# DEGRADABLE INTAKE PROTEIN SUPPLEMENTATION THROUGH THE INCLUSION OF UREA IN FINISHING DIETS CONTAINING DISTILLERS GRAINS. EFFECTS ON FEEDLOT CATTLE PERFORMANCE, RUMINAL FERMENTATION, AND FEED DIGESTIBILITY.

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## Dedication

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## Abstract

Degradable intake protein (DIP) represents the proportion of protein that is potentially fermented in the rumen. Ruminal DIP balance is calculated by the difference between DIP supply and requirements. The former is a function of dry matter intake and dietary DIP, and represents nitrogen (N) available for synthesis of microbial crude protein (MCP), which is used as a measurement of microbial growth or production of new microbial cells. Synthesis of MCP basically requires ammonia-N ($NH_3$-N), carbon skeletons, and energy. While the last two are mainly derived from fermentation of dietary carbohydrates, dietary N represents the main $NH_3$-N source. Consequently, DIP requirements represent rumen-degradable N needs for MCP synthesis, and are a function of available fermentable carbohydrates. High dietary inclusion of grain as well as more extensive grain processing methods can result in increased ruminal availability of rapidly-fermentable carbohydrates, which in turn may result in increased DIP requirements. In addition, corn-based diets may not supply adequate amounts of DIP because corn protein is considered to be approximately 60% undegradable. Despite great protein content and because of great undegradable protein concentration, small to moderate dietary inclusion of corn distillers grains (DG) may also result in DIP deficit. Experiments 1 and 2 described in Chapter 2 evaluated the effect of adding urea, a highly rumen-degradable N source, to a high-concentrate, moderate-DG-containing diet on feedlot cattle performance, ruminal fermentation, and feed digestibility. Results from both experiments indicate that due to a DIP deficit generated by the un-supplemented diet, the addition of urea resulted in enhanced ruminal fermentation and feed digestibility,

and consequently improved animal performance. Because rates of degradation of carbohydrates and conventional urea do not match, beneficial effects may arise from the use of slow-release urea (SRU) sources over conventional urea when added to DIP-deficient diets. Therefore, experiments 1 and 2 described in Chapter 3 evaluated the effect of increasing DIP concentration through the inclusion of one of two SRU sources in comparison with the inclusion of conventional urea in DG-containing feedlot diets on ruminal fermentation and feed digestibility. Likely due to lack of DIP deficit with the un-supplemented diet, results from these experiments do not demonstrate potential beneficial effects of SRU sources over conventional urea. Several confluent factors are discussed that may explain lack of need of urea supplementation in Chapter 3 experiments. Because previous studies have demonstrated improved ruminal fermentation, feed digestibility, and animal performance when supplementing conventional urea to rapidly-fermentable, moderate-DG-containing diets, more research is warranted to evaluate the use of SRU in diets for which a DIP deficit is expressed.

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# CHAPTER 1

# REVIEW OF LITERATURE

## 1. NITROGEN CLASSIFICATION

Nitrogen destined for use by ruminants can be classified based on (a) origin: dietary, microbial, or endogenous; (b) source: protein or non-protein N (NPN); or (c) site of digestion: rumen (rumen-degradable N) or lower tract (rumen-undegradable N).

True proteins and NPN such as peptides, free amino acids (AA), nitrogenous bases from plant nucleic acids, and urea represent various forms of dietary N. Other sources of dietary NPN are ammonia-nitrogen ($NH_3$-N) and nitrates. Fermented feedstuffs such as silages are common sources of $NH_3$-N, which results from microbial activity during the ensiling process (Yan and Agnew, 2004). Stressed and/or N-fertilized forages may contain variable amounts of nitrates (Bolan and Kemp, 2003), also contributing to dietary NPN pool.

Dietary crude protein concentration is estimated by multiplying N concentration in the diet or feed by 6.25; this calculation assumes that all measured N is in the form of proteins and that N concentration in proteins is 16%. Although widely accepted, neither of these assumptions is correct. As mentioned before, while a proportion of dietary N is in the form of protein (true protein) another proportion is in the form of NPN. In addition, there is variation in N content of proteins. For example, Van Soest (1994) cited N

concentrations of 14.4, 15.8, or 16.8% for proteins measured in corn leaves, alfalfa, or broad bean (*Vicia faba*), respectively**.**

Endogenous N includes mucoproteins and recycled N in the form of urea, both entering the rumen via saliva and, in the case of urea, also by diffusion through the rumen wall (van der Walt and Meyer, 1988). Rumen and intestinal epithelial cells as well as gastric, pancreatic, and intestinal enzymes also contribute to endogenous protein and NPN (e.g. small peptides, AA, and nucleic acids from epithelial cells) pools.

As part of the feed digestion process by ruminal and intestinal microorganisms, microbial populations grow leading to synthesis of microbial cells and microbial enzymes, contributing to the microbial N pool. Rumen microbial cells, bacteria, and protozoa contain 9.9 (Isaacson et al., 1975), 7.8, and 6.4% N (Ørskov, 1992), respectively; however, not all microbial N is in the form of proteins. Baldwin (1970) and Hespell and Bryant (1979) reported true protein concentration of 47.5 to 55.8%, and nucleic acid concentration of 9.3 to 27.6% on a dry matter basis. Ørskov (1992) reported 15.2 g of nucleic acids per 100 g of total microbial N. As stated previously, N concentration of proteins may differ from the generally assumed value of 16%; concentrations of 15% or 7.1% N are common for microbial protein or for proteins of the microbial cell wall, respectively (Van Soest, 1994). The term *ruminally synthesized Microbial Crude Protein* (MCP) refers to total microbial N multiplied by 6.25, considering 80% as true protein and 20% as NPN in the form of nucleic acids (NRC, 2000).

Rumen-degradable N represents the N that is potentially degraded in the rumen by ruminal microbes. Consequently, it can be used by ruminal microorganisms to synthesize microbial proteins; the latter being used by the ruminant after digestion and absorption in the small intestine. The NRC suggests the terms Rumen Degradable Protein (RDP) and Degradable Intake Protein (DIP) for dairy and beef cattle, respectively, to refer to dietary rumen-degradable N. Therefore, both terms include true protein as well as some NPN. On the contrary, rumen undegradable N designates the N that escapes from ruminal degradation, which can be digested and absorbed in the lower gastrointestinal tract. Because all NPN is likely digested in the rumen, the N to which the terms dietary Rumen Undegradable Protein (RUP; commonly applied in dairy cattle) and Undegradable Intake Protein (UIP; commonly applied in beef cattle) refer to is all in the form of true protein. In other words, RUP or UIP are considered to be 100% true protein.

