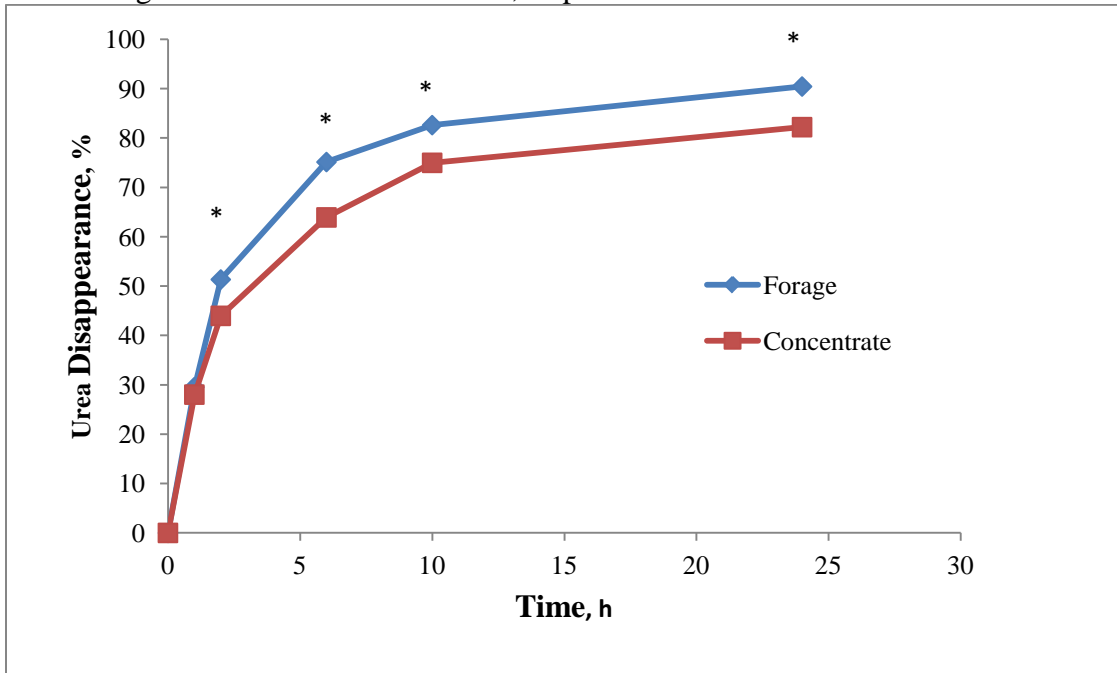
* Treatments differ at indicated time point (P < 0.05)

CHAPTER 4. THE EFFECTS OF CRUDE PROTEIN CONCENTRATION AND NON PROTEIN NITROGEN SOURCE ON NITROGEN METABOLISM IN HOLSTEIN STEERS

INTRODUCTION

Urea is a highly concentrated source of CP that is commonly used to provide degradable intake protein (DIP) to ruminants. Urea is rapidly broken down to ammonia in the rumen by the action of bacterial urease (Satter and Slyter, 1974). Ammonia is used by rumen microbes to produce microbial proteins and is required by many rumen bacteria including cellulose degraders (Hungate, 1966). The supply of DIP to the rumen microbes is therefore important to ruminal degradation of feed and a deficiency in DIP or ammonia in the rumen has been shown to depress diet digestibility (Griswold et al., 2003). Urea is an inexpensive way of providing DIP to the ruminant. However, regular feed grade urea degrades rapidly to ammonia in the rumen and may result in accumulation of ammonia (Pisulewski et al., 1981). Excess rumen ammonia is absorbed into the blood where it is normally detoxified to urea in the liver. A portion of this urea may be recycled back to the rumen while the remainder is excreted in the urine (Lapierre and Lobley, 2001). Therefore increasing the urea content of the diet inevitably leads to increased urinary excretion of urea and consequent increased environmental N pollution and a reduction in efficiency of N use (Marini and Van Amburgh, 2005). Limiting the degradable N supply to the rumen will reduce urinary N excretion, but may result in a deficiency in DIP in the rumen, potentially causing a depression in diet digestibility. Controlling the release rate of urea may allow ruminal ammonia concentration to be maintained at a level adequate for optimal rumen microbial activity and to maintain diet digestibility, while at

the same time, avoiding abrupt increases rumen ammonia concentration associated with excessive excretion of urinary urea. Therefore, the objective of this study was to determine the effect of replacing feed grade urea with slow release urea on N retention and excretion under conditions of limiting and adequate CP intake.

MATERIALS AND METHODS

Experimental design

The experiment was conducted using eight growing Holstein steers with an average initial body weight of 265 ± 18 kg to evaluate N balance, N excretion and rumen and blood variables. The experimental design was a replicated 4×4 Latin square with a 2×2 factorial treatment structure. Treatment factors were dietary CP concentration and the non protein N source (NPN) used. Dietary CP concentration was either 10.9 or 12.1 % CP. The NPN source was either slow release urea (OPTIGEN®II, batch #280752-2) or regular feed grade urea (UREA). The hypothesis was that when CP is limiting (10.9 % CP), N balance and diet digestibility will be depressed to a greater extent for UREA than OPTIGEN®II, and when CP is adequate (12.1 % CP), OPTIGEN®II will reduce urinary N excretion and thus improve N retention and efficiency when compared to UREA. All diets were formulated to be isoenergetic and isonitrogenous within each CP concentration (Table 4.1). Additionally, all diets were formulated to contain equivalent concentrations of NPN (20.5%) as a percentage of total dietary CP. The experiment consisted of four 21-day periods each consisting of 13 days adaptation followed by 7 days of N balance and 1 day of blood and rumen sampling. Diets were limit fed to equalize intake and offered twice daily (0800 and 1700) throughout the.

Nitrogen Balance

At the beginning of each N balance period, steers were moved from holding pens (2.4 x 2.4 m) into individual metabolism tie stalls (1.2 x 2.4 m), each with its own feed bunk and water supply. During the N balance period, total fecal and urine output were quantified before each morning feeding. Feces were weighed, sub-sampled, and immediately frozen (-20°C). Total urine output was continuously collected and kept separate from feces by fitting each steer with a rubber urine funnel under continuous vacuum (Huntington, 1989). Urine collection vessels contained sufficient H_3PO_4 to ensure a final pH of 3.0 or less. A daily sub-sample of the acidified urine was frozen (-20°C). Daily N balance was calculated as the difference between daily N intake and daily excretion of N in urine and feces. After sampling on d 7, each steer was returned to its original holding pen and the evening feeding took place as usual.

Blood and Rumen Sampling

On the following morning (d 8) blood and rumen sampling was initiated. Each animal was fitted with an indwelling jugular catheter (BD Angiocath, Product number: 382259) for blood samples and dosed intraruminally with approximately 500 mg of Cr-EDTA (Binnerts et al., 1968) to determine rumen liquid volume and outflow rate (Udén et al., 1980). Blood and rumen fluid samples were then collected from each animal at 0 h (before feeding, just after Cr-EDTA dosing), and 1, 2, 4, 6, 8 and 10 h after feeding. Blood samples were collected via the jugular catheter into heparinized syringes (35 mL). Plasma was harvested by centrifugation at 3,000 x g for 20 min at 4°C and stored at -20°C for subsequent analysis of plasma urea and glucose. Rumen fluid samples (100 mL) were taken from the ventral rumen using a suction strainer (Raun and Burroughs, 1962)

adapted for use in cattle (50 cm length, 19 mm diameter, 1.6 mm mesh). Rumen fluid (25 mL) was preserved with 5 mL of 25 % (w/v) metaphosphoric acid (Erwin et al., 1961) for analysis of VFA. An additional 50 mL of rumen fluid was collected for chromium analysis. All rumen fluid samples were immediately frozen at -20ºC until analysis.

Laboratory analyses

Feed and orts samples were dried in a forced-air oven at 100°C for 24 h to determine dry matter composition. Organic matter was determined according to the methods of the AOAC (Method 942.05). Samples of feed, feces, orts, and urine were analyzed for total N by combustion using a Vario Max CN elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Additionally, urine composites were used to determine output of urinary purine derivatives by HPLC according to the methods of Shingfield and Offer (1999). Ammonia concentrations in rumen fluid were determined by the glutamate dehydrogenase procedure (Kun and Kearney, 1974) adapted to a Konelab 20XTi clinical analyzer (Thermo Fisher Scientific Inc., Beverly, MA). Plasma urea concentrations were determined by colorimetric assay using a Technicon AAII Autoanalyzer (Marsh et al., 1965).

Rumen fluid samples were thawed and centrifuged at 39,000 x g for 20 min and the pellet discarded. The concentration of chromium in rumen fluid was determined by atomic absorption analysis on a PerkinElmer AAnalyst 200 atomic absorption spectrometer (PerkinElmer, Waltham, MA). VFA concentrations in rumen fluid were determined by gas-liquid chromatography (Ottenstein and Bartley, 1971) on an HP6890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) with a Supelco 25326 Nukol

fused silica capillary column, 15 m x 0.53 mm x 0.5 µm film thickness (Sigma/Supelco, Bellefonte, PA).

Calculations and Statistical analysis

Daily N retention was calculated as the difference between N intake and N excretion (feces plus urine). Liquid passage rate, rumen liquid volume and liquid flow rate were calculated from ruminal chromium concentration according to Udén et al. (1980).

Data were analyzed as a 4 x 4 replicated Latin square design using PROC MIXED of SAS. Plasma urea and ruminal ammonia and VFA data were analyzed as a split plot in time with steer as the main plot and time (h) as the subplot. The following model was used for dependant variables:

$$Y_{ijkl} = \mu + C_i + R_j + A_k + B_l + AB_{kl} + T_m + AT_{km} + BT_{lm} + ABT_{klm} + E_{ijklm}$$

where:

μ = Overall mean

Y_{ijkl} = Observation

C_i = Fixed effect of period

R_j = Random effect of animal

A_k = Fixed effect of CP level

B_l = Fixed Effect of NPN source

AB_{kl} = Effect of DIP level x NPN source interaction

T_m = Fixed effect of time

AT_{km} = CP level by time interaction (Split plot only)

BT_{lm} = NPN source by time interaction (Split plot only)

ABT_{klm} = Interaction between CP level, NPN source and time (Split plot only)

E_{ijklm} = Residual Error

Differences among treatments were considered to be significant when $P < 0.05$, whereas when $P > 0.05$ but < 0.10 differences were considered to indicate a trend.

RESULTS

Dry matter intake was similar for all treatments (Table 4.2). Dry matter (71.3 vs. 69.4 %, $P = 0.024$) and OM (72.4 vs. 70.3 %, $P = 0.018$) digestibility were higher for the 12.1 % CP diets. Source of NPN did not affect DM or OM digestibility. As designed, N intake was higher for the 12.1 % CP diets (123.8 vs. 109.5 g / d, $P < 0.001$) and tended ($P = 0.07$) to be higher for UREA than OPTIGEN®II diets. Fecal N output was similar in all treatments. Urinary N excretion and N retention were higher in the 12.1 % CP than the 10.9 % CP diets (Urinary N: 44.2 vs. 37.8 g / d, $P = 0.03$; N retention: 35.8 vs. 26.1 g / d, $P = 0.02$). When expressed as a percentage of N intake, 12.1 % CP diets had a higher percent of intake N excreted in the urine (36.5 vs. 30.7 %, $P = 0.04$). Source of NPN had no effect on urinary N excretion or N retention. Purine derivatives, expressed as the sum of allantoin and uric acid, were unaffected by treatment as was allantoin and

creatinine excretion. Uric acid excretion was higher for 10.9 % CP than for 12.1 % CP diets (2.99 vs. 1.89 mmol/kg DMI, P = 0.001).

There was no effect of dietary CP concentration on liquid passage rate, rumen liquid volume or liquid flow rate (Table 4.3). Liquid passage rate was higher for OPTIGEN®II than UREA diets (8.59 vs. 7.27 % / h, P = 0.006). Similarly, liquid flow rate was higher for OPTIGEN®II than UREA diets (2.99 vs. 2.69 L/h, P < 0.05). There was an interaction between dietary CP concentration and NPN source on rumen liquid volume (P = 0.004). Ruminal liquid volume was higher for UREA at 12.1 % CP and similar between OPTIGEN®II and UREA at 10.9 % CP. Plasma urea concentration was higher for UREA than OPTIGEN®II in both 12.1 % CP (4.9 vs. 4.2 mM, P < 0.001) and 10.9% CP (4.7 vs. 4.3 mM, P < 0.001) diets. However, there was an interaction between dietary CP concentration and NPN source on plasma urea concentration. UREA had a greater plasma urea concentration than OPTIGEN®II at 10.9 % but was similar at 12.1 % CP. The average ruminal ammonia concentration was higher for 12.1 % CP diets than 10.9 % CP diets (9.5 vs. 6.7 mM, P < 0.001) and higher for UREA than OPTIGEN®II diets (8.6 vs. 7.5 mM, P < 0.001). Ruminal ammonia for all treatments peaked at 1 h post feeding (Figure 4.1). There was an interaction (P = 0.001) between dietary CP concentration and NPN source on ruminal total VFA concentration. UREA had a higher VFA concentration at 12.1 % CP while OPTIGEN®II had a higher VFA concentration at 10.9 % CP. Proportion (mol/100 mol) of acetate was similar between OPTIGEN®II and UREA at 12.1% CP but was higher for OPTIGEN®II at 10.9% CP (P = 0.03). There was an interaction (P = 0.006) between dietary CP concentration and NPN source on the proportion of propionate. The proportion of propionate was higher for UREA than

OPTIGEN®II at 12.1 % CP but did not differ at 10.9 % CP. There was an interaction between dietary CP concentration and NPN source on the proportion of butyrate (P < 0.0001) and isobutyrate (P = 0.05). The proportion of butyrate and isobutyrate were higher for OPTIGEN®II at 12.1 % CP and higher for UREA at 10.9 % CP. There were no differences between treatments in the proportion of valerate. There was a tendency for an interaction (P = 0.08) between CP concentration and NPN source on the proportion of isovalerate where the proportion of isovalerate was greater for OPTIGEN®II at 12.1 % CP and greater for UREA at 10.9 % CP. Additionally, OPTIGEN®II had a higher proportion of isovalerate tat UREA (P = 0.003). The ratio of acetate to propionate (A:P) was higher for OPTIGEN®II than for UREA (4.25 vs. 4.10, P = 0.003). Additionally, there was a tendency for an interaction (P = 0.08) between dietary CP concentration and NPN source on A:P with the difference between OPTIGEN®II and UREA being greater at 12.1 % CP than at 10.9 % CP.

DISCUSSION

The goal of the slow release of N from Optigen®II when compared to urea is to improve the capture of N in the rumen by more closely timing the availability of N and fermentable carbohydrate for microbial use. This may happen by two conceivable modes of action. When ruminal N is limiting, it may prolong the time period at which ruminal ammonia concentration is adequate to support bacterial growth, resulting in increased capture of N from Optigen®II. Alternatively, when ruminal N is adequate, slowing the release of urea from Optigen®II may reduce peak ruminal ammonia concentration, resulting in less ruminal absorption of ammonia. Therefore, to evaluate urea sources, it is important to investigate under conditions of both limiting and adequate N intake.

By design, DMI was not different between treatments. Reduced diet digestibility in the 10.9 % CP diets could be related to N deficiency in the rumen (Marini and Van Amburgh, 2005). However, average ruminal ammonia concentration (10.9 % CP: 6.7 mM) indicates that ruminal ammonia availability may not have been limiting in these diets (Satter and Slyter, 1974). Köster et al. (1996) showed that ruminal OM degradation was maximal at rumen ammonia concentrations of 1.36 mM in beef cows fed a low quality forage diet supplemented with increasing levels of DIP from sodium caseinate.

Increased digestibility of 12.1 % CP diets could have been related to the inclusion of SBM at the expense of cracked corn in these diets. The inclusion of SBM in the high CP diets may have provided stimulatory growth factors such as peptides, nucleotides, branched chain fatty acids and other unidentified growth factors to the rumen microbial population, resulting in increased diet digestibility (Dehority, 2003; Van Soest, 1994). Also, it is unclear how these changes in total tract digestibility were partitioned within the gastrointestinal tract, although the SBM effect described above would be expected to exert its effect ruminally.

The 12.1 % CP diets increased urinary N excretion and also increased N retention. Other authors have also reported increased N retention and urinary N excretion with increasing N intake (Archibeque et al., 2002; Wickersham et al., 2008a). Marini and Van Amburgh (2005) reported that progressively increasing dietary CP from 8 to 22% CP, utilizing a protein supplement containing N from SBM and urea in a 5.25:1 ratio compared with up to 4:1 in the current study, resulted in a curvilinear increase in N retention with a maximum at approximately 19% CP using Holstein replacement heifers. However, the same authors found an exponential increase in plasma urea N which has

been shown to be directly related to urinary urea excretion (Gonda and Lindberg, 1994). In the current experiment, N retention was somewhat higher (10.9 % CP: 26.1 vs. 23.0 g/d; 12.1 % CP: 35.8 vs. 25.5 g/d) than predicted by the equations of Marini and Van Amburgh (2005) and plasma urea values for both concentrations of CP were greater than those reported by Marini and Van Amburgh (2005) at similar levels of CP (10.9 % CP: 4.2 vs. 1.1 mM; 12.1 % CP: 4.9 vs. 1.5 mM). Differences in N retention and plasma urea values between these experiments may be related to the basal diet used in each. The apparent accumulation of ammonia in the rumens of steers fed the diets of the current study indicates that availability of ruminal N may not have limited microbial protein production. Therefore, the increase in N retention for 12.1 % CP diets may be explained by the increase in nutrient availability due to increased diet digestibility, potentially driven by SBM inclusion, resulting in increased energy and protein available for protein deposition at the tissue level (Greathouse et al., 1974; Ludden et al., 2002; Vasconcelos et al., 2009). It is thought that an increase in diet digestibility may lead to a reduction in fecal N output by increasing the digestibility of N in the feed and by limiting fermentable substrate to the hind gut (Bierman et al., 1999). The increase in diet digestibility in the current study did not significantly reduce total fecal N excretion in the current study, even though the percentage of N excreted in the feces was numerically lower in the 12.1% CP diets.

Utilizing urinary purine derivative excretion to estimate microbial protein production is based on the assumption that the nucleic acids digested in the small intestine are entirely microbial in origin. Dietary purines are considered to form a negligible part of intestinally absorbed nucleic acids as most ruminant feeds contain

relatively low concentrations of nucleic acids, most of which are degraded by the microbes in the rumen (Chen and Ørskov, 2004). Microbial purines are completely converted to uric acid during absorption through the intestinal mucosa and a variable amount of uric acid is converted to allantoin in the liver (Chen and Ørskov, 2004). Purine derivatives excreted in the urine originate from microbial purines absorbed in the small intestine as well as those from the animals tissues as a result of regular tissue turnover (Chen et al., 1992). However, the endogenous contribution to urinary purine derivative excretion has been determined to be negligible compared to the amount of purines from microbial origin in ruminants (Chen et al., 1990d).

Urinary excretion of purine derivatives (Allantoin + uric acid) was unaffected by treatment in the current study. However, the 12.1 % CP diets resulted in lower total excretion of uric acid than the 10.9 % CP diets. It is thought that the sum of uric acid and allantoin (PD) is more important than uric acid or allantoin separately, as a predictor of microbial protein production, due to the variable conversion rate of uric acid to allantoin in the liver. It is also possible that changes in N retention may alter the contribution of endogenous purines to urinary purine derivative excretion. However, large variations in energy and protein supplied intragastrically to sheep had no significant effect on urinary excretion of purine derivatives (Lindberg and Jacobsson, 1990). The average PD excretion (13.5 mmol / kg DMI) is less than the value reported by Valadares et al. (1999) with high producing dairy cattle (21.0 mmol / kg DMI) but similar to the value reported by Devant et al. (2000) with growing beef cattle (14.9 mmol / kg DMI). Production of microbial protein is principally limited by the availability of fermentable energy and available N under practical dietary conditions (Clark et al., 1992). Köster et al. (1996)

indicated that maximum microbial protein production occurred at a higher concentration of ruminal ammonia (6.87 mM) than was required for maximum ruminal OM degradability (1.36 mM). Ruminal ammonia concentration averaged 6.7 mM for 10.9% CP diets in the current study possibly indicating that ruminal N was not limiting to microbial protein production, as reflected by the absence of differences in urinary excretion of purine derivatives. However, the availability of fermentable energy in the rumen under these circumstances is not known. Therefore fermentable energy may have been limiting microbial protein production (Clark et al., 1992), which may explain the lack of differences in purine derivative excretion between levels of dietary CP and between NPN sources. Alternatively, it is possible that recycling of urea to the rumen was able to compensate for the deficit in CP in the 10.9 % CP diets.

It is unclear why OPTIGEN®II diets resulted in increases in liquid passage rate and liquid flow rate and why OPTIGEN®II increased rumen liquid volume at 12.1 % CP and not 10.9 % CP. Dry matter intake, digestibility and passage rate are known to be intimately associated (Bull et al., 1979; Staples et al., 1984). However, diet digestibility and intake were not affected by NPN source in this study. Osmotic activity of dietary additives may also affect liquid flow rate (Potter et al., 1972), with increased liquid flow rate for substances that are more osmotically active. However, the slow release nature of OPTIGEN®II resulted in lower rumen ammonia concentrations and would thus be expected to exert less osmotic pressure than a similar amount of dietary urea. Reticulorumen motility, including frequency (Sissons et al., 1984) and duration (Okine et al., 1989) of contractions, as well as mastication, rumination and associated salivation (Ulyatt et al., 1984) may also affect liquid passage rate, however, we did not measure

ruminal motility or salivation. It is unclear how Optigen®II feeding influences these variables or if it will occur on other diets.

The 12.1 % CP diets resulted in higher rumen ammonia and plasma urea concentrations, possibly due to increased supply of rumen degradable N. It has been demonstrated that increased supply of dietary N and specifically rumen degradable N has a direct effect on rumen ammonia concentration and subsequently on levels of urea in the blood (Armentano et al., 1993; Castillo et al., 2001). OPTIGEN®II diets resulted in lower rumen ammonia and decreased plasma urea concentrations, especially at 10.9% CP, due to its controlled-release properties demonstrating why it is less likely than UREA to cause toxicity at higher dietary inclusion rates. The difference in plasma urea between OPTIGEN®II and UREA was greater at 10.9 % CP. This interaction is reflected numerically by the ruminal ammonia concentrations. Ruminal ammonia may have been in excess of microbial requirements in the current study (Köster et al., 1996; Satter and Slyter, 1974). When ruminal N availability is closer to being limiting (i.e. 10.9% CP), changes in the pattern of degradation of NPN may have a greater impact on the resulting rumen ammonia and plasma urea concentrations, due to a reduction in the available N pool. Volatile fatty acid concentration was higher for UREA than OPTIGEN®II at 12.1 % CP and higher for OPTIGEN®II than UREA at 10.9 % CP. The A:P was higher for OPTIGEN®II at both dietary CP concentrations. Fiber degrading bacteria such as *Ruminococcus albus*, *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens* are known to produce acetate as a major product of fermentation while producing no propionate (Van Soest, 1994). In addition, structural carbohydrate degradation generally takes place more slowly than degradation of many proteins and non structural carbohydrates (Jouany,

54

1991). These bacteria also have an obligate requirement for ammonia (Van Soest, 1994).

It is possible that OPTIGEN®II provided a more favorable supply of ruminally available

N to fiber degrading bacteria, thereby resulting in increased appearance of their

fermentation by products (i.e. acetate). Males and Purser (1970) investigated the effect of

urea supplementation on VFA production in sheep fed a diet of shelled corn, corn cobs

and alfalfa meal and found that urea supplementation resulted in increased acetate and

decreased propionate production resulting in an increase in A:P. The 12.1 % CP UREA

diets may have allowed sufficient N to escape the rumen to the blood and consequently

allow urea recycling to prolong rumen N availability when compared to the 10.9 % CP

UREA treatment, possibly explaining why many of the VFA variables responded to

OPTIGEN®II only when ruminal N was limiting (i.e. 10.9 % CP diets).

CONCLUSIONS

Increasing CP intake resulted in the expected changes in diet digestibility, N

retention and excretion. However, based on ruminal ammonia concentrations, and purine

derivative excretion, ruminally available N may not have been limiting, negating the

potential benefits of reducing the rate of degradation of NPN in the rumen. As a result,

there were no significant effects of NPN source on N retention or excretion. However,

the reduction in rumen ammonia and plasma urea concentrations as well as the changes in

ruminal VFA metabolism for OPTIGEN®II diets indicates that the slow release

properties had some effect on ruminal and systemic urea metabolism. In this experiment,

the effect of NPN source on recycling of urea N back to the rumen, as well as other urea

kinetic variables, is not known. It is possible that reducing the rate of release of urea in

the rumen mimics a lower N diet when compared to urea, resulting in increased urea

recycling. Alternatively, it is possible that urea N recycling to the rumen may negate any advantages that slow release urea might have to offer.   Therefore it is important to characterize the effect that OPTIGEN®II has on urea kinetics in cattle so that we are better able to interpret experimental results.

Table 4.1. Diet ingredients given as % of total DM in steers fed 12.1 % and 10.9 % CP diets with urea or Optigen®II

Feedstuff	12.1 % CP		10.9 % CP	
	Urea	**Optigen®II**	**Urea**	**Optigen®II**
Fescue Hay	45.56	45.52	45.64	45.60
Cracked Corn	45.56	45.52	47.68	47.64
Soybean meal	2.12	2.12	0.00	0.00
Urea	0.88	0.00	0.79	0.00
Optigen®II	0.00	0.97	0.00	0.87
Molasses	4.40	4.39	4.40	4.40
Limestone	0.88	0.88	0.88	0.88
Vitamin premix [a]	0.04	0.04	0.04	0.04
Trace mineral premix [b]	0.56	0.56	0.56	0.56
% CP [c]	12.90	12.61	11.40	11.00

[a] Vitamin premix composition: 8811ppm vitamin A, 1762ppm vitamin D, 1100ppm vitamin E
[b] Trace mineral premix composition: 0.06% Ca, 56.34% Cl, 36.53% Na, 1.2% S, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 ppm I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, 5520.2 ppm Zn
[c] Analyzed CP concentration of experimental diets

Table 4.2. Dry matter intake, diet digestibility, N balance and urinary excretion of purine derivatives in steers fed 12.1% and 10.9 % CP diets with urea or Optigen®II

	Treatment					Effects, P > F		
	12.1 % CP		**10.9 % CP**					**CP x**
Item	**CP Urea**	**Optigen®II**	**CP Urea**	**Optigen®II**	**SEM**	**CP**	**Source**	**Source**
DMI, kg/d	5.39	5.34	5.40	5.41	0.26	0.58	0.79	0.69
DMI, % BW	1.97	1.94	1.97	1.97	0.05	0.34	0.17	0.31
Digestibility, %								
DM	71.33	71.35	68.96	69.87	1.88	0.02	0.56	0.57
OM	72.32	72.39	69.93	70.70	1.83	0.02	0.60	0.66
N Balance, g/d								
N intake	125.6	122.0	111.3	107.7	5.74	<0.001	0.07	0.98
Fecal N	43.7	44.0	47.8	44.3	4.22	0.14	0.29	0.22
Urine N	44.9	43.6	40.0	35.5	4.55	0.03	0.31	0.58
N retention[a]	37.0	34.5	23.5	28.8	5.04	0.02	0.72	0.31
Urinary purine derivatives, mmol/kg DMI								
Purine derivatives [b]	12.47	12.95	14.73	13.96	1.68	0.12	0.89	0.54
Allantoin	10.79	10.85	11.64	11.08	1.47	0.58	0.80	0.75
Creatinine	10.00	10.52	10.44	11.69	1.66	0.45	0.41	0.73
Uric acid	1.68	2.10	3.09	2.88	0.59	0.001	0.72	0.27

[a] N retention = N intake – N output (Fecal N + Urine N); [b] Purine derivatives = Allantoin + Uric acid
[b] Purine derivatives = allantoin + uric acid

Table 4.3. Liquid passage rate, plasma and rumen variables in steers fed 12.1 % and 10.9 % CP diets with urea or Optigen®II

	Treatment					Effects, P > F		
	12.1% CP Urea	**12.1% CP Optigen®II**	**10.9% CP Urea**	**10.9% CP Optigen®II**	**SEM**	**CP**	**Source**	**CP x Source**
Liquid passage rate, %/h	6.74	8.59	7.80	8.60	0.96	0.22	0.006	0.23
Rumen liquid volume, L	40.33	34.12	34.96	36.53	2.21	0.22	0.062	0.004
Liquid flow rate, L/h	2.68	2.84	2.69	3.14	0.35	0.30	0.049	0.31
Plasma Urea, mM[a]	4.97	4.75	4.50	3.83	0.47	< 0.001	<0.001	0.010
Plasma Glucose, mM[a]	4.27	4.32	4.41	4.26	0.12	0.466	0.396	0.093
Rumen Ammonia, mM[a]	9.90	9.04	7.35	6.02	0.54	< 0.001	<0.001	0.33
VFA concentration, mM	62.8	55.7	54.5	59.4	2.2	0.19	0.56	0.001
VFA, mol / 100 mol								
Acetate	70.7	70.6	70.0	71.2	0.9	0.95	0.07	0.03
Propionate	17.6	16.5	17.2	17.2	0.3	0.29	0.002	0.006
Butyrate	10.0	11.1	10.9	9.9	0.8	0.65	1.00	< 0.0001
Isobutyrate	0.44	0.48	0.45	0.42	0.04	0.12	0.96	0.05
Valerate	0.43	0.44	0.45	0.40	0.07	0.69	0.45	0.35
Isovalerate	0.86	0.93	0.86	0.89	0.09	0.36	0.003	0.08
Acetate:Propionate	4.08	4.31	4.12	4.18	0.09	0.36	0.003	0.08

[a]Treatment by time interactions were not significant (P > 0.10).

Figure 4.1. Plasma urea concentration (mM) over time in steers fed 12.1 % and 10.9 % CP diets with urea or Optigen®II
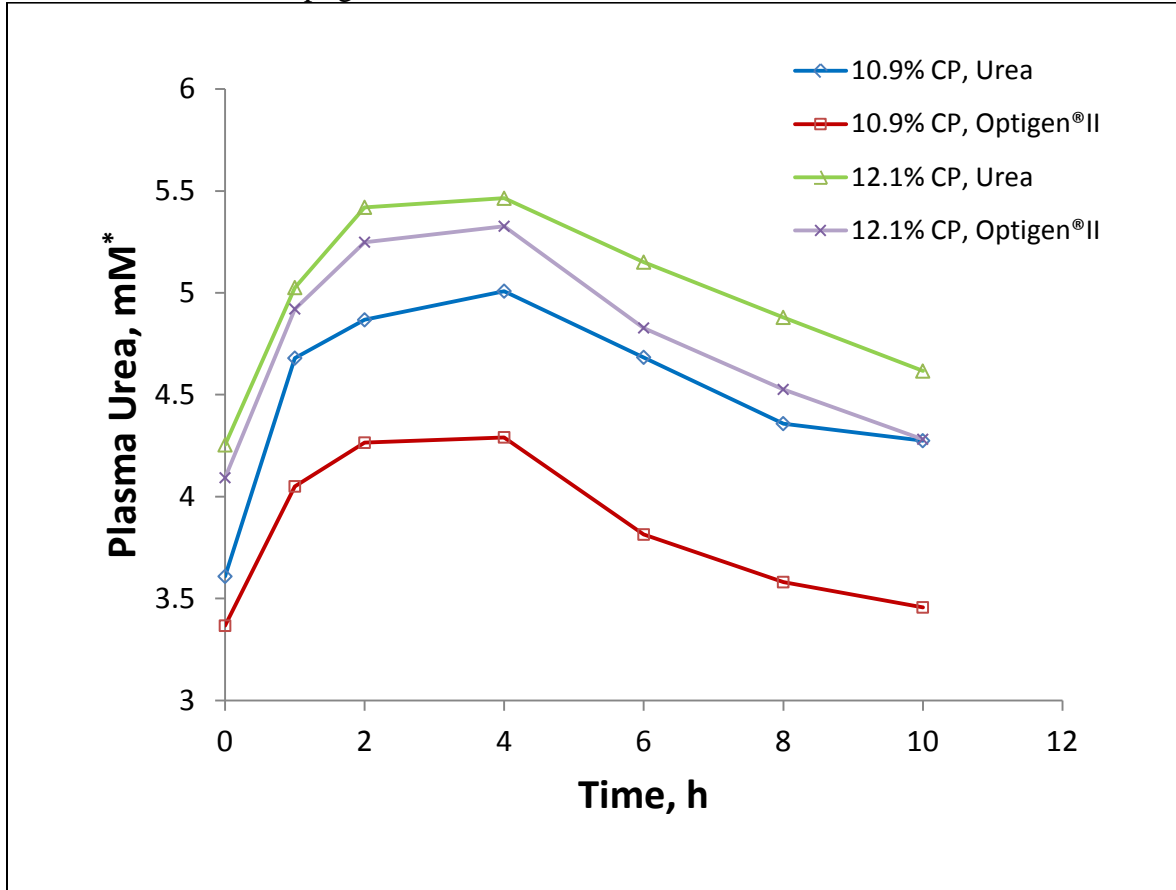
*Pooled standard error = 0.47 mM

CHAPTER 5. THE EFFECTS OF DEGRADABLE NITROGEN LEVEL AND NON PROTEIN NITROGEN SOURCE ON NITROGEN BALANCE AND UREA KINETICS IN HOLSTEIN STEERS

INTRODUCTION

The efficiency of utilization of dietary N by cattle is generally relatively low under normal production conditions (Castillo et al., 2001) with an estimated global average N-efficiency in cattle estimated at 7.7 % (Van der Hoek, 1998). Urea is used rather inefficiently for production of protein products (Broderick et al., 2009) and due to its wide use in ruminant feeds, may be partially responsible for the overall poor N efficiency in cattle. Low efficiency of utilization of dietary urea has been attributed to the rapid hydrolysis of urea to NH_3 in the rumen by microbial urease which occurs at a higher rate than NH_3 utilization by rumen bacteria, leading to ruminal NH_3 accumulation and the subsequent absorption of ammonia and excretion of urea in the urine (Golombeski et al., 2006; Highstreet et al., 2010). In addition, excretion of urea in the urine has been shown to be particularly sensitive to the concentration of protein, particularly rumen degradable protein (Marini and Van Amburgh, 2005) in the diet. Excessive excretion of urinary urea may be related to high ruminal ammonia concentrations, which result in increased absorption of ammonia from the rumen and subsequent conversion to urea in the liver (Symonds et al., 1981) ultimately leading to increased excretion of urea in the urine. Therefore, attempts have been made to produce a form of urea that would degrade more slowly in the rumen, potentially resulting in increased incorporation into microbial proteins and consequently lower excretion in the urine. Therefore the objectives of this experiment were to determine the effects of

dietary DIP level and slow release urea on N balance and ruminal and systemic urea kinetics in Holstein steers.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted using 8 growing Holstein steers with an average initial body weight of 209 ± 15 kg to evaluate N balance and urea kinetics. The experimental design was a replicated 4 × 4 Latin square with a 2 × 2 factorial treatment structure. Treatment factors were NPN source (slow release urea, OPTIGEN®II batch #280752-2; or regular feed grade urea, UREA) and dietary DIP level (100% and 89% of NRC requirements (NRC, 2000). Degradable intake protein was characterized as 13% of TDN according to level 1 of the NRC (2000). Diets were formulated for urea, and OPTIGEN®II diets were subsequently formulated by isonitrogenous substitution of urea for Optigen®II. However, the DIP level in experimental diets containing Optigen®II were higher in DIP than formulated, resulting in the following four treatment combinations: 114% DIP, OPTIGEN®II;   101% DIP, OPTIGEN®II;   100% DIP, UREA; 89% DIP, UREA (Table 1). Dry matter intakes were set to 2.58% of body weight and were offered twice daily (0800 and 1700). Intakes were updated weekly throughout the study; however, DMI was not adjusted the week prior to each N balance period. Each period consisted of a 19 day adaptation period followed by 7 days of N balance and a single day of blood sampling.