Erroneously, the terms *bypass N* and rumen *undegradable N* or *escape N* are commonly used as synonyms. While the term *bypass* implies avoiding a particular compartment of the gastrointestinal tract, as the passage of ingested diet down a closed esophageal groove into the abomasum of infant ruminants, the term *undegradable* or *escape* implies passing undegraded from a particular compartment (NRC, 1985; Van Soest, 1994).

## 2. NITROGEN DIGESTION IN THE RUMINANT

In ruminants, the sites where N digestion takes place are the reticulo-rumen,

abomasum, small intestine, particularly the duodenum, and large intestine. Basically, N degraded in the reticulo-rumen includes dietary and endogenous N, and a variable amount of microbial N synthesized in the rumen that is also digested *in situ* (e.g. predation of bacterial cells by protozoa); the latter contributes to microbial recycling or turnover and, therefore, decreases efficiency of microbial protein synthesis (Leng and Nolan, 1984; Van Soest, 1994; Russell, 2002). As in the reticulo-rumen, N digested in the lower gastrointestinal tract includes dietary, endogenous and microbial N.

## 2.1. NITROGEN METABOLISM IN THE RETICULO-RUMEN

Metabolism of N in the reticulo-rumen can be first divided into two different events: (a) N degradation and (b) synthesis of microbial protein (Bach et al., 2005). Both events are closely related because the first one provides N, carbon backbones, and even some energy in the form of adenosine triphosphate (ATP) to ruminal microbes to synthesize microbial AA, proteins, and nitrogenous bases for nucleic acids, thus supporting microbial growth. Microbial N provides the majority of protein supplied to ruminants, accounting for 50 to 85% of total AA-N entering the small intestine (Storm et al., 1983b; van der Walt and Meyer, 1988). A third event related to N metabolism in the reticulo-rumen involves the absorption of $NH_3$-N from this compartment and recycling back to the rumen or excretion via kidneys and milk.

### 2.1.1. RUMINAL DIGESTION OF NITROGEN

Dietary true proteins which are potentially degraded in the rumen are hydrolyzed to

small peptides and free AA by microbial proteases and peptidases, thus contributing to the ruminal NPN pool. Fate of resulting free AA is (a) direct incorporation into microbial protein or (b) catabolism (fermentation). However, it is commonly accepted that most of the N in AA passes through the $NH_3$-N pool (catabolism) before being incorporated into bacterial protein (Baldwin and Allison, 1983). The first step in catabolism of AA is deamination to $NH_3$-N and a keto-acid (e.g. $\alpha$-ketoglutarate). Ammonia-N also contributes to the ruminal NPN pool and can be used to synthesize bacterial AA and proteins, or be excreted back into the surrounding medium, providing $NH_3$-N for AA synthesis by other ruminal microbes (Tamminga, 1979; Van Soest, 1994). The keto-acid can be used as a carbon skeleton for microbial AA synthesis, or be subsequently decarboxylated to carbon dioxide ($CO_2$), volatile fatty acids (VFA), branched-chain VFA (BCVFA), methane, and heat of fermentation (Tamminga, 1979; Baldwin and Allison, 1983; Van Soest, 1994). Even though keto-acid decarboxylation also produces ATP, energy yield from AA catabolism is low (Russell, 2002). This yield is estimated at 1 mole of ATP per mole of AA fermented and likely an additional yield of 1 mole of ATP per mole of propionate or methane generated (Tamminga, 1979). Some of the end-products of AA catabolism can be used for the synthesis of new microbial AA (Allison, 1969) or excreted into the ruminal medium. Excreted end-products can serve as substrates for AA synthesis by other microbes or leave the rumen by absorption and/or passage. Therefore, the reason why ruminal microorganisms hydrolyze proteins and further use or degrade AA is that degradation of protein is necessary to provide microbes with required

precursors for their own protein synthesis, such as $NH_3$-N, keto-acids, VFA, BCVFA, $CO_2$, or even intact AA (Tamminga, 1979). However, AA catabolism usually exceeds these requirements, which might be related to the fact that AA degradation provides some ATP which can be utilized by ruminal microbes for their own metabolic processes (Tamminga, 1979).

Rate of protein and peptide hydrolysis is rapid, resulting in peak concentrations of peptides, AA, and $NH_3$-N within 1 to 2 h post feeding (Chen et al., 1987a,b). Degradation of N in the rumen is performed by enzymes synthesized by both bacteria and protozoa; yet, no specific type of microbes occupies the "protein fermenter" niche (Baldwin and Allison, 1983). Many rumen microbes possess proteolytic activity and/or can ferment AA or peptides, but only a few can utilize AA as the only energy source (Baldwin and Allison, 1983). Saccharolytic bacteria such as *Prevotella* (*Bacteroides*) *ruminicola*, *Megasphera elsdenii*, and *Butyrivibrio fibrisolvens* have proteolytic activity, as well as the amylo-/dextrinolytic *Streptococcus bovis* and protozoa (Hungate, 1966; Tamminga, 1979; Baldwin and Allison, 1983; Dijkstra, 1994; Russell, 2002). The first two bacteria listed also exhibit AA-fermenting activity (Baldwin and Allison, 1983; Russell, 2002). Species from the genera *Clostridium* and *Peptostreptococcus* represent a particular type of ruminal microbes named obligate AA-fermenting bacteria. These microbes cannot hydrolyze proteins nor use carbohydrates for ATP production. However, they are able to utilize free AA as their sole energy source, the latter likely related to the presence of energy-conserving mechanisms (Russell, 2002). Because these strains produce ammonia

20-fold faster than other ruminal bacteria, they have a significant effect on $NH_3$-N production even though they are present at low numbers *in vivo* (Chen and Russell, 1988 and 1989; Russell et al., 1988; Russell et al., 1992; Wallace et al., 1995; Russell, 2002).

Even though both bacteria and protozoa are involved in protein degradation in the rumen, the mechanism by which protein is degraded differs between these two groups (Tamminga, 1979). Protein degradation by ruminal bacteria is an extracellular process carried out by bacterial proteases that are bound to the microbial cell (Tamminga, 1979; Hoover and Stokes, 1991). Protein is hydrolyzed to small peptides and free AA. These products are then transported inside the bacterial cell where peptides are further hydrolyzed to AA (Tamminga, 1979). Resulting AA are then incorporated into proteins or fermented. On the other hand, protozoa are capable of engulfing small feed particles, large molecules like proteins and starch, and even bacteria (Coleman, 1975; Van Soest, 1994). Protein hydrolysis takes place inside the protozoal cell; free AA resulting from proteolysis can be (a) incorporated into protozoal protein, (b) fermented, or (c) released into the rumen fluid again (Williams and Coleman, 1988). von Harmeyer (1971), cited by Leng and Nolan (1984), reported that protozoa excrete alanine, glutamic and aspartic acid, proline, and $NH_3$-N as end-products of their metabolism. Possibly related to the fact that protozoa cannot utilize $NH_3$-N for AA synthesis (Onodera et al., 1977), and energy yield from AA catabolism is low (Russell, 2002), Coleman (1975) reported that if the resulting AA are not incorporated into protozoal protein they are often excreted into the surrounding medium rather than being catabolized. Because protozoa prefer insoluble to

soluble protein (Dijkstra, 1994), and due to their inability to use $NH_3$-N for AA synthesis, protein degradation by protozoa results in a proportion of engulfed insoluble protein being excreted back to the rumen fluid as soluble protein in the form of free AA or $NH_3$-N (Bach et al., 2005). As rationalized by Bach et al. (2005), this may partially explain why defaunation decreases $NH_3$-N concentration in the rumen, as observed by Eugene et al. (2004).