All animals were implanted with Synovex®Plus (200mg trenbolone actetate, 28mg estradiol benzoate, Fort Dodge Animal Health, Fort Dodge, IA) in order to increase

muscle deposition and the animals' N requirement. Animals were implanted at the beginning of the first adaptation period and were then re-implanted at the start of the adaptation of the 3rd period (56 days later).

Nitrogen balance

At the beginning of each N balance period, steers were moved from holding pens (3 x 3 m) into individual metabolism tie stalls (1.2 x 2.4 m), each with its own feed bunk and water supply. During the N balance period, total fecal and urine output were quantified before each morning feeding. Feces were weighed, sub-sampled (5%), and immediately frozen (-20^oC). Total urine output was continuously collected and kept separate from feces by fitting each steer with a rubber urine funnel under continuous vacuum (Huntington, 1989). Urine collection vessels contained sufficient H_3PO_4 to ensure a final pH of 3.0 or less. Each day, a 1.5% sub-sample of the acidified urine was frozen (-20^oC). After sampling on d 7 of the N balance, each steer was returned to its original holding pen and the evening feeding took place as usual.

Urea kinetics study

The method used to evaluate urea kinetics was developed by Sarraseca et al. (1998) for use in sheep and was later refined by Lobley et al. (2000). The method was later validated for use in cattle (Archibeque et al., 2002; Marini and Van Amburgh, 2003). The premise of the method is that by continuously infusing animals with $^{15}N^{15}N$ urea, and allowing sufficient time for the concentration of $^{15}N^{15}N$ urea and its metabolite, $^{15}N^{14}N$, to reach steady state, and by measuring the enrichment of the two urea species in the urine, systemic urea metabolism may be determined. Urine was collected for a full 24 h period in order to account for the potential effect that meal feeding (non-steady

state) might have on the enrichment of N species in the urine (Wickersham et al., 2009). The parameters estimated by the urea kinetics method include the following: Urea entry rate (UER) is the rate of appearance of urea N in the blood. It represents the urea synthesis rate occurring in the liver (Lobley et al., 2000). This urea (UER) can suffer only one of two fates: It can be excreted in the urine or it can enter the gastrointestinal tract. Urinary urea excretion (UUE) is the rate of excretion of urea in the urine. Entry into GIT (GER) is the rate of entry of urea N into the gut (Sarraseca et al., 1998) and is commonly referred to as urea recycling. Return to the ornithine cycle (ROC) represents urea N that is returning from the gut (NH_3 absorbed across the rumen epithelium into the blood) and is re-synthesized to urea in the liver. Urea N excreted in the feces (UFE) is N that originates from urea, which is excreted in the feces. Urea N utilized for anabolism (UUA) is N that originates from urea, which is used for anabolic purposes, mainly via microbial protein. The model also allows the estimation of fractional transfers of urea between body pools (Lobley et al., 2000). The UER to urine (u) is the fraction of UER that ends up in the urine. The fraction of UER that enters the GIT is then denoted by (1-u). The GER to ROC (r) is the fraction of GER that returns to the urea cycle for re-synthesis to urea. The GER to feces (f) is the fraction of GER that ends up in the feces. The GER to UUA is the fraction of GER that is used for anabolic purposes.

At the beginning of each N balance period, animals were fitted with an indwelling jugular catheter for infusion of tracer. The jugular vein was punctured with a 14 ga. thin walled hypodermic needle (Delmed Inc., Canton, MA). A 20 cm length of sterile Tygon tubing (ID: 0.05 in., OD: 0.09 in., Saint-Gobain, Akron, OH) was passed through the needle into the jugular vein. The needle was then removed, taking care to leave the

64

tubing in the jugular vein. An 18 ga. Luer stub adapter (Becton Dickinson, Rutherford, NJ) was then fitted into the Tygon tubing. The Luer stub adapter was modified by cutting grooves into the outer wall of the plastic base. These grooves were used as an anchor to suture the Luer adapter to the skin to secure the catheter in place. The catheter was then covered with gauze with a topical antibiotic secured by a protective cloth flap that was glued to the skin to prevent damage. During the first 78 h of each N balance period, steers were continuously infused via the jugular catheter with a sterile solution of $^{15}N^{15}N$ urea (NLM-233-5, Cambridge Isotope Laboratories, Andover, MA) in physiological saline to provide approximately 0.12 g of $^{15}N^{15}N$ urea per day in an attempt to reach a predicted enrichment of $^{15}N^{15}N$ urea of 0.1 atom percent excess at plateau. The $^{15}N^{15}N$ solution was prepared in sterile 5 L 0.9 % sodium chloride irrigation bags (Baxter, Deerfield, IL) by injecting a sterile $^{15}N^{15}N$ urea solution into the bag through a sterile 0.2 µm syringe filter (Millipore, Corporation, Billerica, MA). Medical grade intravenous infusion pumps (MVP™1 volumetric pump, International Medical Systems, Inc., Huntington, NY) were used to achieve a continuous and constant infusion rate. During the final 24 h of the infusion period urine samples were collected every 6 h and immediately frozen ($-20^{o}C$) to determine enrichment of ^{15}N urea species. Infusates were sampled at the beginning and end of each infusion period to monitor the concentration of marker infused. Fecal samples were collected at the start and end of each infusion and immediately frozen ($-20^{o}C$) to determine the background and final ^{15}N enrichment in the feces respectively.

Blood sampling

On the morning of d 8 each steer was fitted with an indwelling jugular catheter (BD Angiocath, Product number: 382259). Blood samples were collected into heparinized syringes (35 mL) at 0 (before feeding) and 1, 2, 4, 6, 8, and 10h after feeding. Plasma was harvested and immediately frozen (-20°C) for subsequent analysis of plasma urea and plasma ammonia.

Laboratory analyses

Feed, urine and fecal samples were composited by animal and period during the experiment. Feed and orts samples were dried in a forced-air oven at 100°C for 24 h to determine dry matter composition. Organic matter was determined according to the methods of the AOAC (Method 942.05). Urine composites were thawed, mixed and sampled for N analysis. Feed and fecal composites were thawed and mixed in a commercial mixer before being subsampled for N analysis. Samples of feed, fecal and urine composites were analyzed fresh for total N by combustion using a Vario Max CN elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Additionally, urine composites were used to determine output of urinary purine derivatives, as an estimate of microbial protein production (Shingfield and Offer, 1999) by high pressure liquid chromatography.

Urea was purified from urine utilizing cation exchange chromatography (AG 50W-X8, 100-200 mesh hydrogen form, Bio-Rad Laboratories, Richmond, CA). Urea samples and infusates were diluted to a constant concentration of 1.65 mM. The samples were prepared for the Hoffman degradation (Sarraseca et al., 1998) by transferring 2.73 mL of diluted urine sample or infusate into a 12 mL Exetainer tube (Labco Ltd. UK).

Urea samples and LiOBr (Page, 1982) were bubbled with high purity He gas at 60 mL/min for 20 min to remove N_2 from solution. With He gas still flowing, samples were frozen by immersion of the Exetainer in liquid N_2. Exetainers were uncapped and 0.5 mL of LiOBr was added and tubes were recapped. Tubes were then flushed with high purity He at 60 mL/min for 3 min by inserting a 19 ga. (inlet) and a 23 ga. (outlet) hypodermic needle. The needles were then removed (23 ga. first) and the tubes were transferred to a heating block at 65°C for 20-25 minutes to complete the Hoffman degradation. Enrichments of ^{15}N species in resulting N_2 gas and fecal samples were determined according to the methods described by Marini and Attene-Ramos (2006) by high-temperature conversion elemental analysis-isotope ratio mass spectrometry (Finningan Delta V Plus, Thermo Scientific, Bremen, Germany). The method accounted for the correction of $^{15}N^{14}N$ urea in the infusate as well as the amount of $^{15}N^{14}N$ urea produced by the non-monomolecular reaction of $^{15}N^{15}N$ urea and other species (Lobley et al., 2000).

Plasma ammonia was determined by the glutamate dehydrogenase procedure (Kun and Kearney, 1974) adapted to a Konelab 20XTi clinical analyzer (Thermo Fisher Scientific Inc., Beverly, MA). Plasma urea was determined by colorimetric assay using a Technicon AAII Autoanalyzer (Marsh et al., 1965).

Calculations

Nitrogen retention was calculated as the difference between N intake and N output (feces plus urine). Urine urea concentration, ratios of N species ($^{15}N^{15}N$ and $^{15}N^{14}N$ urea) in urinary urea and fecal ^{15}N enrichment were used to calculate urea production, urea recycling and use of urea for anabolic purposes according to the

methods described by Lobley et al. (2000). The calculations utilized are based on doses

and movements of urea expressed as mass/time as follows (Lobley et al., 2000):

$$UER(g\ urea\ N/d) = \left(\frac{E_{D30}}{E_{U30}} - 1\right) * D_{30}$$

Where:

- ED_{30} = Enrichment (APE) of $^{15}N^{15}N$ urea in the infusate

- EU_{30} = Enrichment (APE) of $^{15}N^{15}N$ urea in the urine at plateau

- D_{30} = Dose of $^{15}N^{15}N$ urea, g urea N / d

$$UUE\ (g\ urea\ N/d) = \ Urine\ volume\ (L) * urea\ N\ concentration\ (g/L)$$

$$u = \frac{UUE}{UER}$$

$$\rho = \frac{UUE_{29}}{(UUE_{29} + UUE_{30})}$$

Where:

- UUE_{29} = urinary concentration of $^{15}N^{14}N$ urea at plateau

- UUE_{30} = urinary concentration of $^{15}N^{15}N$ urea at plateau

$$r = \frac{\rho}{(1 - u)}$$

$$UFE \ (g \ urea \ N/d) = Fecal \ 15N \ excretion \ (g/d)$$

$$f = \frac{u * UFE}{(1 - u) * (UUE_{29} + UUE_{30})}$$

$$a = 1 - r - f$$

$$GER \ (g \ urea \ N/d) = UER - UUE$$

$$ROC \ (g \ urea \ N/d) = \rho * UER$$

Statistical analysis

Data were analyzed as a 4 x 4 replicated Latin square design using PROC MIXED of SAS. Plasma urea were analyzed as a split plot in time with steer as the main plot and time (h) as the subplot. The following model was used for dependant variables:

$Y_{ijkl} = \mu + C_i + R_j + A_k + B_l + AB_{kl} + T_m + AT_{km} + BT_{lm} + ABT_{klm} + E_{ijklm}$

where:

μ = Overall mean

Y_{ijkl} = Observation

C_i = Fixed effect of period

R_j = Random effect of animal

A_k = Fixed effect of DIP level

B_l = Fixed Effect of NPN source

AB_{kl} = Effect of DIP level x NPN source interaction

T_m = Fixed effect of time

AT_{km} = DIP level by time interaction (Split-plot only)

BT_{lm} = NPN source by time interaction (Split-plot only)

ABT_{klm} = Interaction between CP level, NPN source and time (Split-plot only)

E_{ijklm} = Residual Error

Actual N intakes differed from what was intended, therefore, data analysis was performed by using contrasts between DIP levels within each NPN source and a contrast between 101% DIP OPTIGEN®II and 100% DIP UREA, which had similar N and DIP intakes. This allowed the determinations of the effect of DIP level using both OPTIGEN®II and Urea and to compare OPTIGEN®II and Urea at approximately 100% DIP. Differences among treatments were considered to be significant when $P < 0.05$, whereas when $P > 0.05$ but < 0.10 differences were considered to indicate a trend towards a significant effect.

RESULTS

The higher DIP diets (114 % OPTIGEN®II and 100 % UREA) differed from lower DIP diets (101 % OPTIGEN®II and 89 % UREA) by addition of SBM and

additional urea or Optigen®II for each diet respectively (Table 5.1). In lower DIP diets (101 % OPTIGEN®II and 89 % Urea), SBM was replaced by cracked corn.

By design, DMI did not differ between treatments (Table 5.2). Dry matter (P = 0.09) and OM (P = 0.1) digestibility tended to be higher for 114 % vs. 101 % OPTIGEN®II and higher for 100 % vs. 89 % UREA (DM: P = 0.06; OM: P = 0.05). At 100 % DIP, OPTIGEN®II had lower DM and OM digestibility than UREA (DM: P = 0.01; OM: P = 0.01). Changes in DIP levels of the diet were reflected in the N intakes and were a consequence of the imposed treatment structure. Fecal N output was not different between treatments. Urinary N excretion was higher for 114 % vs. 101 % OPTIGEN®II (P < 0.001) and higher for 100 % vs. 89 % Urea (P < 0.0001). Urinary N excretion was not different between OPTIGEN®II and UREA at 100% DIP. Nitrogen retention was greater (P = 0.001) at 114 % vs. 101 % OPTIGEN®II; however, N retention was not different between 89 % and 100 % UREA. There was a tendency (P = 0.096) for UREA to have greater N retention than OPTIGEN®II at 100% DIP. Retained N, expressed as a percentage of N intake, was higher (P = 0.04) for 114 % vs. 101 % OPTIGEN®II but was not different between the other treatments.

Plasma ammonia (Table 5.3) was higher (P = 0.003) for 114 % vs. 101 % OPTIGEN®II but was not different between 100 % and 89 % UREA. Plasma ammonia was higher (P = 0.02) for 101% OPTIGEN®II than 100% Urea. Plasma urea was higher (P < 0.0001) for 114 % vs. 101 % OPTIGEN®II and higher (P < 0.0001) for 100 % vs. 89 % UREA. Plasma urea was higher (P = 0.03) for UREA than OPTIGEN®II at 100 % DIP. There was no difference in the urinary excretion of purine derivatives between treatments.

71

Figure 5.1 represents the enrichment of $[^{15}N^{15}N]$ urea (N_{30}) and $[^{14}N^{15}N]$ urea (N_{29}) in urine during the last 24 h of the 78 h infusion of $^{15}N^{15}N$ urea. Both N_{30} and N_{29} urea species reached plateau enrichment by 54 h of infusion as is required by the assumptions of this model. The N_{30} urea species reached plateau at approximately 0.13 % atom percent excess (APE) while the N_{29} species reached plateau at 0.03 % APE.

Table 5.4 contains urea kinetic variables presented as mass movements of urea N in g/d as well as fractional transfers of urea N between various body pools. Urea entry rate was higher (P = 0.002) for 114 % vs. 101 % OPTIGEN®II and higher (P = 0.004) for 100% vs. 89% UREA. Urea entry rate was not different between UREA and OPTIGEN®II at 100 % DIP. Similarly, UUE was higher (P < 0.001) for 114% vs. 101% OPTIGEN®II and higher (P < 0.0001) for 100 % vs. 89 % UREA. Urinary urea excretion was not different between UREA and OPTIGEN®II at 100 % DIP. Urea N returned to the ornithine cycle was higher (P = 0.03) for 114 % vs. 101 % OPTIGEN®II and was not different between 100 % and 89 % UREA or between UREA and OPTIGEN®II at 100% DIP. Increasing DIP level: increased (P = 0.01) the fraction of UER that was transferred to urine (u) for UREA but not OPTIGEN®II; increased (P = 0.003) the amount of GER that went to ROC (r) for OPTIGEN®II; increased (P = 0.01) the fraction of GER in the feces for UREA and reduced the fraction of GER in UUA for both UREA (P = 0.003) and OPTIGEN®II (P = 0.03).

DISCUSSION

Due to the nature of the diet composition, we were only able to compare the effect of DIP level within each NPN source, and can only compare OPTIGEN®II and UREA at the 100 % DIP level. This still provides the important comparison of OPTIGEN®II and

72

UREA at 100% DIP, and determines the effect of replacing the SBM and urea N in the 100 % UREA diet with N from Optigen®II.

In this experiment, higher DIP diets tended to have higher diet digestibility. Increased diet digestibility may be related to increased supply of degradable N to the rumen, resulting in higher microbial activity and thus, improved ruminal degradation of the diet (Köster et al., 1996). Alternatively, the inclusion of SBM in the high DIP diets may have provided stimulatory growth factors such as peptides, nucleotides, branched chain fatty acids and other unidentified growth factors to the rumen microbial population, resulting in increased diet digestibility (Dehority, 2003; Van Soest, 1994) and may also explain the increased digestibility of UREA vs. OPTIGEN®II diets at 100 % DIP. Alternatively, the slow release properties of OPTIGEN®II may have resulted in a reduction in the concentration of ammonia in the rumen at 101 % DIP, supported by the fact that UREA had higher plasma urea at 100% DIP than OPTIGEN®II (Table 5.3). These results infer that at 101 % DIP, rumen ammonia concentrations may have been sufficiently reduced with OPTIGEN®II to become a limiting factor to diet digestibility (Köster et al., 1996). However, we did not measure ruminal ammonia concentrations in the current study.

The changes in DIP levels of the diet were reflected in the N intakes (Table 5.2) and were a consequence of the imposed treatment structure. Fecal N excretion was not affected by treatment even though increased diet digestibility has been shown to reduce fecal N excretion (Bierman et al., 1999).

Excretion of N in urine reflected changes in DIP level of the diet. Urinary N excretion is primarily driven by N intake (Colmenero and Broderick, 2006; Marini and Van Amburgh, 2005) and is particularly sensitive to the rumen degradable N content of the diet (Nennich et al., 2006). Therefore the addition of SBM and either urea or Optigen®II (high DIP diets) may have increased ruminal N supply, ultimately leading to increased excretion of urea in the urine. When comparing UREA and OPTIGEN®II at 100 % DIP, there was no difference in urinary N excretion, suggesting that a certain amount of SBM and urea may be replaced by Optigen®II without significantly affecting urinary N excretion.

Increasing the DIP content of the diet from 89 % to 100 % did not have any effect on N balance for UREA, with increases in N intake being excreted in the urine. However, for OPTIGEN®II, there was an increase in N retention and improved efficiency of N retention when DIP was increased from 101 % to 114 %. The difference between the 101 % and 114 % OPTIGEN®II diets is the inclusion of 1.85 % soybean meal and 0.15 % Optigen®II in the 114 % DIP diet at the expense of cracked corn in the 101 % DIP diet. It is possible that the SBM inclusion resulted in increased N retention and improved N efficiency of the 114 % vs. the 101 % OPTIGEN®II diet by providing a ruminal (Marini and Van Amburgh, 2005) and/or post ruminal (Atkinson et al., 2007) source of protein, or that SBM may have provided stimulatory growth factors to the rumen microbial population, resulting in increased N retention (Dehority, 2003; Van Soest, 1994). However, the 100 % UREA diet included the same amount of SBM and urea but did not increase N retention or improve N efficiency when compared to the 89 % UREA diet. Degradable N may still have been limiting at the 100 % DIP level and

74

improved N retention and efficiency in the 114% OPTIGEN®II diet may have been solely due to improved supply of degradable N to the rumen. Alternately, it is possible that OPTIGEN®II slow release properties resulted in improved synchrony of carbohydrate and N degradation in the rumen (Johnson, 1976), resulting in more efficient capture of degradable N. It suggests a more detailed study including an expanded dose titration of urea and Optigen®II feeding may be warranted to test this hypothesis.

Increasing the supply of degradable N from 89 to 100 % DIP with UREA did not improve N retention, suggesting that supplying additional rapidly degradable urea would not improve N retention. In addition, the reduction in the fraction of recycled urea that was used for anabolic purposes (a) indicates that urea recycling a making up for some part of the deficiency in ruminal N. When comparing UREA and OPTIGEN®II at 100 % DIP, it is possible that, at 100 % DIP, it may not be favorable to reduce the rate of degradation of DIP in the rumen, possibly limiting supply of degradable N to the rumen as suggested by the tendency for increased N retention for UREA at 100% DIP and decreased plasma urea and diet digestibility for OPTIGEN®II at 100 % DIP. At higher concentrations of DIP, as are often used in commercial cattle feeding operations, Optigen®II may result in improved N retention and increased efficiency of N retention related to prolonged supply of degradable N to the rumen, resulting in a lower percentage of N being excreted and a higher percentage being retained (i.e. never entering the circulation) as microbial protein. This theory is supported by the fact that the fraction of urea N that was excreted in the rumen (u) was increased for UREA when DIP was increased but not for Optigen®II. In addition, the fraction of recycled N that was used for anabolism (a), was decreased as DIP was increased, and N retention and efficiency

75

increased simultaneously, indicating that more N from Optigen®II was captured during "1st pass" ruminal metabolism. However, the excretion of urinary purine derivatives, as an indicator of microbial protein production, was not different between treatments (Table 5.3). This would suggest that there was no difference in microbial protein production between treatments. If there was no difference in microbial protein production, then it is possible that increased N retention in 114 vs. 101 % OPTIGEN®II diets was due to the inclusion of soybean meal in the 114% DIP diet and that the soybean meal was used directly by the animals (i.e. absorbed intestinally) and not for microbial protein production. However, a similar increase in N retention was not apparent for 100 % vs. 89 % UREA treatments where the same amount of soybean meal was included. Alternately, it is possible that increased microbial protein production of 114 vs. 101 % OPTIGEN®II was too small to detect via the method used as one might expect some difference in MCP production between diets that varied so widely in CP intake.

Urea entry rate and UUE are driven by N intake and DIP level (Marini and Van Amburgh, 2003). Increasing dietary degradable N leads to higher production (Johnson, 1976) and absorption (Bartley et al., 1976) of ammonia from the rumen, and subsequently higher production of urea in the liver. Higher circulating urea results in increased excretion of urea in the urine (Colmenero and Broderick, 2006). Urea entry rate ranges from approximately 33 % of N intake on the 89 % UREA diet to 42 % of N intake on the 114 % OPTIGEN®II diet. This is within the same range reported by Marini and Van Amburgh (2003) who reported UER of between 35 and 65 % of ingested N in growing cattle and considerably lower than values reported by Huntington et al. (1996) in non-growing cattle. Urea entry rate is dependent on the N and energy content of the diet, as

well as on physiological state (Marini and Van Amburgh, 2003). Steers on this experiment were fed isoenergetically and were all growing and therefore assumed to have similar physiological status. Therefore, changes in urea entry rate are assumed to be a result of changes in N intake.

Urinary urea excretion reflected UER and varied between 15.5 g/d on the 89 % UREA treatment (N intake: 139 g/d) and 36.3 g/d on the 114 % OPTIGEN®II treatment (N intake: 168 g/d). This equates to between 11 and 22% of intake N excreted as urea in the urine. This is comparable to values reported by Marini and Van Amburgh (2003) that between 15 and 71 % of intake N was UUE using diets that varied in N intake between 88 and 204 g/d. Gut entry rate of urea N includes urea that is recycled to the gut via saliva as well as across the gut wall directly (Lobley et al., 2000). Gut entry rate of urea N did not differ between treatments (~31 g/d). However, as a percentage of N intake, GER ranged from 20 % on the 114 % OPTIGEN®II diet to approximately 22 % of intake N on the 89 % UREA diet. It has been postulated that the amount of urea that enters the rumen is more dependent on energy availability in the rumen than it is on the N content of the diet (Huntington, 1989). The diets used in the current study were formulated to be isoenergetic and should have had similar rumen energy availability. Therefore the constant GER between treatments was not unexpected.

Return of urea N to the ornithine cycle (ROC) was higher for 114 % vs. 101 % Optigen®II. The 114 % OPTIGEN®II diets may have elevated rumen ammonia for a prolonged period as suggested by improved N retention and efficiency data as well as elevated plasma urea concentration. This is reflected in ROC. If rumen ammonia concentration is maintained at a higher level, then more ammonia would be returned to

the ornithine cycle for re-synthesis to urea, resulting in higher ROC. The percentage of GER that was ROC was between 18 and 30 %. Archibeque et al. (2001) using beef steers and Sarraseca et al. (1998) using sheep reported a similar range (26 to 41 % of GER). Marini and Van Amburgh (2003) found that increasing daily N intake from 88 to 110 g/d lead to an increase in ROC but did not find any further increase at higher N intakes. In contrast, we only found increased ROC at higher N intakes (168 g/d) for OPTIGEN®II. There were no differences between treatments for UFE or UUA. Increased N retention of 114% vs. 101% DIP diets should have resulted from an increase in microbial protein production, as NPN from Optigen®II must be converted to microbial protein before being useful to the animal. However, this was not reflected in UUA. Increasing dietary N intake has been shown to decrease the quantity of N excreted in the feces that originated from urea (Marini and Van Amburgh, 2003). However, this decrease was not apparent with increased N intake in the current study and the percentage GER to feces was actually lower for the 89 % vs. the 100 % DIP UREA diet.

Although there were higher plasma ammonia concentrations for OPTIGEN®II, the magnitude of the difference may not be biologically significant, and it is important to realize that these are jugular samples. Under normal conditions the liver is able to nearly completely extract ammonia from the hepatic portal vein (Milano et al., 2000), resulting in drastic reduction on blood ammonia levels between the hepatic portal vein and the peripheral circulation. Therefore, jugular plasma ammonia is not the most sensitive indicator of ammonia absorption. Plasma urea, produced from ammonia in the liver is probably a more robust measure.

Predictably, plasma urea was higher for the high DIP treatments for both UREA and OPTIGEN®II. Higher dietary DIP concentrations presumably led to more ammonia production in the rumen and consequently more ammonia entering the blood which is converted to urea, primarily in the liver (Huntington, 1989). The sensitivity of plasma urea to N intake is well known (Archibeque et al., 2002; Bunting et al., 1987; Huntington, 1989). Lower plasma urea of OPTIGEN®II vs. UREA at 100 % DIP may be as a result of OPTIGEN®II slow release properties resulting in lower ammonia concentrations in the rumen, less entry into the blood and subsequently lower plasma urea concentrations.

CONCLUSIONS

Reducing the DIP content of diets below the recommended dietary requirement may lead to decreased N excretion into the environment. However, it may also lead to decreased production related to depressed diet digestibility and decreased N retention. When degradable N is marginal, it may be counterproductive to slow the release of N in the rumen as it may exacerbate a ruminal N deficiency leading to decreased N retention and increased urinary N excretion, in addition to a possible depression in diet digestibility. However, at higher DIP levels, reducing the rate of degradation of N in the rumen may lead to improved N retention by alleviating transient extremes in rumen ammonia concentrations and prolonging favorable concentrations for rumen microbes. In terms of urea kinetics, N intake appears to have a dominant effect on UER, UUE and ROC due to the fact that there was more urea N in the system at higher N intakes. This was also reflected in the plasma urea concentrations. The fact that GER did not differ between treatments suggests that there is limited potential to improve N recycling under these conditions. There were vastly differing blood N fluxes and different diet

79

digestibility and yet GER did not change, potentially due to the constant level of fermentable energy in the diets.

Table 5.1. Diet ingredients given as % of total DM in steers fed 89%, 100% and 114% DIP diets with UREA or OPTIGEN®II

	114% DIP, OPTIGEN®II	101% DIP, OPTIGEN®II	100% DIP, UREA	89% DIP, UREA
Feedstuff		% of DM		
Cracked Corn	47.03	48.92	47.27	49.14
Fescue Hay	23.70	23.76	23.82	23.86
Cottonseed Hulls	22.22	22.27	22.33	22.37
Soybean meal	1.85	0.00	1.86	0.00
Urea	0.00	0.00	1.04	0.95
OPTIGEN®II	1.54	1.39	0.00	0.00
Molasses	1.93	1.93	1.94	1.94
Limestone	0.80	0.80	0.80	0.81
Dicalcium Phosphate	0.19	0.19	0.19	0.19
Calcium Sulfate	0.11	0.11	0.11	0.11
Vitamin Premix [a]	0.04	0.04	0.04	0.04
Trace mineral premix [b]	0.59	0.59	0.60	0.60

[a] Vitamin premix composition: 8811ppm vitamin A, 1762ppm vitamin D, 1100ppm vitamin E
[b] Trace mineral premix composition: 0.06% Ca, 56.34% Cl, 36.53% Na, 1.2% S, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 ppm I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, 5520.2 ppm Zn

Table 5.2. Dry matter intake, diet digestibility and N-balance in steers fed 89%, 100% and 114% DIP diets with UREA or OPTIGEN®II

Item	Treatment				SEM	Contrasts		
	114% DIP OPTIGEN®II	101% DIP OPTIGEN®II	100% DIP Urea	89% DIP Urea		114% vs. 101%, OPTIGEN®II	100% vs. 89 %, Urea	100% Urea vs. 101% OPTIGEN®II
DMI, kg/d	7.11	7.11	7.23	7.20	0.84	0.96	0.81	0.26
Digestibility, %								
DM	59.8	58.2	60.7	58.8	1.60	0.09	0.06	0.01
OM	59.6	58.0	60.8	58.7	1.69	0.11	0.05	0.01
N Balance, g/d								
N intake	167.8	148.8	153.1	138.7	18.10			
Fecal N	67.6	66.0	67.1	65.2	7.95	0.38	0.29	0.55
Urine N	62.2	53.6	54.3	41.7	7.42	0.0006	<0.0001	0.75
Absorbed N	102.9	86.3	91.0	77.2	10.26	<0.0001	0.001	0.18
N Retention [b]	39.6	31.3	34.8	34.1	4.00	0.001	0.73	0.096
Retained N, % of N Intake [a]	23.0	20.0	22.2	24.1	0.91	0.04	0.21	0.12

[a] Data were transformed to obtain a normal distribution for statistical analysis. SEM of original data is reported

[b] N Retention = N Intake – N Output (Fecal N + Urine N)

Table 5.3. Plasma ammonia, urea and urinary excretion of purine derivatives in steers fed 89%, 100% and 114% DIP diets with UREA or OPTIGEN®II

	Treatment					Contrasts [a]		
Item	114% DIP OPTIGEN ®II	101% DIP OPTIGEN ®II	100% DIP Urea	89% DIP Urea	SEM	114% vs. 101%, OPTIGEN ®II	100% vs. 89 %, Urea	100% Urea vs. 101% OPTIGEN ®II
Plasma Ammonia, mM	0.40	0.37	0.36	0.36	0.038	0.003	0.73	0.02
Plasma Urea, mM	2.86	2.36	2.55	1.90	0.120	<0.0001	<0.0001	0.03
Urinary purine derivatives, mmol/kg DMI								
Purine derivatives [b]	22.6	21.2	22.4	21.5	1.72	0.57	0.73	0.65
Allantoin	14.8	14.0	14.1	13.5	1.08	0.58	0.72	0.96
Pseudouridine	0.45	0.44	0.47	0.42	0.04	0.85	0.33	0.51
Uric acid	7.8	7.3	8.3	8.0	0.84	0.66	0.78	0.35
Hypoxanthine	4.2	4.3	3.7	4.5	1.02	0.86	0.22	0.33
Creatinine	14.3	12.5	13.0	12.7	0.83	0.11	0.83	0.67

[a] Main effects interaction with time were non-significant (P > 0.10)
[b] Purine derivatives = Allantoin + uric acid

83

Table 5.4. Urea Kinetics variables in steers fed 89%, 100% and 114% DIP diets with UREA or OPTIGEN®II

Item	114% DIP OPTIGEN®II	101% DIP OPTIGEN®II	100% DIP Urea	89% DIP Urea	SEM	Contrasts 114% vs. 101%, OPTIGEN®II	100% vs. 89 %, Urea	100% Urea vs. 101% OPTIGEN®II
Urea Kinetic variables (g urea N/d)								
Urea entry rate (UER)	70.1	57.8	56.7	45.4	8.09	0.002	0.004	0.75
Urinary urea excretion (UUE)	36.3	26.5	26.6	15.5	2.50	0.0002	<0.0001	0.96
Entry to GIT (GER)	33.8	31.3	30.1	30.0	7.35	0.47	0.97	0.73
Return to ornithine Cycle (ROC) [a]	7.92	6.31	6.16	5.92	1.29	0.03	0.73	0.83
Loss to Feces (UFE)	6.67	6.13	6.16	6.66	0.66	0.11	0.13	0.93
Re-use for anabolism (UUA)	19.6	22.0	20.7	22.0	6.11	0.50	0.72	0.72
Fractional Transfers								
UER to urine (u)	0.54	0.48	0.48	0.34	0.065	0.25	0.01	0.90
UER to GIT (1-u)	0.46	0.52	0.52	0.66	0.065	0.25	0.01	0.90
GER to ROC (r) [a]	0.30	0.18	0.22	0.19	0.033	0.003	0.42	0.20
GER to feces (f) [a]	0.11	0.10	0.09	0.06	0.037	0.60	0.01	0.71
GER to UUA (a) [a]	0.64	0.71	0.68	0.75	0.041	0.03	0.003	0.26

[a] Data were transformed to obtain a normal distribution for statistical analysis. SEM of the original data is reported