Similar to dietary proteins, microbial proteins (e.g. enzymes and structural proteins) resulting from microbial death and protozoal predation, endogenous salivary mucoproteins, and proteins from desquamated rumen epithelial cells are hydrolyzed to peptides and AA, which can then be further utilized by ruminal microbes. Bacteria adhered to the rumen wall, which represent 1 to 2% of the total population (Russell, 2002), are responsible for the fermentation of dead rumen epithelial cells (Leng and Nolan, 1984).

Regarding NPN, fate of dietary, microbial, and endogenous small peptides and free AA is similar to that described for small peptides and free AA resulting from degradation of dietary, microbial, and endogenous proteins. As part of the NPN pool, dietary nitrates and dietary and endogenous urea, are rapidly degraded to $NH_3$-N by ruminal microbes (Leng and Nolan, 1984; van der Walt and Meyer, 1988). Urea is hydrolyzed to $NH_3$-N and $CO_2$ by the extremely active ruminal urease. No specific bacterial species occupies this particular niche (Hungate, 1966). Smith (1975) reported that dietary, endogenous, and microbial nucleic acids were degraded to $NH_3$-N, purines, pyrimidines, and their

derivatives by ruminal microbes *in vitro*. However, the latter three types of products accumulated only transiently *in vivo*, which suggests that they were absorbed through or metabolized by the rumen wall, or incorporated or degraded further by ruminal microbes *in vivo*.

Based on the information described in the previous paragraphs, sources of ruminal $NH_3$-N are:

- Catabolism of dietary, microbial, and endogenous AA
- Catabolism of dietary, microbial, and endogenous nucleic acids
- Hydrolysis of dietary and endogenous urea
- Degradation of dietary nitrates
- Dietary $NH_3$-N

On the other hand, routes for $NH_3$-N disappearance from the rumen are:

- Passage to the lower tract
- Absorption through the rumen wall
- Utilization for synthesis of bacterial AA in the rumen

### 2.1.2. SYNTHESIS OF MICROBIAL PROTEIN

In the rumen, ingested feed undergoes microbial fermentation, resulting in microbial growth, that is, production of new microbial cells. These microbial cells are then utilized as sources of AA and other nutrients by the ruminant. Many waste products

of microbial metabolism (e.g. VFA) can also be utilized by the animal for its own metabolism (Harfoot, 1978). Because microbial cells contain about 55% true protein (Hespell and Bryant, 1979), the production of new microbial cells is usually associated with "production of microbial protein". However, it must also be noted that other N-containing compounds as well as microbial polysaccharides and lipids are also synthesized as a part of microbial growth.

Protein synthesis involves the highly regulated concatenation of AA, sequence that is encoded in the microbial DNA. Amino acids utilized for the synthesis of microbial protein can be pre-formed AA or newly synthesized AA from $NH_3$-N and carbon skeletons. Most ruminal bacteria utilize $NH_3$-N as their principal source of N, even when the medium contains a complete mixture of AA (Bryant and Robinson, 1961, 1962, 1963). In this regard, ruminal bacteria differ from most heterotrophic bacteria in which AA synthesis is inhibited when preformed AA are available (Fry, 1964; Allison, 1969). Potentially due to peptides having a special function in bacterial nutrition (Pittman and Bryant, 1964), rate of uptake of peptides by ruminal microbes is faster than that of free AA; thereby, free AA do not represent a significant extracellular intermediate (Chen et al., 1987a). Bryant and Robinson (1961) and Allison (1969) suggested that there might be little or no survival value in maintaining systems to transport AA inside the microbial cells in an environment where AA concentration is almost always low. Therefore, the lack of inhibition of $NH_3$-N utilization for microbial protein synthesis by free AA availability might be related to the low activity (or absence) of that type of system.

Even though $NH_3$-N was demonstrated to be the main source of N for protein synthesis, incorporation of AA directly into microbial protein may account for more than 20% of microbial-N (Baldwin and Allison, 1983). However, the ability of preformed AA to stimulate growth differs among microbial groups. Immediately after ingestion, soluble carbohydrates (sugars) and proteins are available for degradation, ready to be used by fast-growing bacteria such as sugar-fermenting bacteria. On the other hand, fiber-digesting bacteria and their enzymes are not yet established on feed particles, thus utilizing little of the AA available from soluble proteins. By the time fiber-digesting bacteria begin to grow, and while they digest the more resistant forage components, AA are scarce; $NH_3$-N derived from AA catabolism is primarily available (Hungate, 1966). Therefore, it makes sense that small peptides and AA are relatively more important for the nutrition of fast-growing soluble, sugar-fermenting bacteria like *Prevotella* (*Bacteroides*) *ruminicola* and *Megasphera elsdenii*, while $NH_3$-N plays a more important role in protein synthesis of the relatively slow-growing, fiber-digesting bacteria such as *Ruminococcus albus* and *R. flavefaciens* (Bryant and Robinson, 1963; Hungate, 1966). Starch-digesting bacteria such as *Bacteroides amylophilus*, *Succinivibrio dextrinosolvens*, and *Streptococcus bovis* may represent an intermediate situation between fast and slow-growing bacteria, in terms of the importance of $NH_3$-N or AA for their nutrition. Because protozoa are not able to use $NH_3$-N for AA synthesis (Onodera et al., 1977), direct incorporation of AA into protozoal protein plays a crucial role. Compared with free AA, peptide-AA seem to be more efficiently used, incorporated faster, and/or contribute

more to the non-protein, non-ammonia N incorporated into cells of microbes whose growth is stimulated by preformed AA (Pittman and Bryant, 1964; Wright, 1967; Chen et al., 1987a).