Figure 5.1. Enrichment of $^{15}N^{15}N$ urea (N30) and $^{14}N^{15}N$ urea (N29) in urine during the 78 h infusion of $^{15}N^{15}N$ urea
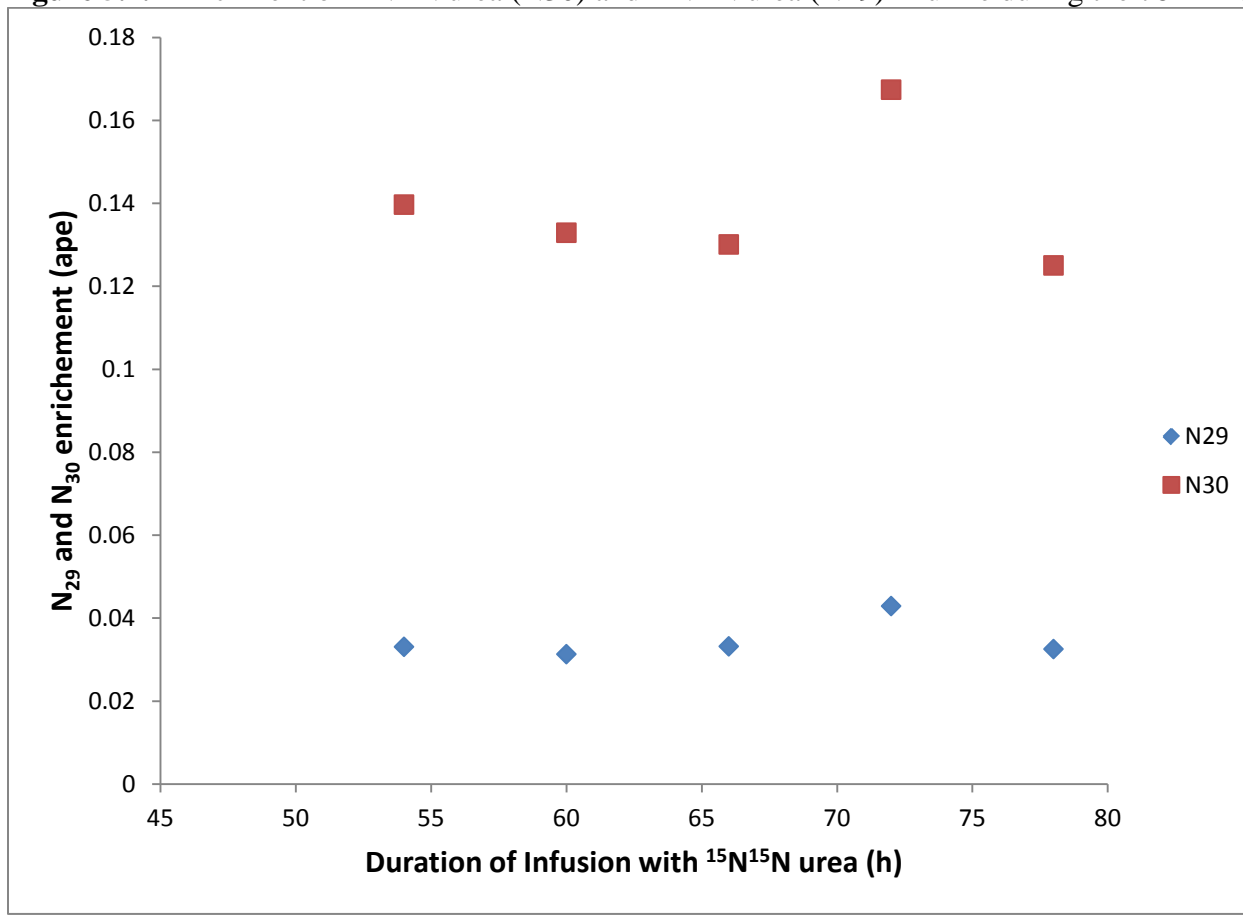
Figure 5.2. Plasma ammonia over time in steers fed 89%, 100% and 114% DIP diets with UREA or OPTIGEN®II
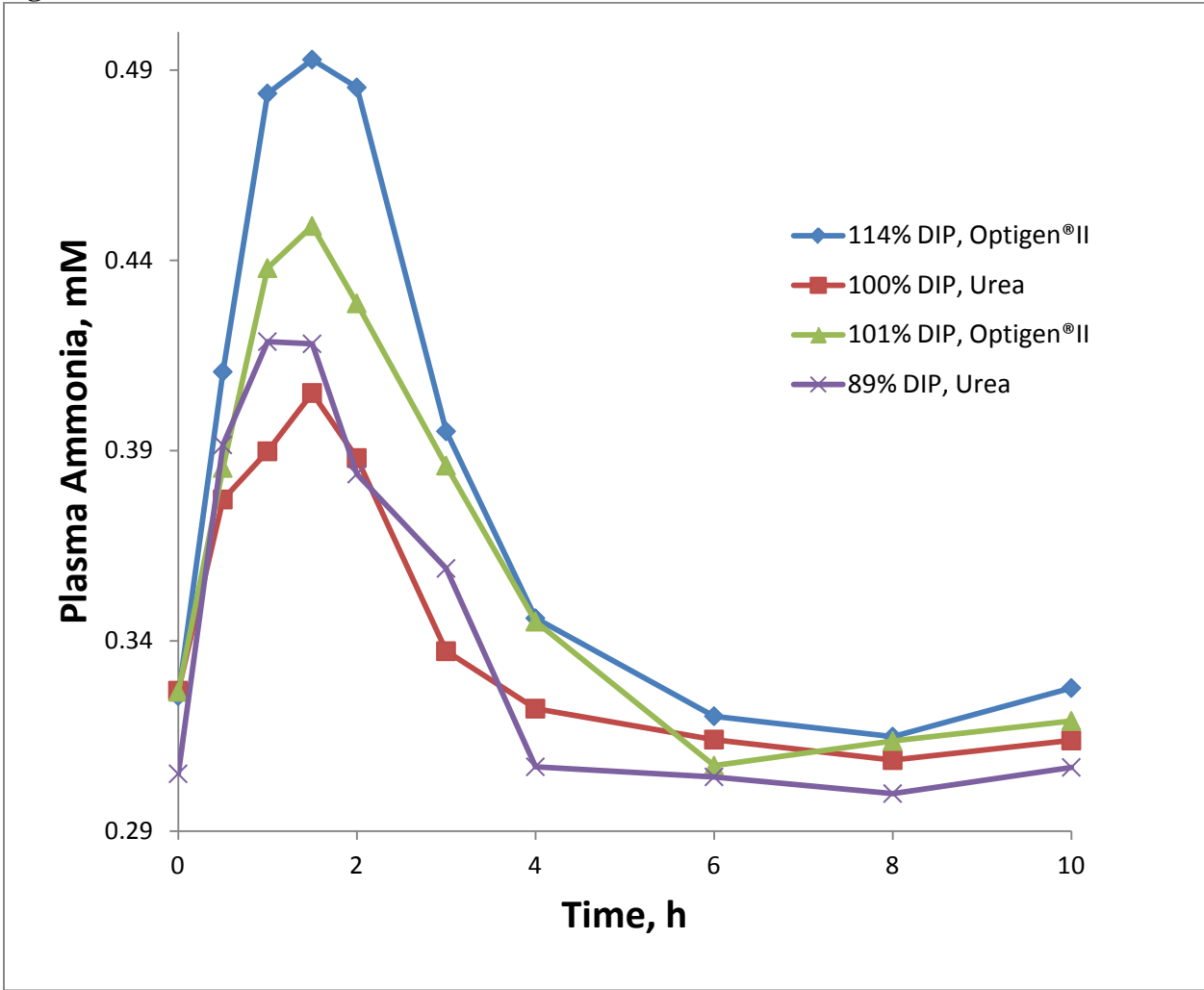
Figure 5.3. Plasma Urea over time in steers fed 89%, 100% and 114% DIP diets with UREA or OPTIGEN®II
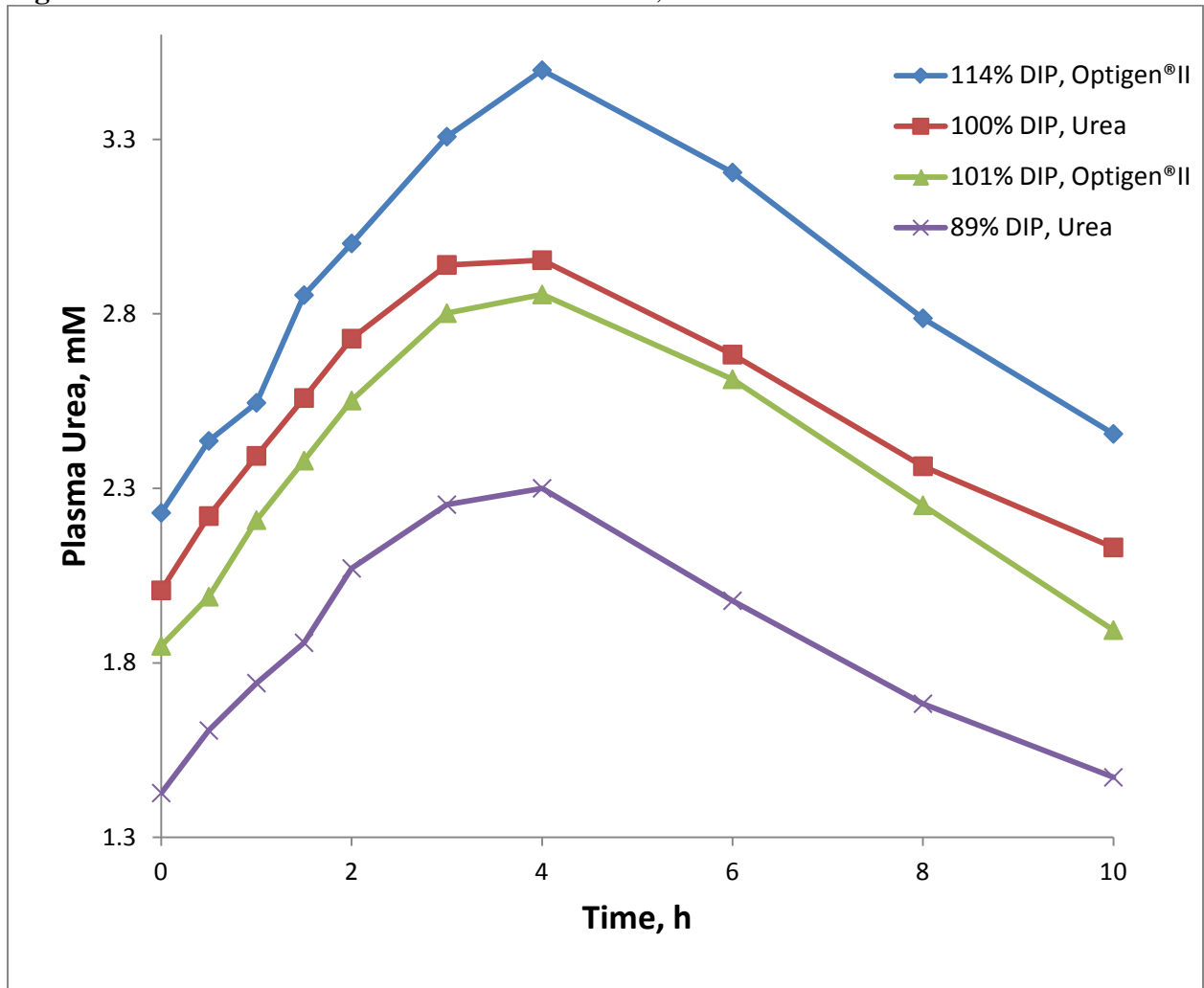
CHAPTER 6. EFFECTS OF REPLACING SOYBEAN MEAL N WITH NPN FROM UREA OR OPTIGEN®II ON INTAKE AND PERFORMANCE OF RECEIVING CATTLE

INTRODUCTION

The feed intake of newly received feedlot cattle is generally low during the first 2 weeks after arrival at the feedlot. Depressed feed intake is often associated with the combined stress of weaning, transportation, disease challenge and feed and water deprivation before arriving at the feedlot (Fluharty et al., 1994), as well as depressed fermentative ability in the rumen (Cole and Hutcheson, 1990). There is evidence that increasing the N content of the diet may lead to improved recovery of appetite in newly received feedlot cattle (Cole and Hutcheson, 1990; Fluharty and Loerch, 1995). It is possible that supplying steers with a readily available and concentrated source of degradable N may allow the fermentative ability of the rumen to recover and thus lead to improved feed intake. Urea is a highly concentrated source of CP that can be used to supply ruminally available N to cattle and it is economically favorable to replace the more expensive and traditionally used protein sources such as soybean meal in feedlot diets. The highly concentrated nature of urea may means the supplemented DIP takes very little space in the ration and may allow inclusion of more energy in the diet, resulting in higher energy intake when DMI is depressed. However, urea has been shown to depress intake in cattle when fed at inclusion rates exceeding 1% of DM (Kwan et al., 1977; Reid, 1953). Wilson et al. (1975) demonstrated that slowly infusing urea into the rumen instead of bolus dosing alleviated the negative effects of urea on DMI when urea inclusion exceeded 1% of DM. Therefore slow release urea may be a suitable means to

supply DIP to receiving cattle to stimulate intake while avoiding the negative effects on intake associated with regular feed grade urea.

MATERIALS AND METHODS

Experimental design

The experiment was conducted over 42 d using 288 Angus crossbred steers with an initial body weight of 271 ± 3.3 kg to evaluate receiving cattle feed intake and growth. The experimental design was a randomized complete block design with a 2 x 4 factorial treatment structure. Cattle were blocked by weight and receiving date and within block were randomly allocated to 1 of 8 treatment combinations (6 pens per treatment, 6 head per pen). Treatment factors were four incremental levels of urea inclusion replacing soybean meal N and two sources of dietary urea. Steers were fed 0.45, 0.9, and 1.35% of DM as urea. Urea isonitrogenously replaced an equivalent amount of SBM N in the diet. The sources of urea were feed grade urea (UREA) and slow release urea (OPTIGEN®II). The hypothesis was that replacing DIP from SBM with DIP from urea would maintain DMI up to 0.9 % of DM inclusion in the urea diet but that 1.35 % inclusion of urea will depress DMI for UREA but not OPTIGEN®II.

Receiving and processing

Cattle were purchased through local auction by an order buyer and arrived in 3 lots. The first lot consisted of 157 hd and arrived 10 d before processing. The second lot consisted of 73 hd and arrived 6 d before processing. The last lot consisted of 58 hd and arrived 3 d before processing. Upon arrival at the research unit, cattle were offloaded to holding pens with unlimited access to fescue hay and water. Steers were kept in holding

90

pens for the amount of days specified above until processing and sorting onto treatment for the experiment. Processing and sorting of cattle occurred over a 2 d period. On d 1, all cattle were weighed, ear tagged, bled, and vaccinated (Bovishield Gold 5, Somubac, One shot, Ultrachoice, Pfizer Animal Health, New York, NY). On d 2, cattle were reweighed, dewormed (Pfizer Dectomax, Pfizer Animal Health, New York, NY) and implanted with Revalor S (120 mg trenbolone acetate, 24 mg estradiol; Intervet, Millsboro DE) growth promoting implants. Steers were then sorted onto treatments and feeding of the experimental diets commenced. All steers were given booster vaccinations (Bovishield Gold 5, Somubac, Ultrachoice, Pfizer) and reweighed at 14 d.

Diets, feeding and sample collection

Diets (Table 6.1) were formulated to be isoenergetic (NEg = 1.02 Mcal), isonitrogenous (CP = 14%) and supplying the same amount of MP (87.7% of requirement) according to the NRC requirements (NRC, 2000). Three premixes were utilized to mix all of the experimental diets including a control, urea and Optigen®II based premix. These premixes were then added to the experimental TMR at variable rates in order to achieve the required treatment combinations. Cattle were fed *ad libitum* and diets were offered once daily. Total DM offered was measured daily. Feed ingredients were sampled weekly and DM content was used to adjust ingredient composition of the diet. In addition, weekly feed ingredient samples were frozen (-5°C) and composited for the entire experiment to determine dietary nutrient composition (Table 6.2). Each week before feeding, feed refusals were removed from the bunk, weighed and composited by treatment. Animals were weighed on d 0, 1, 14, 41 and 42. In addition, blood samples were collected by jugular venipuncture into 7 mL heparinized

Vacutainers® (#367676, BD, Franklin Lakes, NJ) on day 1 and 42. Plasma was collected by centrifuging at 4,000 x g for 20 min and stored frozen (-5°C) until analysis.

Laboratory analyses

Feed and orts composite samples were dried in a forced-air oven at 100°C for 24 h to determine DM composition. Neutral detergent fiber and ADF were determined according to Van Soest et al. (1991) using an Ankom A200 Fiber Analyzer (Ankom Technology, Macedon, NY). Crude protein (N x 6.25) was determined by analysis of N content with a Leco FP-528 (Leco Corp., St. Joseph, MI) according to method 990.03 of the AOAC (2005). Mineral concentrations were determined using a HNO_3/HCl microwave digestion followed by analysis with an inductively coupled plasma (ICP) radial spectrometer (Thermo IRIS HX, Thermo Fisher Scientific Inc., Waltham, MA). Plasma urea concentrations were determined by colorimetric assay using a Technicon AAII Autoanalyzer (Marsh et al., 1965).

Calculations and Statistical Analysis

Total DMI was calculated by subtracting total orts DM from DM offered to each pen on a weekly basis. Weight gain over the entire experiment was calculated as the difference between the average weight on d 0 and 1 and the average of d 41 and 42. Data were analyzed as a randomized complete block design with a 2 x 4 factorial treatment structure using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) using pen as the experimental unit. Differences among treatments were considered to be significant when $P < 0.1$, whereas when $P > 0.1$ but < 0.15 differences were considered to indicate a trend.

RESULTS AND DISCUSSION

The nutrient composition of the experimental diets is presented in Table 6.2. Although the experimental diets were formulated to be isonitrogenous, the OPTIGEN®II premix had a lower CP concentration than the UREA and control premixes, resulting in a progressive reduction in diet CP concentration between 0 and 1.35 % inclusion rates. The effects of replacing multiple levels of soybean meal N with urea N from feed grade urea and Optigen®II are presented in Table 6.3. There were no differences in initial body weight (BW) and no effect of treatment on final BW. Dry matter intake, BW gain and gain to feed (G:F) data were divided up into 2 periods. The first period consisted of the first 14 days representing the period of highest stress and incidence of depressed intake (Cole and Hutcheson, 1990; Eck et al., 1988; Fluharty and Loerch, 1997). The second period consisted of the final 4 weeks of the experiment. There were no treatment effects on DMI at any point in the experiment suggesting that UREA and OPTIGEN®II were able to support DMI as effectively as SBM. Depressed feed intake is characterized by Galyean and Hubbert (1995) as being below 1.5 % of BW. In the current experiment, DMI in the first two weeks averaged 2.3 % of BW and therefore a severe depression in feed intake was not evident. Similarly, Eck et al. (1988) reported that there was no difference in feed intake between receiving cattle supplemented with urea, cottonseed meal (CSM) or a 50:50 mixture on corn gluten meal (CGM) and blood meal (BM) fed at both 10.5 and 12.5 % CP but reported increased intake across treatments at 12.5% CP. Therefore, increasing CP concentration led to increased DMI regardless of whether the N source was highly degradable (urea) or not (CSM, BM). However, increasing CP concentration has also been shown to increase morbidity in receiving cattle (Fluharty and

Loerch, 1995; Galyean et al., 1999), possibly related to the effects of ammonia emissions from feed on incidence of respiratory disease. Therefore research is needed to characterize the DIP and UIP requirements of newly received feedlot cattle in order to maximize early intake and production while minimizing morbidity. Contrary to the hypothesis, inclusion of 1.35 % urea did not depress DMI. Generally, a urea concentration in the diet exceeding 1 % of DM has caused a reduction in DMI (Kwan et al., 1977; Reid, 1953). However, when diets are available *ad libitum* and when urea is mixed into the TMR, urea appears to be tolerable at higher levels in the diet without causing depression in DMI (Broderick et al., 1993; Kertz, 2010). There was no effect of treatments on period 1 or total ADG or F:G, indicating that substituting SBM N with N from urea or Optigen®II up to 1.35% urea equivalent did not have any adverse effects on production parameters. However, there was a tendency for OPTIGEN®II to have higher BW gain and G:F than UREA in the second period (BW gain: 1.41 vs. 1.33 kg/d, *P* = 0.11; G:F: 194 vs. 185 g/kg, *P* = 0.14) despite the lower CP intake for the OPTIGEN®II vs. UREA diets. Improved weight gain and feed efficiency for OPTIGEN®II in the second period may indicate that although Optigen®II did not offer any advantage in terms of stimulating intake in receiving cattle, it may improve the efficiency of utilization of NPN in early receiving cattle under these conditions by improving production without any increase in feed intake. Similarly, in finishing Angus steers fed a steam flaked corn diet, Bourg et al. (2012) did not find any difference in performance or carcass composition but feeding a lipid coated slow release urea resulted in a tendency for improved G:F than for an isonitrogenous amount of urea. In contrast, Taylor-Edwards et al. (2009) fed multiple levels (0.4, 0.8, 1.2 and 1.6 % of DM as urea) of urea and a

polymer-coated slow release urea to beef cattle. The authors reported an interaction with the quantity of urea in the diet, where urea resulted in better growth at low levels of supplementation whereas slow release urea was superior at higher levels of supplementation with the exception of the highest level (1.6 %) with a similar trend being evident for feed efficiency. There were no differences between treatments on initial or final plasma urea concentration. In contrast, other authors have reported that slow release urea reduced blood urea concentration when compared to feed grade urea (Cherdthong et al., 2011; Taylor-Edwards et al., 2009c). Huntington et al. (2006a) reported no difference in blood urea concentration between feed grade urea and a slow release urea-calcium mixture in steers fed high forage and high concentrate diets *ad libitum*. However, when a series of blood urea samples were used to calculate the area under the resulting urea curve, as an estimate of total urea absorbed, a significantly higher quantity of urea was absorbed into the blood for urea when compared to slow release urea. It is possible that feeding diets *ad libitum*, which allows animals to consume feed throughout the day, may reduce the large increases in post feeding blood urea concentration related to meal feeding of feed grade urea. However, total urea absorbed may still be increased for feed grade urea vs. slow release urea (Huntington et al., 2006b), possibly indicating increased utilization of N from slow release urea.