*De novo* synthesis of AA involves various pathways and substrates (Allison, 1969). Synthesis of microbial AA requires $NH_3$-N, carbon skeletons, ATP, and, in the case of sulfur-containing AA, a sulfur source. As discussed in the previous section, dietary, endogenous, and microbial AA catabolized in the rumen represent various sources of $NH_3$-N. In addition, fermentation of other NPN sources such as dietary and endogenous urea, dietary, endogenous, and microbial nucleic acids, and dietary nitrates also contribute to the $NH_3$-N pool (Leng and Nolan, 1984). Many anaerobic bacteria, including the ruminal *Methanobacterium ruminantium*, can fix N gas (Li Pun and Satter, 1975; Leng and Nolan, 1984), reducing it to $NH_3$-N by a high energy-demanding reaction that is catalyzed by nitrogenase, a molybdenum-iron-containing enzyme which is repressed by increased $NH_3$-N concentrations (Halbleib and Ludden, 2000). However, it was demonstrated that contribution of atmospheric N fixed by rumen microorganisms to $NH_3$-N pool is negligible, even in animals fed low protein diets for a sustained period of time, for which $NH_3$-N ruminal concentration was expected to be low (Li Pun and Satter, 1975).

Due to its lipophilic nature, dissociated ammonia ($NH_3$) can pass across bacterial cell membranes by facilitated diffusion (Russell, 2002). Active transport of ammonium ion ($NH_4^+$), a highly inefficient process, has not been demonstrated in ruminal bacteria

(Russell, 2002). In most ruminal bacteria, assimilation of $NH_3$-N inside the microbial cell is mainly mediated by NAD-linked glutamate dehydrogenase (Allison, 1969; Russell, 2002). Therefore, biosynthesis of a specific AA occurs via transamination from glutamate to the appropriate carbon skeleton (Russell, 2002).

Carbon backbones for AA synthesis are mainly derived from intermediates in glucose metabolism such as the keto-acids pyruvate, oxaloacetate, and α-ketoglutarate, acetyl CoA, and succinyl CoA (Russell, 2002) as well as end-products like $CO_2$ and VFA (Allison, 1969). Therefore, synthesis of microbial protein depends on carbohydrate availability. Keto-acids derived via AA deamination, and $CO_2$ and VFA resulting from the decarboxylation of these keto-acids during AA catabolism also provide carbon for synthesis of new AA. The typical example of cross-feeding between cellulolytic and AA-fermenting ruminal bacteria represents a case of VFA incorporation into microbial protein. Cellulolytic bacteria cannot synthesize branched-chain AA *de novo*; therefore, they rely on AA-fermenting bacteria to provide them with BCVFA as precursors for the synthesis of branched-chain AA (Russell, 2002). One possible pathway through which acetate is incorporated into carbon backbones is reductive carboxylation of acetate to pyruvate by pyruvate synthase (Allison, 1969). Additionally, carboxylation of intermediates and end-products of glycolysis such as phosphoenolpyruvate and pyruvate, leads to the incorporation of $CO_2$ to carbon skeletons (Allison, 1969; Russell, 2002).

Inorganic sulfur from diet and drinking water as well as sulfur derived from the catabolism of methionine and cysteine contribute to the sulfur pool for synthesis of

sulfur-containing AA (Hungate, 1966). As in the case of carbon skeletons, energy required for AA biosynthesis is mainly derived from carbohydrate fermentation (Allison, 1969), even though fermentation of AA can provide some ATP. On the other hand, obligate AA-fermenting bacteria obtain all required ATP from the catabolism of AA (Russell, 2002).

#### 2.1.2.1. ESTIMATION OF MICROBIAL PROTEIN SYNTHESIS. NITROGEN REQUIREMENTS FOR RUMINAL MICROBES. EFFICIENCY OF MICROBIAL PROTEIN SYNTHESIS.

Based on the information discussed in the previous paragraphs, the dependence of microbial protein synthesis and consequently microbial growth, on carbohydrate availability is clear. If ATP from carbohydrate fermentation is not sufficient to drive protein synthesis, AA will be fermented as an energy source and $NH_3$-N will accumulate (Nocek and Russell, 1988; Russell et al., 1992; Van Soest, 1994). As expressed by Coleman (1986), protozoa will not utilize AA for biosynthesis unless sufficient energy is available to support growth and demand those AA for biosynthesis. On the other hand, total carbohydrate fermentation and microbial growth can be decreased by reduced degradable N availability (Stokes et al., 1991). The importance of adequate degradable N and readily fermentable carbohydrates availability on microbial growth has been reported by Hoover and Stokes (1991).

Several methods, such as the total purine method (Zinn and Owens, 1986), the

purine derivatives method, and incorporation of $^{15}N$ (Firkins et al., 1987), have been developed to estimate MCP flow from the rumen to the lower tract. Additionally, the NRC model (2000) provides calculations to estimate daily MCP synthesis based on RDP or ***DIP requirements*** for microbial growth. Level 1 of NRC model (2000) for beef cattle estimates DIP requirements as total digestible nutrients (TDN) intake multiplied by ***microbial efficiency***. While the latter refers to the units of MCP synthesized per unit of TDN ingested, TDN intake refers to the daily amount of nutrients (carbohydrates, lipids, and proteins) digested along the whole digestive tract. A similar expression to represent MCP synthesis is based on organic matter (OM) fermented or digested in the rumen, where microbial efficiency is then defined as units of MCP produced per unit of OM fermented in the rumen (Hoover and Stokes, 1991; Van Soest, 1994).

Adequacy of dietary DIP is calculated through the DIP balance in the rumen. The DIP balance is estimated as the difference between ***DIP supply*** and DIP requirements. Level 1 of NRC model (2000) estimates DIP supply as dry matter intake (DMI) multiplied by DIP concentration in the diet. The model assumes that, when present, DIP deficiency (DIP requirements > DIP supply) is overcome by degradable N supplementation. Therefore, the amount of MCP synthesized in the rumen per day is represented by daily DIP requirements, which in turn are calculated as a function of availability of digestible nutrients. However, using TDN to determine DIP requirements or MCP synthesis ignores the fact that ruminal microbes can only utilize ruminally available nutrients, only glycerol from the lipid fraction can be used as energy, and only

some microbes are able to grow on AA (Chen and Russell, 1988, 1989; Russell et al., 1988; Russell et al., 1992; Russell, 2002). In fact, most ruminal microorganisms are only able to derive energy from carbohydrates (digestible N-free extracts and digestible crude fiber; Nocek and Russell, 1988). That is likely why calculations of MCP synthesis based on digestible carbohydrates are less variable than those based on rumen-digestible OM (Nocek and Russell, 1988). To overcome some of the limitations mentioned above, Level 2 of the NRC model (2000) estimates DIP requirements or MCP synthesis by considering only the amount of carbohydrates digested in the rumen.

Based on the formula suggested by Level 1 of the NRC model (2000), DIP requirements –or MCP synthesis– can potentially be altered by factors affecting TDN intake or amount of OM digested in the rumen as well as microbial efficiency. For example, intake-affecting factors such as diet composition, environmental factors, physiological stage, and health status may impact DIP requirements. Clark et al. (1992) summarized results from 39 and 28 experiments in which dairy cows were fed 157 and 102 diets, respectively. Amount of OM apparently and truly digested in the rumen and microbial N flow to the small intestine increased while OM digestibility, expressed as OM digested divided by OM intake (OMI), decreased with increasing OMI. The negative association between OM digestibility and OMI may be due to reduced digestibility resulting from shorter residence time in the rumen at greater intakes. The authors partially attributed the increased passage of MCP to the small intestine with increasing OMI to the greater amount of energy supplied by increased OM ingested and fermented in the rumen.

Similarly, growth of ruminal microbes and extent of OM digestion in the rumen can be affected by availability of microbial growth factors such as branched-chain AA and therefore BCVFA, cobalt, sulfur, phosphorous, highly fermentable carbohydrates, and rumen-degradable N (Hungate, 1966). Cobalt is required for synthesis of vitamin B12, which is in turn involved in the propionic acid production pathway; sulfur is required for synthesis of sulfur-containing AA and phosphorous is required for synthesis of microbial nucleic acids.

Two reasons can be mentioned to explain the positive relationship between ruminal availability of fermentable carbohydrates and MCP synthesis. First, based on data from both *in vitro* and *in vivo* studies, there is general agreement that rate of digestion of carbohydrates is the major factor controlling energy available for microbial growth (Hoover and Stokes, 1991). Rate of digestion of carbohydrates is affected by solubility and complexity of the chemical structure. Consequently, rate of digestion of starches, pectins, and sugars is faster compared with that of cellulose and hemicellulose (Hoover and Stokes, 1991). Second, increased proportion of rapidly fermentable carbohydrates may result in reduced rumen pH and consequently in a greater pool of $NH_3$-N for MCP synthesis, as the rumen wall is less permeable to the ionized form ($NH_4^+$) present with lower rumen pH (Cabrita et al., 2006). Stokes et al. (1991) reported an increase in DM and neutral detergent fiber (NDF) digestibility, and VFA and MCP synthesis when increasing dietary non-structural carbohydrate (NSC) concentration from 25 to 54% of DM in a continuous culture system. Similarly, McCarthy et al. (1989) observed increased

microbial N flow from the rumen to the lower tract with increasing amounts of starch and OM fermented in the rumen of Holstein cows.

Additionally, NSC and DM digestion, VFA production, and amount of MCP synthesized per day were positively associated with availability of rumen-degradable N (McCarthy et al., 1989; Herrera-Saldana et al., 1990a; Hoover and Stokes, 1991; Stokes et al., 1991). As expressed by Russell (2007), if the growth medium is N-limited and the energy source is in excess, which results in anabolic rate (ATP utilization, ADP regeneration, and protein biosynthesis) being slower than catabolic rate (carbohydrate fermentation and ATP production), many bacteria spill energy, and the few that do not may die. While energy spilling negatively affects microbial efficiency (see later), reduced microbial activity and even death of ruminal microbes reduces overall MCP synthesis in the rumen. The ruminal bacteria *Prevotella (Bacteroides) ruminicola* does not have energy-spilling mechanisms and consequently cannot survive if N is limiting and glucose is in excess (Russell, 2007). Russell (1993) reported that when N availability was limiting, *P. ruminicola* could not use ATP for protein synthesis, resulting in insufficient amount of ADP to fuel glycolysis. In this situation, carbohydrate fermentation shifted to the methylglyoxal shunt which does not generate ATP or require ADP. Methylglyoxal affected membrane physiology by decreasing its electrical potential and reducing intracellular potassium concentration, and its accumulation was correlated with reduced microbial cell number and increased cell death (Russell, 1993). Another ruminal bacterium that does not spill energy and can be killed by excess carbohydrate is

*Fibrobacter succinogenes*, but this toxicity is not due to methylglyoxal accumulation (Russell, 2007).

Protein solubility, NPN content, and structure of proteins are some of the factors that affect ruminal degradability of N sources. From the early studies of McDonald and Hall (1957) and Henderickx and Martin (1963), N solubility has been positively associated with N ruminal degradability (Crawford et al., 1978; Sniffen, 1980; Blethen et al., 1990; Chaudhry and Webster, 2001), mainly with short term (1 to 3 h) ruminal digestion (Nocek, 1988). Ruminal fluid is a complex mineral buffer (Blethen et al., 1990) and solubility of N is influenced by chemical composition of the buffer, ionic strength, temperature, and pH (Waldo and Goering, 1979). Therefore, several solvents, such as water, McDougal's artificial saliva, Burrough's mineral mixtures, and ruminal fluid, have been employed to determine N solubility (Nocek, 1988). The principle underlying the effects of protein solubility on ruminal degradability of protein is the ability of rumen microbes to digest and use N to synthesize their own protein being dependent on the interaction between microbial proteases/peptidases and substrate. The solubility of the substrate influences this enzyme-substrate interaction (Blethen et al., 1990) because accessibility of proteins to proteases is much greater if the protein is in solution (NRC, 1985). In other words, contact between proteases/peptidases and the substrates is mediated by water (Chen et al., 1987a). Because NPN is part of the soluble N fraction in feed components and is readily available for microbial use (Van Soest, 1994), its concentration positively relates to solubility of the N-containing fraction and to ruminal

degradation of feed N sources.

Despite the positive association between N solubility and N ruminal degradability, differences in ruminal degradability exist among proteins of similar solubility, or vice versa (Nugent and Mangan, 1978; Mahadevan et al., 1980; Nugent et al., 1983; Wallace and Kopecny, 1983, NRC, 1985). Nugent and Mangan (1978) reported different rates of rumen proteolysis for casein, fraction I leaf protein, and bovine serum albumin despite similar buffer solubility. Treatment of bovine serum albumin with dithiothreitol, which breaks some of the disulfide bonds that cross-link the protein, caused a significant increase in its rate of rumen proteolysis. Additionally, Mahadevan et al. (1980) observed that buffer-soluble and buffer-insoluble proteins of soybean meal were hydrolyzed at almost identical rates. It was concluded that hydrolysis and extended N ruminal digestion are related to the protein configuration and structure rather than solubility (Nugent and Mangan, 1978; Wallace and Kopecny, 1983; Nocek, 1988).

Besides rumen-degradable N, addition of preformed AA in particular may also stimulate growth of ruminal microbes. While fast-growing microbes may benefit from AA supplementation by direct incorporation into microbial protein, cellulolytic bacteria may benefit by the indirect supply of BCVFA, derived from the fermentation of branched-chain AA by other ruminal microbes (Dijkstra et al., 1998).