CONCLUSIONS

Degradable intake protein from SBM in receiving diets was effectively replaced by up to 1.35 % urea as feed grade urea or Optigen®II without any adverse effects on intake or production. Contrary to the hypothesis, 1.35 % inclusion of urea in the diet did not reduce DMI in receiving cattle under these circumstances.

Table 6.1. Ingredient compositions of experimental diets fed to receiving steers to evaluate source of degradable intake protein

Substitution rate:	0%	0.45%	0.9%	1.35%
Ingredient		Inclusion, % of DM		
Corn silage	73.50	73.50	73.50	73.50
Fescue hay	10.00	10.00	10.00	10.00
Soybean meal	15.00	10.28	5.43	0.71
Amino Plus	0.00	2.52	5.13	7.65
Cracked Corn	0.00	0.99	2.01	3.00
Urea [a]	0.00	0.45	0.90	1.35
Tallow	0.00	0.76	1.53	2.29
Limestone	0.85	0.85	0.85	0.85
Trace mineral premix [b]	0.60	0.60	0.60	0.60
Vitamin premix [c]	0.05	0.05	0.05	0.05

[a] Formulation is based on urea weight. For Optigen®II treatments, urea and tallow was be replaced by Optigen®II on an isonitrogenous and isoenergetic basis.
[b] Trace mineral premix composition: 0.06% Ca, 56.34% Cl, 36.53% Na, 1.2% S, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 ppm I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, 5520.2 ppm Zn
[c] Vitamin premix composition: 8811ppm vitamin A, 1762ppm vitamin D, 1100ppm vitamin E

Table 6.2. Nutrient composition of experimental diets in steers fed multiple levels of urea from feed grade urea or Optigen®II

Item	Optigen®II				Urea			
	0	0.45%	0.90%	1.35%	0	0.45%	0.90%	1.35%
DE, Mcal/kg	3.09	3.09	3.08	3.07	3.09	3.09	3.09	3.09
% of DM								
DM	49.1	49.1	49.1	49.2	49.1	49.1	49.0	49.0
NDF	38.4	38.3	38.2	38.1	38.4	38.3	38.3	38.2
ADF	23.9	23.8	23.8	23.8	23.9	23.8	23.8	23.8
CP	13.9	13.3	12.8	12.2	13.9	13.8	13.6	13.5
TDN	68.2	68.2	68.2	68.2	68.2	68.2	68.2	68.2
Ca	0.73	0.76	0.79	0.81	0.73	0.75	0.76	0.78
P	0.37	0.35	0.34	0.33	0.37	0.35	0.34	0.33
Mg	0.16	0.16	0.15	0.15	0.16	0.16	0.15	0.15
K	1.30	1.23	1.15	1.07	1.30	1.24	1.17	1.10
Na	0.39	0.35	0.31	0.28	0.39	0.37	0.35	0.33
S	0.18	0.17	0.16	0.15	0.18	0.16	0.15	0.14
PPM								
Cu	26	25	25	24	26	25	25	25
Fe	199	200	202	204	199	200	202	203
Mn	72	72	72	72	72	65	57	51
Zn	65	69	74	78	65	62	58	54
Mb	0.9	0.9	0.8	0.8	0.9	0.9	0.8	0.8

Table 6.3. Effects of replacing multiple levels of soybean meal N with feed-grade urea or Optigen®II on BW, DMI and performance of receiving feedlot steers

| | Optigen®II | | | | Urea | | | | SEM | *P*-value | | |
	0	0.45%	0.90%	1.35%	0	0.45%	0.90%	1.35%		Source	Level	Source x Level
Initial BW, kg	261	261	259	256	260	257	257	259	1.6	0.42	0.18	0.15
Final BW, kg	317	315	316	310	315	315	310	313	3.0	0.56	0.48	0.48
Day 0 to 14:												
DMI, kg/d	6.06	5.77	5.87	5.62	5.82	6.10	5.72	5.89	0.191	0.72	0.69	0.34
BW gain, kg/d	1.32	1.16	1.21	1.20	1.27	1.42	1.17	1.21	0.093	0.51	0.57	0.32
G:F, g/kg	217	198	204	214	217	232	202	208	12.9	0.51	0.72	0.40
Day 14 to 42												
DMI, kg/d	7.29	7.31	7.32	7.24	7.25	7.45	6.92	7.24	0.162	0.53	0.46	0.39
BW gain, kg/d	1.35	1.41	1.47	1.38	1.32	1.34	1.33	1.32	0.066	0.11	0.77	0.84
G:F, g/kg	186	194	203	192	184	181	193	183	8.2	0.14	0.37	0.92
Day 0 to 42												
DMI, kg/d	6.85	6.75	6.80	6.66	6.74	6.97	6.49	6.76	0.159	0.80	0.56	0.37
BW gain, kg/d	1.34	1.29	1.36	1.30	1.30	1.38	1.26	1.26	0.061	0.61	0.84	0.48
G:F, g/kg	195	190	200	196	193	198	194	187	6.3	0.55	0.86	0.57
Initial plasma urea, mM	3.39	3.39	3.31	3.41	3.14	3.56	3.48	3.55	0.142	0.56	0.41	0.40
Final plasma urea, mM	3.59	3.50	3.24	3.69	3.50	3.42	3.36	3.61	0.167	0.77	0.22	0.92

CHAPTER 7. THE EFFECTS OF BASAL DIET AND THE SUPPLY OF DEGRADABLE INTAKE PROTEIN ON GROWTH OF STEERS

INTRODUCTION

Due to increased population and income levels, particularly in developing countries, worldwide production of meat and milk will have to double within 50 years (Dijkstra et al., 2011). This will require a massive increase in the productive output from animals without any appreciable increase in land availability. However, maximizing production from ruminants is often associated with an increase in excretion of waste products that may be harmful to the environment (VandeHaar and St-Pierre, 2006). Overfeeding degradable N may result in excessive losses of N in the urine (Marini and Van Amburgh, 2005) while underfeeding it may reduce digestion and microbial protein production (Köster et al., 1996; Satter and Slyter, 1974). Reducing dietary DIP concentration has been suggested as a means to reduce N excretion; however, farmers are unlikely to adopt such measures if they lead to a reduction in production. Excessive losses of urinary N in cattle supplemented with urea as a DIP source has been associated with a rapid degradation rate of urea in the rumen, and its subsequent loss in the urine (Brito and Broderick, 2007). It is thought that reducing the rate of degradation of urea in the rumen may reduce urinary N excretion by increasing the capture of ruminal NH_3, or may allow for improved production at higher DIP concentrations without increasing N excretion. Finally, it is unclear whether slow release urea will react differently to basal diets of different forage to concentrate ratios, as previous work performed by this group (unpublished) indicated that basal diet affected the ruminal disappearance rate of urea and slow release urea in situ. Therefore, the objective of this experiment was to determine

whether urea and Optigen®II interact differently in diets varying in concentrate level and DIP content.

MATERIALS AND METHODS

Experimental design

The experiment was conducted over 70 d using 240 Angus crossbred steers with an initial body weight of 287 ± 1.4 kg implanted with Revalor S (120 mg trenbolone acetate, 24 mg estradiol; Intervet, Millsboro DE) to evaluate growth. The experimental design was a randomized complete block design with a 2 x 2 x 2 factorial treatment structure. Cattle were blocked by weight and within block were randomly allocated to 1 of 8 treatment combinations (6 pens per treatment, 5 head per pen). Treatment factors were the basal diet (40 % or 70 % concentrate), the DIP level in the diet, and the source of supplemental DIP. The source of DIP in the diet was either regular feed grade urea (UREA) or slow release urea (OPTIGEN®II) and the level of DIP in the diet was either 90 % (Low DIP) or 120 % (High DIP) of the animals' DIP requirement (NRC, 2000). The DIP requirement was defined as 13% of TDN. Diets were then formulated using urea and OPTIGEN®II diets were generated by the isonitrogenous and isoenergetic substitution of urea and EnergyBOOSTER100 for Optigen®II. The hypothesis was that Optigen®II will be used more effectively than urea at 90 % DIP and that DIP may be used more efficiently on the 70% concentrate diet.

Diets, feeding and sample collection

The 40% and 70% concentrate levels in the experimental diets (Table 7.1) were achieved by adding a combination of wheat straw and corn stalks (40% Concentrate) or

high moisture shelled corn (70% Concentrate) to a corn silage-based diet. The level of

DIP and DIP source in the diet were achieved by adding either UREA or OPTIGEN®II at

different levels for the low and high DIP diets. OPTIGEN®II and UREA diets were

formulated to be isonitrogenous within similar combination of DIP level and basal diet

and a small amount of powdered fat (Energy Booster 100, Milk Specialties Global, Eden

Prairie, MN) was added to urea diets to equalize energy intake between DIP sources.

Cattle were fed *ad libitum* and diets were offered once daily. Total DM offered was

measured and recorded daily. Feed ingredients were sampled weekly and DM content

was used to adjust ingredient composition of the diet. In addition, weekly feed ingredient

samples were frozen (-5°C) and composited for the entire experiment to determine

dietary nutrient composition (Table 7.2). Each week before feeding, feed refusals were

removed from the bunk, weighed and composited by treatment, and a sample was frozen

(-5°C). Animals were weighed on d 0, 1, 69 and 70. In addition, blood samples were

collected by jugular venipuncture into 7 mL heparinized Vacutainers® (#367676, BD,

Franklin Lakes, NJ) on day 70. Plasma was collected by centrifuging at 4,000 x g for 20

min and stored frozen (-5°C) until analysis.

Laboratory analyses

Feed and orts composite samples were dried in a forced-air oven at 100°C for 24

h to determine DM composition. Neutral detergent fiber and ADF were determined

according to Van Soest et al. (1991) using an Ankom A200 Fiber Analyzer (Ankom

Technology, Macedon, NY). Crude protein (N x 6.25) was determined by analysis of N

content with a Leco FP-528 (Leco Corp., St. Joseph, MI) according to method 990.03 of

the AOAC (2005). Mineral concentrations were determined using a HNO_3/HCl

microwave digestion followed by analysis with an inductively coupled plasma (ICP) radial spectrometer (Thermo IRIS HX, Thermo Fisher Scientific Inc., Waltham, MA). Plasma urea concentrations were determined by colorimetric assay using a Technicon AAII Autoanalyzer (Marsh et al., 1965).

Calculations and Statistical Analysis

Total DMI was calculated by subtracting total orts DM from DM offered to each pen on a weekly basis. Weight gain over the entire experiment was calculated as the difference between the average weight on d 0 and 1 and the average of d 69 and 70. Data were analyzed as a randomized complete block design with a 2 x 2 x 2 factorial treatment structure using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) using pen as the experimental unit. Differences among treatments were considered to be significant when $P < 0.05$, whereas when $P > 0.05$ but < 0.15 differences were considered to indicate a trend.

RESULTS AND DISCUSSION

The nutrient composition of the experimental diets is presented in Table 7.2. The DE and TDN content of the diets was equivalent within concentrate level and were not different between DIP sources or levels. By design, CP concentration was higher for high DIP vs. low DIP diets. In addition, CP concentration was higher for the 70% concentrate diet than 40% concentrate diet.

Growth measurements were divided into three periods: Period 1 (P1) was d 0 – 28; period 2 (P2) was d 29 to 56; period 3 (P3) was d 57 – 70. Total refers to the entire experiment (d 0 – 70). The main effects of dietary concentrate level and DIP level and

source on performance of feedlot steers are presented in Table 7.4. The effects of concentrate level, and DIP level and source on growth performance are presented in Table 7.3. There was a tendency for UREA to have a higher DMI than OPTIGEN®II in P1 (8.00 vs. 7.77 kg/d, P = 0.06). However, the increased intake did not lead to any improvement in gain or efficiency of gain thus, the increased intake would have a negative environmental impact. In comparison to 40% concentrate, the 70 % concentrate diet increased DMI in all three experimental periods as well as over the whole experiment (P1: 8.32 vs. 7.45 kg/d, P < 0.0001; P2: 9.86 vs. 7.66 kg/d, P < 0.0001; P3: 10.61 vs. 8.58 kg/d, P < 0.0001; Total: 9.38 vs. 7.76 kg/d, P < 0.0001). Increased DMI of 70% concentrate diet is likely due to a combination of factors. The 70% concentrate diets likely had a higher palatability than the 40% concentrate diets, due to the inclusion of less palatable ingredients in the 40% concentrate diet, such as wheat straw and corn stalks. In addition, the 70% diet was in a more condensed form, i.e. it would not cause as much rumen fill as a similar amount of DM from the high forage diet. Finally, the 70% concentrate diet had a higher CP concentration (Table 7.2) and increasing the protein content of ruminant diets has been shown to increase DMI (Köster et al., 1996). The 70% concentrate diet also resulted in increased BW gain than 40% concentrate in all periods (P1: 1.90 vs. 1.34 kg/d P < 0.0001; P2: 1.75 vs. 1.09 kg/d, P < 0.0001; P3: 1.56 vs. 1.21 kg/d, P = 0.0001; Total: 1.77 vs. 1.21 kg/d, P < 0.0001). Increased growth is likely due to increased intake of both energy and protein, resulting from increased DMI as well as increased nutrient density in the 70% concentrate diet (Gorocica-Buenfil and Loerch, 2005). It is likely that there was sufficient forage in the 70% concentrate diet (65% corn silage) that rumen health was maintained, allowing steers to take advantage of

the higher nutrient density in the 70% concentrate diets (Koenig et al., 2003). Feed efficiency (G:F) was also better for the 70% concentrate vs. the 40% concentrate diet in P1 (228.5 vs. 179.5 g/kg, P < 0.0001), P2 (177.4 vs. 154.9 g/kg, P < 0.0001) and Total (189.1 vs. 156.4 g/kg, P < 0.0001). Increased nutrient concentration may be responsible for the increase in G:F for 70% vs. 40% concentrate diets. Higher DE and protein concentrations (Table 7.2) indicate that the 70% concentrate diets had more usable nutrients per unit of feed, resulting in improved gain per unit feed. In addition, increased growth rate of 70% concentrate leads to higher dilution of maintenance energy and protein requirements (Rotz, 2004), allowing a higher percentage of the nutrients available to be used for growth rather than maintenance (Tedeschi et al., 2006), resulting in increased feed efficiency.

When compared to low DIP, high DIP resulted in increased DMI across all time periods (P1: 8.09 vs. 7.68 kg/d, P = 0.002; P2: 9.05 vs. 8.47 kg/d, P < 0.0001; P3: 9.89 vs. 9.30 kg/d, P = 0.0004; Total: 8.82 vs. 8.31, P < 0.0001). Intake of DIP has been shown to be directly related to DMI, especially when diets deficient in ruminally available N are supplemented with DIP (Köster et al., 1996; Valkeners et al., 2008). Increases in DMI may be related to the effect of DIP supply on diet digestibility. It has been shown that supplementing diets deficient in DIP can increase diet digestibility (Slyter et al., 1979). The high DIP diets also resulted in higher BW gain (P1: 1.79 vs. 1.45 kg/d, P < 0.0001; P2: 1.51 vs. 1.33 kg/d, P = 0.0002; Total: 1.61 vs. 1.38 kg/d, P < 0.0001) and G:F (P1: 220.2 vs. 187.8 g/kg, P < 0.0001; P2: 165.0 vs. 154.9, P = 0.046; Total: 181.2 vs. 164.2 g/kg, P < 0.0001) in P1, P2 and Total. Increased growth and efficiency in high DIP diets, as with the 70% concentrate diets, is likely related to

105

increased supply of nutrients, due to increases in diet intake and likely diet digestibility. Increased nutrient supply can result in higher growth rate and more efficient growth due to dilution of maintenance energy and protein requirements (Rotz, 2004).

There was an interaction (P = 0.007) between DIP source and basal diet on BW gain in P2 (Figure 7.1). At 40% concentrate, UREA resulted in higher BW gain than OPTIGEN®II, while at 70% concentrate; OPTIGEN®II had higher BW gain than UREA. The same interaction was evident over the whole experiment (Total: P = 0.04). In addition, a similar interaction (Figure 7.2) was evident on G:F for P2 (P = 0.018) and Total (P = 0.07). It is unclear why slow release urea would be more effective in high concentrate diets. One might expect slower release of ruminal NH_3 to favor the slower degrading diet with a high forage inclusion (40% concentrate). It is possible, and somewhat likely, that the growth of 40% concentrate-fed animals was limited by energy intake. Therefore, it is possible that UREA resulted in a higher ruminal NH_3 concentration than OPTIGEN®II, resulting in improved digestibility of the forage component of the diet (Köster et al., 1996), due to the fact that most of the cellulolytic bacteria have a strict requirement for NH_3 (Van Soest, 1994) and may respond in increases in ruminally available ammonia. Increased forage digestibility, in turn, may have led to increased energy availability, and thus, improved growth and feed efficiency for UREA compared to OPTIGEN®II at 40% concentrate. On the 70% concentrate diet, steer growth was more likely to be limited by protein supply than on the 40% concentrate diet. Therefore, improved capture of DIP from OPTIGEN®II may have led to increased microbial protein production (Cherdthong et al., 2011), resulting in improved performance for OPTIGEN®II at 70% concentrate.

There was a tendency for an interaction (Figure 7.3; P = 0.068) between DIP source and DIP level on DMI of steers in P3. Dry matter intake was essentially the same between OPTIGEN®II and UREA at low DIP but was higher for UREA at high DIP. It is unclear why UREA would increase DMI at high DIP but not at low DIP, as improved supply of ruminal ammonia would likely be more effective at stimulating DMI when DIP is limiting (Köster et al., 1996). There was a tendency for an interaction (Figure 7.4) between basal diet and DIP level on G:F in P2 (P = 0.11) and P3 (P = 0.10) which became significant over the entire experiment (Total: P = 0.008). The interaction indicates that increasing DIP concentration was more effective at improving G:F at 40% concentrate than at 70% concentrate. The 40% concentrate diets had lower average CP concentration than the 70% concentrate diets (Table 7.2). Therefore, 40% concentrate diets were more likely to respond to DIP or protein supplementation than were the 70% concentrate diets. The increase in feed efficiency was likely related to improved digestibility of the diets in response to DIP supply, as the BW gain and DMI were not affected.

Plasma urea concentration was higher for high DIP (P < 0.0001), 70% concentrate (P = 0.033) and tended to be higher for Optigen®II (P = 0.054). There was no interaction between treatments on plasma urea concentration. Higher plasma urea concentration for high vs. low DIP diets was expected, as DIP intake is known to increase ruminal ammonia and subsequently, plasma urea concentration (Wickersham et al., 2008b). The 70% concentrate diets had higher plasma urea concentration, which was probably related to the total CP concentration in the 70% vs. the 40% concentrate diet. Crude protein concentration was higher for 70% concentrate diets and was probably responsible for

increased plasma urea on those diets (Marini and Van Amburgh, 2005). Finally, the increase in plasma urea concentration for Optigen®II over UREA may seem counterintuitive, as slow release urea has been shown to decrease plasma urea concentrations (Huntington et al., 2006b). However, blood samples were taken from the steers at the same time as they were weighed. On weigh days steers were away from feed for between 2 and 6 h by the time the blood samples were taken. Therefore, it may be that the prolonged release of urea from Optigen®II led to elevated ruminal ammonia and therefore plasma urea concentrations in animals away from feed, whereas steers fed urea may have already peaked in plasma urea concentration before blood sampling, and thus the peak plasma urea was not identified.

CONCLUSIONS

As expected, the 70% concentrate diets resulted in improved DMI, BW gain and feed efficiency when compared to the 40% concentrate diets due to its higher available nutrient density. High DIP diets also resulted in increased DMI, growth and feed efficiency suggesting that the low DIP diets were indeed DIP deficient and showing that reducing DIP intake to 90% of requirements has negative consequences for production and feed efficiency. There is some indication that Optigen®II may improve production and feed efficiency in higher concentrate diets but not high forage diets.

Table 7.1. Ingredient compositions of experimental diets fed to receiving steers to evaluate source of degradable intake protein

Ingredient	Optigen®II				Urea			
	40% Concentrate		70% Concentrate		40% Concentrate		70% Concentrate	
	Low DIP	High DIP	Low DIP	High DIP	Low DIP	High DIP	Low DIP	High DIP
Corn Silage	71.18	70.31	65.18	64.31	71.18	70.31	65.18	64.31
Wheat straw	12.00	12.00	0.00	0.00	12.00	12.00	0.00	0.00
Corn Stalks	12.00	12.00	0.00	0.00	12.00	12.00	0.00	0.00
High Moisture Corn	0.00	0.00	30.00	30.00	0.00	0.00	30.00	30.00
Amino Plus [a]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Urea	0.00	0.00	0.00	0.00	0.75	1.50	0.75	1.50
Energy booster 100 [b]	0.00	0.00	0.00	0.00	0.12	0.24	0.12	0.24
Optigen®II	0.87	1.74	0.87	1.74	0.00	0.00	0.00	0.00
Trace mineral premix [c]	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Limestone	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium Sulfate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin Premix [d]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

[a] Heat treated soybean meal, AG Processing Inc., Omaha, NE
[b] Powdered fat, Milk Specialties Global, Eden Prairie, MN
[c] Trace mineral premix composition: 0.06% Ca, 56.34% Cl, 36.53% Na, 1.2% S, 68.9 ppm Co, 1837.7 ppm Cu, 119.9 ppm I, 9290.2 ppm Fe, 4792.3 ppm Mn, 18.5 ppm Se, 5520.2 ppm Zn
[d] Vitamin premix composition: 8811ppm vitamin A, 1762ppm vitamin D, 1100ppm vitamin E

Table 7.2. Nutrient composition of experimental diets

	Optigen®II				Urea			
	40% Concentrate		70% Concentrate		40% Concentrate		70% Concentrate	
	Low DIP	High DIP	Low DIP	High DIP	Low DIP	High DIP	Low DIP	High DIP
DE, Mcal/kg	2.83	2.94	3.28	3.39	2.82	2.97	3.27	3.42
% of DM								
DM	52.4	52.8	47.8	48.2	52.5	52.8	47.8	48.1
NDF	50.2	49.9	33.3	33.0	50.2	49.7	33.3	32.8
ADF	29.5	29.4	18.2	18.1	29.6	29.1	18.2	17.8
CP	10.3	12.9	11.1	13.6	9.7	13.5	10.5	14.2
Ca	0.49	0.52	0.30	0.34	0.53	0.51	0.35	0.32
P	0.32	0.31	0.38	0.37	0.31	0.31	0.37	0.37
Mg	0.14	0.14	0.13	0.13	0.14	0.14	0.13	0.13
K	0.99	0.99	0.80	0.79	0.99	0.98	0.79	0.78
Na	0.05	0.38	0.01	0.34	0.05	0.39	0.01	0.35
S	0.23	0.24	0.22	0.24	0.26	0.24	0.25	0.24
TDN	65.5	67.2	75.2	77.0	65.4	67.7	75.2	77.5
PPM								
Cu	19.2	21.2	15.6	17.6	23.0	18.3	19.4	14.7
Fe	158.9	166.6	114.9	122.7	171.4	170.6	127.5	126.6
Mn	55.8	60.6	40.0	44.8	59.9	55.9	44.1	40.1
Zn	67.9	68.2	61.0	61.3	61.6	66.9	54.6	60.0
Mb	0.80	0.80	0.45	0.45	0.77	0.81	0.42	0.46

Table 7.3. Effects of DIP source, concentrate level and DIP level on performance of feedlot cattle

| | Optigen®II | | | | Urea | | | | SEM | P-value | | | | | |
| | 40% Concentrate | | 70% Concentrate | | 40% Concentrate | | 70% Concentrate | | | Source | Diet | DIP | Source x Diet | Source x DIP | Diet x DIP |
	Low DIP	High DIP	Low DIP	High DIP	Low DIP	High DIP	Low DIP	High DIP							
Day 0 to 28															
DMI, kg/d	7.12	7.42	8.09	8.46	7.34	7.93	8.18	8.57	0.172	0.06	<0.0001	0.002	0.29	0.54	0.79
BW gain, kg/d	1.12	1.48	1.72	2.10	1.22	1.54	1.75	2.05	0.055	0.36	<0.0001	<0.0001	0.24	0.45	0.96
G:F, g/kg	157.4	199.3	212.9	248.1	167.3	194.1	213.7	239.2	5.56	0.83	<0.0001	<0.0001	0.42	0.12	0.61
Day 29 to 56															
DMI, kg/d	7.77	7.37	10.28	9.48	7.99	7.51	10.16	9.52	0.167	0.55	<0.0001	<0.0001	0.36	0.87	0.25
BW gain, kg/d	0.96	1.09	1.70	1.89	1.03	1.30	1.64	1.75	0.060	0.70	<0.0001	0.0002	0.007	0.74	0.57
G:F, g/kg	130.1	140.4	180.0	184.7	136.7	162.8	172.6	172.4	6.98	0.64	<0.0001	0.046	0.018	0.58	0.11
Day 57 to 70															
DMI, kg/d	8.52	8.36	10.76	10.30	9.06	8.40	11.24	10.13	0.217	0.16	<0.0001	0.0004	0.66	0.068	0.23
BW gain, kg/d	1.16	1.35	1.60	1.53	1.03	1.30	1.49	1.61	0.117	0.54	0.0001	0.14	0.66	0.41	0.22
G:F, g/kg	139.3	156.5	155.5	142.0	123.4	143.0	147.4	143.5	11.48	0.27	0.42	0.55	0.49	0.71	0.10
Day 0 to 70															
DMI, kg/d	7.78	7.46	9.63	9.07	8.16	7.62	9.72	9.09	0.156	0.15	<0.0001	<0.0001	0.35	0.51	0.47
BW gain, kg/d	1.06	1.30	1.69	1.90	1.11	1.39	1.65	1.84	0.040	0.71	<0.0001	<0.0001	0.04	0.79	0.31
G:F, g/kg	142.5	166.6	186.5	197.9	145.7	170.7	182.1	189.7	3.82	0.62	<0.0001	<0.0001	0.07	0.79	0.008
Plasma urea, mM	1.47	2.05	1.65	2.46	1.49	1.94	1.49	2.06	0.114	0.054	0.033	<0.0001	0.15	0.26	0.30

*P-values for interaction term: Source x Diet x DIP are not presented due to lack of significant differences (P > 0.10)

Table 7.4. Least squares means for main effects of diet, and supply of DIP on performance of growing cattle

Item	Basal Diet		DIP concentration		Source of NPN		SE
	70%	40%	90%	120%	Optigen®II	Urea	
Day 0 to 28							
DMI, kg/d	8.32***	7.45	7.68**	8.09	7.77*	8.00	0.086
BW gain, kg/d	1.90***	1.34	1.45***	1.79	1.60	1.64	0.028
G:F, g/kg	228.5***	179.5	187.8***	220.2	204.4	203.6	2.78
Day 29 to 56							
DMI, kg/d	9.86***	7.66	8.47***	9.05	8.72	8.80	0.083
BW gain, kg/d	1.75***	1.09	1.33***	1.51	1.41	1.43	0.030
G:F, g/kg	177.4***	142.5	154.9*	165.0	158.8	161.1	3.49
Day 57 to 70							
DMI, kg/d	10.61***	8.58	9.30***	9.89	9.48	9.71	0.109
BW gain, kg/d	1.56***	1.21	1.32	1.44	1.41	1.36	0.059
G:F, g/kg	147.1	140.5	141.4	146.3	148.3	139.3	5.74
Day 0 to 70							
DMI, kg/d	9.38***	7.76	8.31***	8.82	8.48	8.65	0.078
BW gain, kg/d	1.77***	1.21	1.38***	1.61	1.49	1.50	0.020
G:F, g/kg	189.1***	156.36	164.2***	181.24	173.38	172.05	1.91

* P < 0.1
** P < 0.05
*** P < 0.001

Figure 7.1. Interaction between basal diet and DIP source (Source) on body weight gain in growing steers
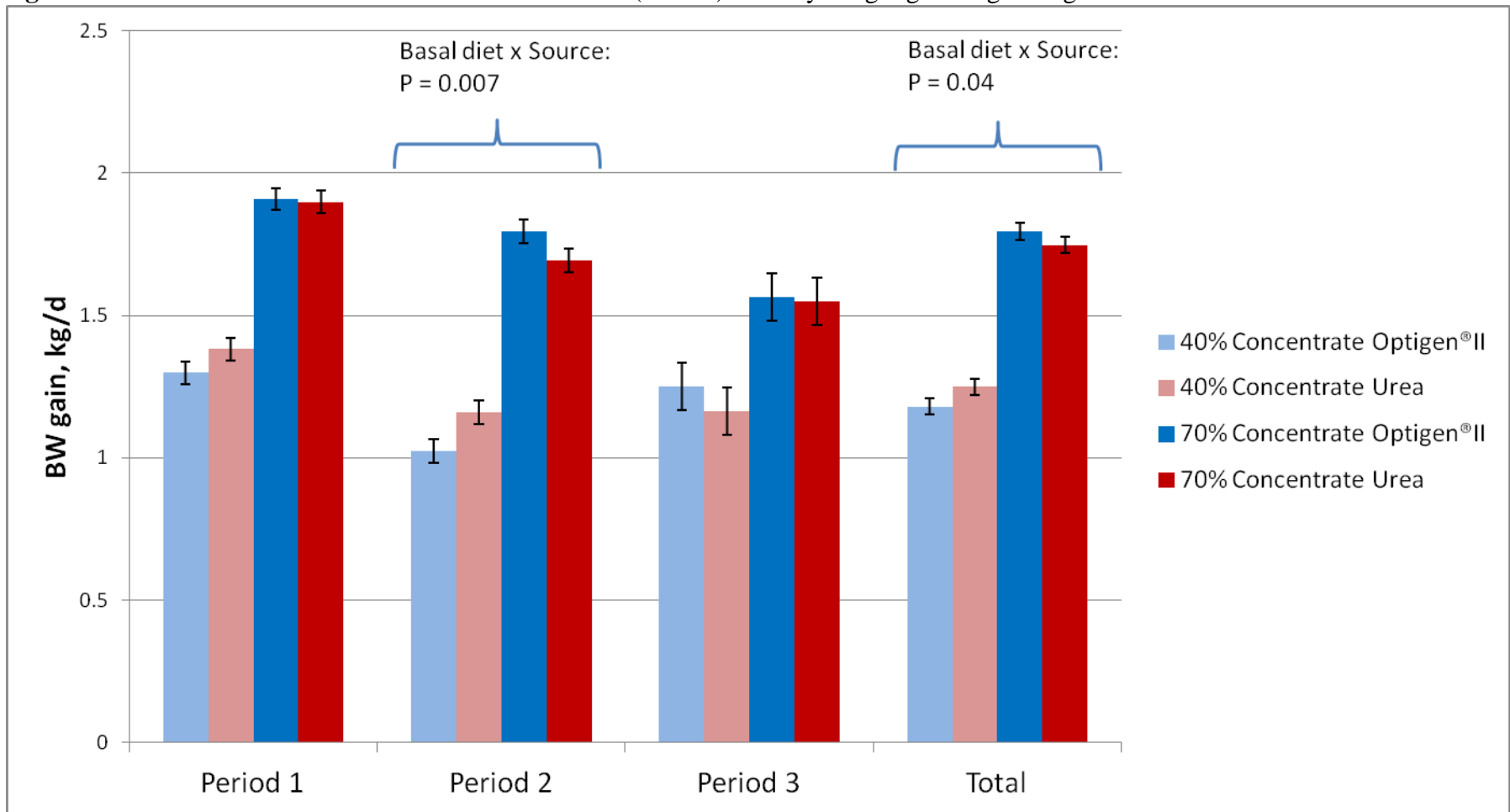
Figure 7.2. Interaction between basal diet and DIP source on feed efficiency (Gain:Feed, g/kg) of growing steers
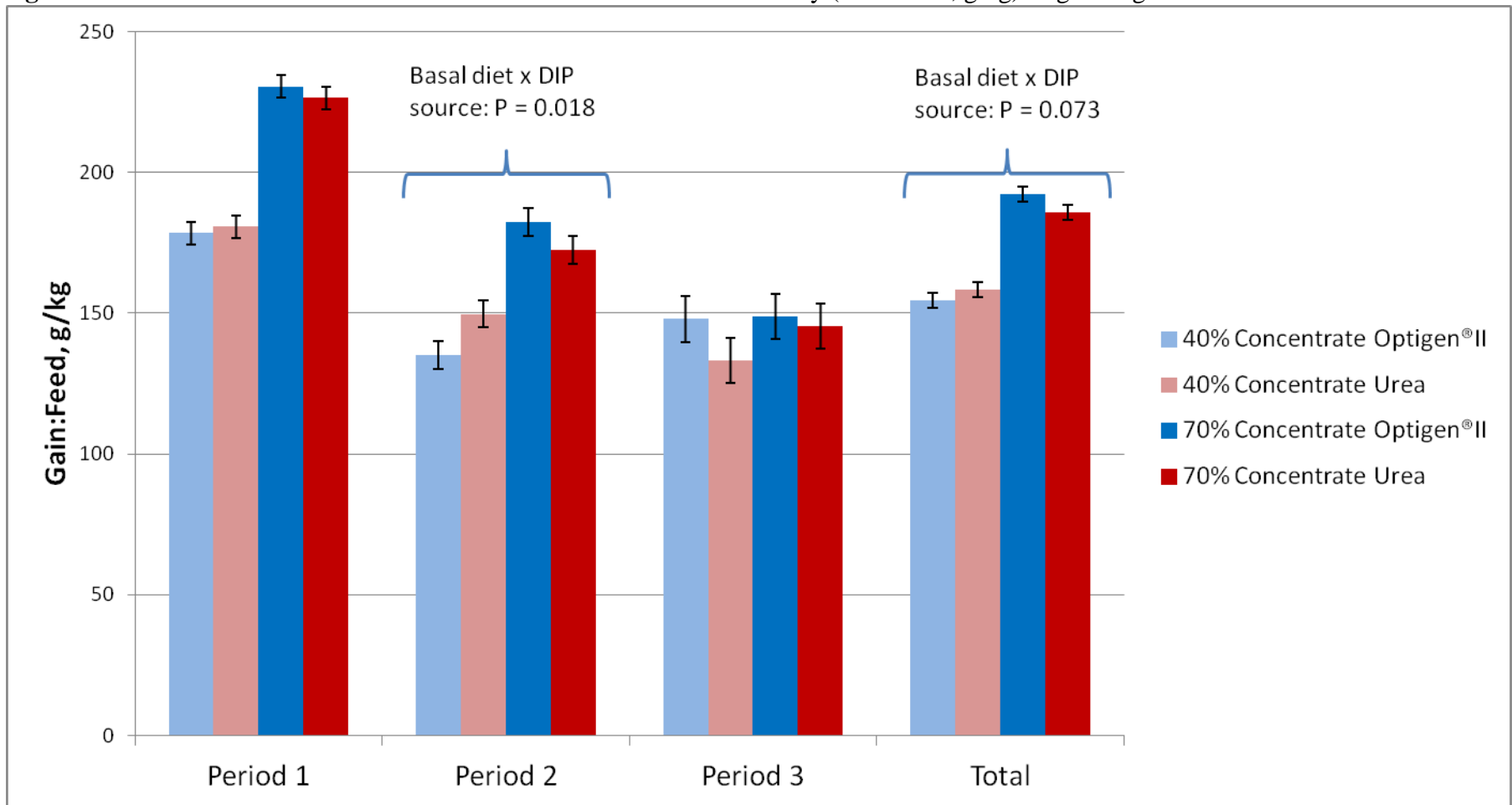
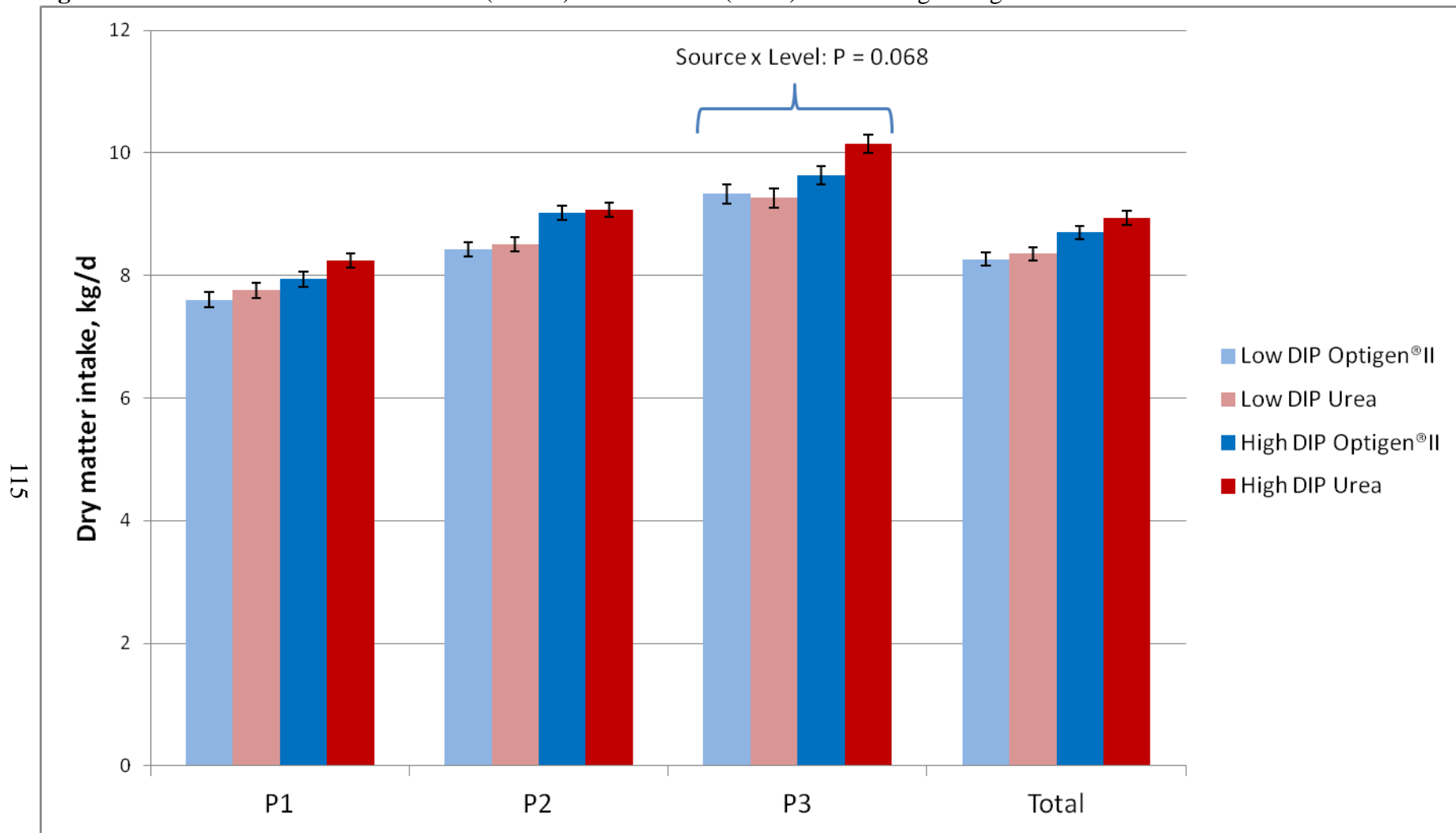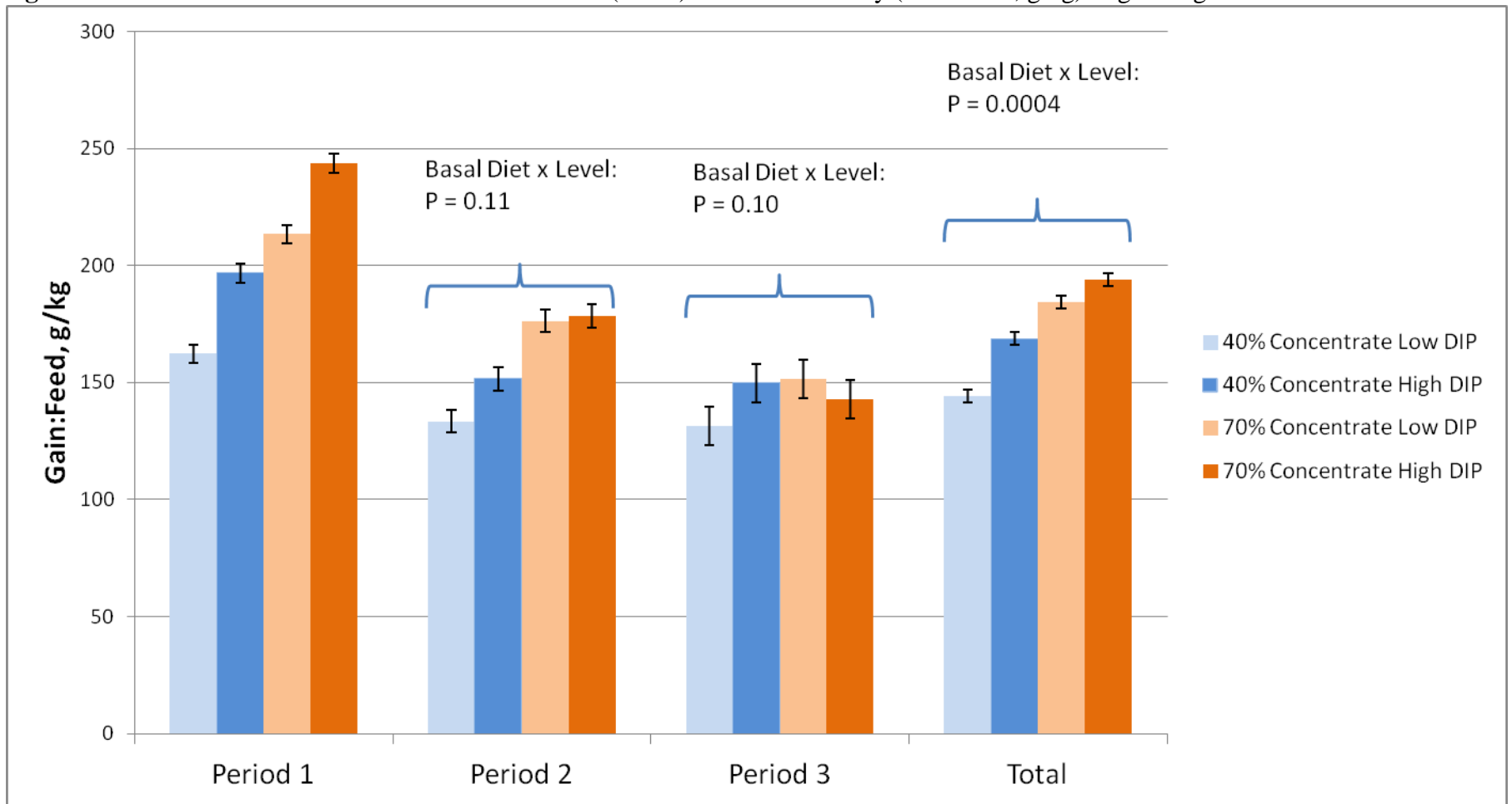**Figure 7.3.** Interaction between DIP source (Source) and DIP level (Level) on DMI in growing steers