Based on the information summarized in the previous paragraphs, factors that can potentially affect DIP requirements by affecting TDN intake or OM fermented in the

rumen are:

- DMI or OMI
- DM or OM digestibility
    - Growth of ruminal microbes
        - Availability of growth factors such as BCVFA and minerals, and availability of rumen-fermentable carbohydrates and N.

On the other hand, DIP requirements can be altered by factors that influence microbial efficiency such as (1) ruminal pH, (2) ionophores, (3) dietary fat, (4) microbial outflow rate from the rumen to the lower tract, (5) type of substrate fermented and rate of fermentation of the diet, (6) availability of rumen-degradable N, and (7) type of microbe.

***Ruminal pH***

Ruminal pH can affect the amount of ATP available for microbial growth thus affecting microbial efficiency. Russell and Dombrowski (1980) observed a reduction in the amount of microbial cells synthesized per gram of glucose fermented with decreasing ruminal pH. Similarly, Strobel and Russell (1986) observed a decrease in MCP synthesized per unit of substrate fermented when pH decreased from 6.7 to 5.5. As VFA and/or lactic acid concentration in the rumen increases, ruminal fluid pH decreases. Decreasing pH results in increased proportion of un-dissociated VFA which are lipophilic and thereby able to cross the microbial cell membrane (Russell and Wilson, 1996). Following a concentration gradient, un-dissociated VFA enter the microbial cell, where

pH is higher (close to neutrality) than that of ruminal fluid, resulting in un-dissociated VFA to release protons in the interior of the cell (Russell and Wilson, 1996). To avoid a decrease in internal pH due to proton accumulation and maintain a membrane potential, the microbial cell is forced to pump out those protons against a pH and proton gradient. Therefore, ATP is used to pump protons outside the cell and, as the extracellular pH becomes acidic, more energy is needed to expel protons. This process diverts ATP from growth to non-growth related functions, thus increasing energy spilling (Strobel and Russell, 1986; Russell, 2007). Consequently, less MCP is produced per unit of OM fermented. In addition, VFA anions (dissociated VFA) accumulate within the microbial cell because once deprotonated, VFA become hydrophilic and they cannot cross the cell membrane (Russell and Wilson, 1996). Internal accumulation of the end product (VFA) may inhibit the VFA-ATP-producing reactions, which may result in decreased ATP availability, thus further affecting microbial growth. In this regard, Klemps et al. (1987) observed that the acetic acid-ATP-producing reactions (pyruvate oxidoreductase and acetate kinase) were strongly inhibited by increased internal acetate concentrations.

### *Ionophores and dietary fat concentration*

Despite their potential effect on ruminal pH drop mitigation, ionophores reduce overall microbial efficiency (Zinn, 1988; Van Soest, 1994) by increasing energy spilling by sensitive bacteria (Russell and Strobel, 1989; Callaway et al., 2003; Russell and Houlihan, 2003). Similarly, increased energy spilling may also be the mechanism through which elevated dietary fat concentration, mainly polyunsaturated fatty acids (PUFA),

reduces microbial efficiency. The latter might be induced by a disruption of the lipid bilayer structure of the microbial cell membrane produced by the typical twisted chain of PUFA, which alters cell membrane physiology resulting in ion leakage, uncoupling of the proton-motive force, and reduced ATP pool available for microbial growth (Maia et al., 2007, 2010). On the other hand, Zinn (1988) reported increased microbial efficiency with increased dietary yellow grease concentration, potentially due to a direct energy-sparing effect of supplemental fat on *de novo* synthesis of fatty acids or to an indirect effect through increased ruminal turnover. The possible direct effect is supported by the early work of Erwin et al. (1963) and Marwaha et al. (1972) but challenged by that of Maczulak et al. (1981) and by the fact that bacterial lipids do not contain any PUFA (Polan et al., 1964) or contain small amounts (Van Soest, 1994) predominantly represented by *de novo* synthesized branched- and odd-chain fatty acids (Goldfine, 1982). This observation suggests that cellular incorporation of PUFA is minimal (Kim et al., 2009).

### *Microbial outflow rate*

Microbial outflow rate refers to the speed at which ruminal microbes are removed from the rumen towards the lower tract, in the liquid phase as well as attached to indigested feed particles. Therefore, microbial outflow rate is affected by ***rate of passage*** and ***dilution rate***. The former refers to the speed at which feed leaves the rumen (mass/time) and differs from the *fractional rate of passage* which refers to the proportion of total feed that leaves the rumen per unit of time (%/time). Similarly, dilution rate

indicates the rate at which the liquid phase leaves the rumen (volume/time) while the *fractional dilution rate* refers to the proportion of total rumen volume leaving this compartment per unit of time (%/time). On the other hand, retention time in a certain compartment is defined as the reciprocal of fractional passage rate. Passage rates are regulated by feed intake, processing (particle size and surface area), and type of feed (e.g., forage or grain; Russell et al., 1992). While a slow rate of passage is usually associated with low DMI and/or low-quality diets, dilution rate is positively associated with rumination and saliva production as well as water intake. In this regard, different studies reported an increase in both fractional rate of passage and dilution rate in cattle with increasing DMI (Sniffen and Robinson, 1987) or DMI expressed as a percentage of body weight (BW) and dietary forage concentration (Evans, 1981a,b).

Theoretically, the relationship between microbial efficiency and outflow rate is positive until maximal microbial efficiency is achieved, which would occur when outflow rate and microbial division rate are equal (Ørskov, 1992). Beyond that point, very high outflow rates may cause plateauing and even decreased microbial efficiency through loss of unfermented feed and competition between outflow and microbial cell generation time (Van Soest, 1994). The net result of outflow rate being greater than microbial division rate is microbes becoming washed out[1] from the rumen. Because of slow doubling times (15 to 24 hours), protozoa are more prone to be washed out from the rumen than bacteria (Russell, 2002). However, they associate to the slow-moving particle fraction, thus

[1] Washed out microbes refers to microbes whose cell generation time is slower than the outflow rate; therefore, they are removed from the rumen before reproducing and their populations are diminished.

prolonging their residence time in the rumen.

Isaacson et al. (1975) observed an increase in microbial efficiency, expressed as grams of microbial cells produced per mole of glucose fermented, from 42.2 to 60.2 and 83.9 g/mol when dilution rate increased from 2 to 6 and 12.5 %/h, respectively, in a continuous culture system of mixed bacteria. Similarly, Meng et al. (1999) reported a quadratic increase in microbial efficiency with increasing dilution rate from 2.5 to 20 %/h in continuous culture fermenters with mixed ruminal bacteria. In addition, the efficiency of protein synthesis by individual cultures of *Megasphaera elsdenii* was also increased with increasing dilution rate (Russell and Baldwin, 1979). The basis underlying this relationship is that slow outflow rate increases microbial maintenance costs by increasing microbial population age and microbial turnover, the latter referring to growth, death, and recycling of microbial materials by other microbes inside the rumen. Many protozoa degenerate and lyse before leaving the rumen; therefore, contributing to microbial protein turnover or recycling (Russell, 2002). Additionally, Holotrichs cannot control their rate of sugar uptake and internal starch storage, and consequently, they can degenerate and burst inside the rumen (Hungate, 1966; Dijkstra, 1994; Russell, 2002). Predation of bacterial and even protozoal cells by protozoa (Hungate, 1966; Dijkstra, 1994; Van Soest, 1994; Russell, 2002) also contributes to N recycling in the rumen.