Figure 7.4. Interaction between basal diet and DIP level (Level) on feed efficiency (Gain:Feed, g/kg) in growing steers

117

CHAPTER 8: CONCLUSIONS AND IMPLICATIONS

In order for a slow release urea product to provide a benefit to ruminant production it has to result in improvement in productivity, i.e. gain, production efficiency, or the efficiency of N utilization. Improved production may result in improved performance or yield from a similar level of N intake and with similar N excretion, thus resulting in lower N excretion per unit of production. Alternatively, using slow release urea may improve efficiency of N usage by reducing the total amount of urea required for a certain level of production, thus reducing both N intake and excretion per unit product.

There is some indication from N balance experiments, that when fed above the NRC requirements for DIP, Optigen®II may result in improved N retention. This occurred without the concomitant decrease in N efficiency that is commonly reported when the DIP content of diets is increased. Increased production would lead to increased productive output per unit N excretion, potentially reducing N output per unit of product. However, it appears that it may not be favorable to feed Optigen®II when DIP is limiting, as reduced degradation rate of urea may exacerbate a ruminal N deficiency resulting in reduced diet digestibility, decreased N retention and increased urinary N excretion when compared to urea.

Degradable intake protein from SBM in receiving diets was effectively replaced by up to 1.35 % urea from Optigen®II or urea without any adverse effects on intake or production. There was no overall advantage to using Optigen®II to supply urea.

In growing cattle the reduction in productive performance when dietary DIP was reduced by just 10 % below requirements shows that reducing DIP may not be the best

strategy for reducing N pollution from growing beef cattle, as farmers will be unlikely to adopt a practice that results in the loss of production. However, there is some indication that Optigen®II may result in superior production when added to high concentrate diets based on corn silage and high moisture corn. However, on low concentrate diets containing corn silage, and low quality roughages like wheat straw and corn stalks, urea diets resulted in improved production over Optigen®II. However, it is unclear whether this interaction between basal diet and urea source would occur with diets based on other ingredients.

There is a relatively wide range in both rate and extent of ruminal disappearance of urea from Optigen®II from different batches and between different diets. It is important, when interpreting results from experiments with Optigen®II, that rate and extent of degradation of the product under evaluation are considered. The extent of degradation of the products that were used in these experiments (Chapter 4 – 7) was similar ($\pm$ 80%), however, the rate of degradation differed somewhat. In Chapter 4 and 5, the product (# 280752-2) had a somewhat higher degradation rate, while the products used in Chapter 6 (#321245-3) and 7 (#400569-4) had progressively slower degradation rates. In meal fed ruminants, Optigen®II consistently reduced ruminal ammonia and plasma urea concentrations, thus making Optigen®II less likely to cause toxicity than urea. Finally, the total extent of ruminal urea disappearance from Optigen®II ($\pm$ 80%) has to be kept in mind when considering the results of these experiments. In some cases, where Optigen®II outperformed feed grade urea, it is important to realize that although urea and Optigen®II may have been fed isonitrogenously, ruminal availability of Optigen®II may have been less than that of urea. Therefore, if the extent of ruminal

119

availability of urea from Optigen®II were improved, the potential for increased N efficiency may be greater than demonstrated with the current product. However, this statement would have to be evaluated experimentally if such a product were to become available.

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121

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PERSONAL INFORMATION

Surname: Holder

First name(s): Vaughn Barry

Date of Birth: 31 October 1981

Birth Place: Sasolburg, South Africa

Title: Mr.

Gender: Male

Present employment: PhD candidate / Research Assistant in animal nutrition at the Department of Animal and Food Sciences, University of Kentucky.

ACADEMIC INFORMATION

2007 – Current: Ph.D.: Pursuing a Ph.D. in Animal Science (Ruminant Nutrition) at the University of Kentucky, Lexington, Kentucky. Major Professor: Dr David Harmon.

Research Focus: Evaluation of the effect of slow release urea on N metabolism in cattle.

2005 – 2007: M.Sc.: M.Sc. (Agric) Animal Science, Nutrition, University of Pretoria. Completed in July 2007.

Thesis title: The effects of specific *Saccharomyces cerevisiae* strains and monensin on rumen fermentation *in vitro*

In fulfillment of the research component of the M.Sc. degree I completed a 10 month research internship at Alltech Biosciences Center, Nicholasville, KY. Research advisor: Dr. J.M. Tricarico.

Undergraduate degree: B.Sc. Animal Science, Department of Animal and Wildlife Sciences, University of Pretoria.

Completed B.Sc. in December 2004.

High School education: (grade 12) completed at Highveld Park High School, Secunda, South Africa (1999)

Additional qualifications:

- Artificial Insemination of Cattle (South African Department of Agriculture, 2002)
- Wool Classer (BKB Ltd.) (2004)
- Business presentations (Dynamic presentations cc)(2004)

WORK EXPERIENCE

2007-present – Research assistant at the department of Animal and Food Sciences, University of Kentucky.

The focus of my PhD work has been intensive the study of N metabolism in cattle. In the course of my experimental work, I have conducted multiple studies investigating N balance, rumen metabolism, and digesta kinetics comparing urea and Optigen®II at multiple protein levels. Additionally we conducted some stable isotope infusions studying systemic urea metabolism and characterizing urea synthesis, recycling and

excretion. We also studied the in situ degradation of urea and various batches of Optigen®II and conducted an experiment to determine the effect of basal diet on the degradation characteristics of both urea and Optigen®II. Finally we are currently investigating the effect of Optigen®II under more practical conditions by conducting a large (+- 300hd) receiving and growing steer study comparing Optigen®II and urea under varying conditions.

I addition to my own research, I have had the opportunity to help other students and research scientists in our research group in a variety of different studies and have been exposed to the various methodologies involved therein, including:

- Nitrogen balance studies on dairy cows.

- Surgical preparation of research animals including rumen cannulation (performed myself) and surgical implantation of indwelling jugular catheters, carotid artery, mesenteric artery, mesenteric vein, hepatic vein and portal vein catheterizations.

- Subcutaneous and liver biopsies on live animals for studies on fat metabolism in beef cows.

- Washed rumen studies to study ruminal nutrient absorption in response to endophyte intoxication

- Various grazing and confinement feeding studies with beef cattle.

2006-2007 - Technical assistant at the University of Pretoria Nutrilabs (Feed analysis laboratory). Responsibilities:

- Kjeldahl CP analysis

- Gross Energy determinations using bomb calorimetry

- Preparation of Liver Samples for mineral analysis

- Ether extract fat determination

- In Vitro digestibility determination

- Neutral detergent (NDF) and Acid detergent (ADF) fiber analysis

- Directed an undergraduate nutritional studies lab which involved conducting

  digestibility trials with sheep in metabolism crates.

- Involved in egg production trials for determining inclusion levels of non-

  traditional raw materials in the diets of layers.

2005 - 10 month research internship under Dr Juan Tricárico at Alltech Biosciences

center, Nicholasville, KY.

During my research at Alltech I acquired the following skills:

- Strict aseptic and anaerobic microbiology techniques necessary for culturing pure strain and mixed culture bacteria and fungi
- The use of gas pressure fermentation measurements to analyze ruminant feed additives
- Setting up, operation and data analysis from continuous culture fermentations with rumen fluid.
- Development of quality control procedures for analysis of feed additives (Yea Sacc).

TEACHING EXPERIENCE

2011 - Teaching Assistant for ASC 378: Animal Nutrition and Feeding. Involved

preparing class material and conducting the laboratory section of the class which focused

on diet formulation in multiple species.

2006 - Teaching Assistant for VGE 301: Nutritional Science. Responsible for the laboratory section which involved organizing, supervising and helping the students conduct a digestibility trial with sheep in metabolism crates and subsequent laboratory analyses required to complete the nutrient digestion calculations.

SCHOLARSHIPS AND AWARDS

Achieved 8 Distinctions in final year of B.Sc. Animal Science Degree (2004)

Best final year student in Animal Breeding (2004)

Best final year student in Animal Nutrition (2004)

Best Final year student in Animal Science (2004) from the South African Society of Animal Sciences

University of Pretoria Post-Graduate, Master's Bursary (2006)

D M Joubert University achievement award (2006)

University of Kentucky graduate school dissertation enhancement award (2010)

PUBLICATIONS

Abstracts

The effect of live yeast and monensin supplementation on rumen fermentation in vitro.

V Holder, J Tricãrico, L Erasmus. Abstract submitted and presented as a poster at the 41st Congress of the South African Society of Animal Sciences (2006).

Effects of crude protein concentration and non protein N source on N metabolism in Holstein steers. V Holder, S.W El-Kadi, J Tricãrico, E Vanzant, K McLeod, D Harmon. Abstract submitted and presented as a poster at the joint annual meeting ADSA-CSAS-ASAS in Monteal, Quebec, Canada (2009).

The effects of degradable N level and degradation rate on N balance and urea kinetics in Holstein steers.   V.B. Holder, J.M. Tricarico, D.H. Kim, N.B. Kristensen and D.L. Harmon.   Abstract presented as an oral presentation at the 2011 animal science joint annual meeting in New Orleans, LA.

Basal diet affects ruminal in situ degradation rate of urea and Optigen®II in steers. V. B. Holder, J. S. Jennings, J. M. Tricarico and D. L. Harmon. Abstract accepted for poster presentation at the animal science joint animal science meeting in Phoenix, AZ in 2012.

Manuscripts in preparation

The effects of specific *Saccharomyces cerevisiae* yeast culture strains on growth of anaerobic rumen bacteria in pure culture. V. B. Holder and J. M. Tricarico. Intend to submit to the Journal of Microbiology and Biotechnology, 2012.

Effects of crude protein concentration and slow release urea on nitrogen metabolism and digestion kinetics in Holstein steers. V. B. Holder, S.W El-Kadi, J Tricãrico, E Vanzant, K McLeod, D Harmon. Intend to submit to Journal of Animal Science, 2012.

The effects of degradable nitrogen level and degradation rate on nitrogen balance and urea kinetics in Holstein steers. V.B. Holder, J.M. Tricarico, D.H. Kim, N.B. Kristensen and D.L. Harmon. Intend to submit to Journal of Dairy Science, 2012.

Basal diet affects ruminal in situ degradation rate of urea and Optigen®II in steers. V. B. Holder, J. S. Jennings, J. M. Tricarico and D. L. Harmon. Intend to submit to Journal of Animal Science, 2012.

Effects of replacing soybean meal nitrogen with non protein nitrogen from urea or slow release urea on intake and performance of receiving cattle. V. B. Holder, J. S. Jennings and D. L. Harmon. Intend to submit to Journal of Animal Science, 2012.

The effects of basal diet and the supply of degradable intake protein on growth of steers. V. B. Holder, J. S. Jennings and D. L. Harmon. Intend to submit to Journal of Animal Science, 2012.

REFERENCES

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