Increased microbial maintenance expenditures resulting from slow outflow rates are reflected partly in greater heat of fermentation and represent a loss of microbial protein, but not necessarily a loss of VFA, for the animal (Russell and Hespell, 1981). Results

from several studies summarized by Ørskov (1992) demonstrated that the proportion of ATP used to maintain rumen microbes decreased from 0.65 to 0.12 when increasing dilution rate from 2 to 13.6%/h. In addition, Meng et al. (1999) reported that protozoal numbers decreased with increasing dilution rate; when dilution rate reached 11.5%/h most of the protozoa were washed out of the fermenters, at which point reduced predation rates of bacteria by protozoa were expected. Recycling of lysed microbial cells within the rumen leads to decreased efficiency of MCP synthesis because of a direct reduction in the amount of microbial protein reaching the small intestine. At slow outflow rate, OM fermentation must continue to maintain mature microbial cells; yet, microbial rate of multiplication is largely reduced (Van Soest, 1994). The net effect is a reduction in MCP produced per unit of OM fermented. In addition, slow outflow rate leads to selection in favor of slow-growing microbes; outflow rate sustains bacteria that reproduce at a rate consistent with a given outflow rate. When bacteria grow slowly, a large proportion of the energy is used for maintenance; conversely, during fast growth, rate of energy utilization is fast, and maintenance makes up a small proportion of the total budget (Russell et al., 1992). In sum, fast rate of outflow results in increased microbial efficiency because mean age of microbial population is decreased and younger microbial population is subject to lower death and predation rates (Van Soest, 1994; Meng et al., 1999; Russell, 2002). Fast outflow rate also permits selection in favor of fast-growing microbes (Meng et al., 1999), which reduces the proportion of energy used for maintenance.

### *Type of substrate fermented and rate of fermentation of the diet*

The amount of ATP derived per mole of substrate fermented and thus available for microbial growth varies among types of substrate fermented in the rumen, which consequently affects the amount of MCP synthesized per unit of OM fermented. The highest ATP yield results from fermentation of glucose derived from polysaccharides (fiber and starch), while intermediate and lowest yields result from fermentation of soluble carbohydrates (sugars) and proteins, respectively (Van Duinkerken et al., 2011). Due to their fast rate of degradation, hexoses derived from sugars influence fermentation towards synthesis of lactic acid, which produces only 2 ATP/mole compared with 4 ATP/mole derived from acetate or propionate formation (Russell, 2002; Van Duinkerken et al., 2011). Low ATP yield from protein fermentation might be related to the fact that, unlike hexoses, fermentation of AA does not involve glycolysis but only keto-acid decarboxylation, and that about 16% of a mole of protein (that ascribed to N content) is not involved in ATP generation.

Fermentation rate is an inherent property of the feed (Russell et al., 1992) which determines the amount of feed energy per unit of time for microbial growth. Thus, fermentation rate is positively associated with microbial efficiency (Van Soest, 1994) as it facilitates faster microbial growth and more diluted microbial maintenance requirements. Therefore, the type of substrate fermented not only affects the amount of ATP available for microbial growth but also how ATP is partitioned between growth and maintenance by the resulting rate of fermentation of the diet. Stokes et al. (1991)

observed a quadratic increase in bacterial efficiency with increasing concentrations of dietary NSC in a continuous culture system. Similarly, Herrera-Saldana et al. (1990a) reported an increase in microbial efficiency when replacing milo with barley in a cottonseed meal-containing diet fed to lactating Holstein cows. However, when compiling data from several *in vivo* studies, no significant association between fermentability of the diet and microbial efficiency was reported by Hoover and Stokes (1991) and Cabrita et al. (2006). These contradictory results may be partially explained by the effects of other factors affecting microbial efficiency, which are in turn modified when changing diet fermentability. For example, greater VFA and/or lactate ruminal concentrations resulting from increased rate of fermentation may reduce ruminal pH as well as ATP available for microbial growth, which can offset the beneficial effects of increased fermentation rate on microbial efficiency. Similarly, if DMI is reduced with increased energy density of the diet, the beneficial effects of the latter on microbial efficiency can be overridden by the negative effects of decreased rate of passage.

### *Availability of rumen-degradable N*

Availability of rumen-degradable N for microbial growth is another factor that is known to affect microbial efficiency (Russell et al., 1992). Extensive fermentation of carbohydrates can occur in cultures with inadequate N, but microbial growth will be limited resulting in low MCP synthesized per unit of carbohydrate fermented (Hespell, 1979). Hoover and Stokes (1991) summarized results from several studies both *in vitro* and *in vivo* in which a positive association between dietary DIP concentration and grams

of MCP synthesized per kilogram of carbohydrate digested was demonstrated. Energy dissipation or energy spilling, which impacts microbial maintenance costs, might be the mechanism by which microbial efficiency is reduced under conditions of limited rumen-degradable N supply. As expressed by Fox et al. (2004), the impact of maintenance on cell yield is most apparent when energy is limiting, but bacteria are more prone to spill energy when energy supply exceeds concentration of other nutrients needed for microbial growth. Van Kessel and Russell (1996a) observed that bacterial non-growth energy dissipation increased 10 fold in a $NH_3$-N-limiting media with excessive carbohydrate supply. When energy-excessive batch cultures were provided with $NH_3$-N, microbial growth rate increased, the difference in anabolic and catabolic rates was smaller, and less energy was spilled. *Streptococcus bovis* is one of the ruminal bacteria species that has been more extensively studied in terms of its very active energy-spilling mechanism, which is mediated by membrane ATPase activity and a futile cycle of protons through the cell membrane (Russell, 2007). When rate of ADP and inorganic phosphorous (Pi) utilization and ATP production through carbohydrates fermentation was faster than rate of ATP utilization and ADP and Pi regeneration through biosynthesis, Pi concentration decreased; this change in Pi concentration increased rate of ATP hydrolysis (Russell, 2007). Increased ATP hydrolysis is consistent with an increase in membrane potential, thus increasing proton conductance (or decreasing membrane resistance). Therefore, protons in the media may enter the cell to be then pumped out when excess ATP is hydrolyzed; however, the pathway through which protons cross the cell membrane is still

a matter of speculation (Russell, 2007). Energy spilling mechanisms have also been studied in ruminal bacteria from the genus *Clostridium* and methanogens (Russell, 2007).

***Type of microbe***

Due to differences in maintenance cost, efficiency of microbial growth and protein synthesis in the rumen is affected by the type of microbes present in the rumen (Van Duinkerken et al., 2011). For example, *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* have low maintenance expenditures compared to other species (Russell and Hespell, 1981). Derived from the work of Russell and Baldwin (1979), maintenance requirements were estimated to be 0.05 and 0.15 g carbohydrates/g bacteria/h for particle- and liquid-associated bacteria, respectively (Van Duinkerken et al., 2011), or for fiber- and non-fiber-digesting bacteria, respectively (Russell et al., 1992; Fox et al., 2004). However, the ruminal microbes studied by Russell and Baldwin (1979) were not categorized as being free or attached, and no clear association arises between the primary fermentation niche and maintenance expenditure.

In general, microbial efficiency ranges from 8 to 13 g of microbial protein/ 100 g TDN intake; the latter value is considered as the maximum microbial efficiency by the NRC model (2000). Given observations and discussions above, greater efficiency values are ascribed to forage-based diets and lesser values are ascribed to grain-based diets. Based on potential negative effects of decreased ruminal pH on microbial efficiency, Level 1 of the NRC model (2000) estimates microbial efficiency as 13% affected by a coefficient called the “effective NDF adjusted” (eNDFadj) which depends on dietary

effective NDF (eNDF) concentration. This value refers to the proportion of the NDF that is sufficiently large (> 1.18 mm; NRC, 2000) to be effective at stimulating chewing, rumination, and salivation, and consequently at mitigating ruminal fermentation-induced pH drop. Therefore, dietary concentration of eNDF is used by the model to predict ruminal pH and estimate eNDFadj coefficient. The model assumes that at 20% dietary eNDF, microbial efficiency is maximized. Therefore, for diets containing as much or more than 20% eNDF the coefficient value is 1, while a reduction of 0.025 units per each percentage unit of dietary eNDF below 20% is integrated in the model.

### 2.1.3. ABSORPTION AND METABOLISM OF AMMONIA-N. ENERGETIC COST OF UREA SYNTHESIS.

The portion of $NH_3$-N that is not incorporated into microbial N is absorbed through the walls of the digestive tract to the portal vein. The lipophilic un-ionized form ($NH_3$) is rapidly absorbed by simple diffusion through the apical membrane of the rumen wall while the protonated form ($NH_4^+$) is probably absorbed via a $K^+$ channel (Abdoun et al., 2007). Less is known about transport across the basolateral membrane.

Several factors affect the absorption of $NH_3$-N through the rumen wall. The close relationship between availability of N and energy in the form of carbohydrates for microbial growth discussed in Section 2.1.2 suggests that, as expressed by Reynolds and Kristensen (2008), $NH_3$-N absorption from the rumen to the portal vein is influenced by amount supplied and rumen degradability of dietary protein and carbohydrate. Studies

reviewed by Reynolds and Kristensen (2008) demonstrated a decrease in $NH_3$-N absorption through the rumen wall under conditions of increased starch ruminal digestion or supply. Additionally, Abdoun et al. (2007) reported that permeability of the ruminal membrane is 175 times higher for $NH_3$ compared with that for $NH_4^+$, which may explain why ruminal pH is positively associated with N absorption because it increases the $NH_3/NH_4^+$ ratio.

Because $NH_3$-N is extremely toxic in non-hepatic tissues (Abdoun et al., 2007), it is transported by the portal vein to the liver, specifically to the periportal hepatocytes, where most of it is transformed by the ornithine cycle into urea, which is less toxic than $NH_3$-N (Visek, 1968). If any $NH_3$-N reaches the perivenous hepatocytes, it is converted to glutamine, which is transported back to periportal hepatocytes where the amino-group is removed and metabolized to urea (Abdoun et al., 2007). Absorbed $NH_3$-N can also be incorporated into glutamic acid, glutamine, and carbamyl phosphate and from there into non-essential AA, purines, and pyrimidines (Armstrong and Hutton, 1975), even though the proportion of $NH_3$-N that follows this path is small.

When urea is synthesized from $NH_3$-N absorbed from the digestive system, net energy cost is 1.5 molecule of ATP per molecule of urea synthesized. This cost arises from the following reactions. During hepatic synthesis of urea, 2 molecules of ATP are hydrolyzed to 2 molecules of adenosine di-phosphate (ADP) and 2 atoms of Pi, while another ATP is hydrolyzed to 1 molecule of adenosine mono-phosphate (AMP) and Pi. Since a phosphate group from an extra ATP is needed to regenerate 2 ADP from 1 AMP,

the hydrolysis of ATP to AMP is equivalent to the hydrolysis of 2 ATP to 2 ADP. Therefore, it can be said that the synthesis of 1 molecule of urea requires 4 molecules of ATP. In addition, urea cycle produces 1 molecule of cytosolic fumarate which is transformed to malate by cytosolic fumarase. Because mitochondrial membrane is permeable to malate, the latter enters the mitochondria where it is transformed to oxaloacetate in a TCA reaction, producing 1 molecule of $NADH^+$ (Newsholme and Leech, 1983). When each pair of electrons from each molecule of $NADH^+$ flows along the electron transport chain, 10 protons are pumped out of the mitochondrial matrix; on the other hand, 4 protons are required for the synthesis of 1 molecule of ATP and its transport out to the cytosol (Stryer et al., 2002). Therefore, each molecule of $NADH^+$ produces 2.5 molecules of cytosolic ATP via oxidative phosphorylation (Stryer et al., 2002). Consequently, the balance between 4 ATP required and 2.5 ATP produced results in a net energy cost of urea synthesis of 1.5 ATP. As expressed by Milano et al. (2000), the actual magnitude of that cost *in vivo* is still debatable. These authors observed that a significant increase in $NH_3$-N supply to the liver did not impair glucose or AA supply to non-splanchnic tissues.

Synthesized urea can enter the digestive tract at the rumen and hind gut level, where it is hydrolyzed by microbial ureases, reinitiating the $NH_3$-N cycle. Urea is recycled back into the rumen via saliva and absorption across the rumen wall. Absorption occurs by simple as well as facilitated diffusion via transporters located in the basolateral and apical membranes (Abdoun et al., 2007). Even though $NH_3$-N from recycled urea can

be used for microbial growth in both the rumen and hind gut, microbial protein produced in the latter does not have the chance to be digested in the lower gut (Abdoun et al., 2007) and is excreted to the environment. Consequently, microbial protein of hind gut origin is not available to the host. Besides entering the digestive tract, urea is excreted in urine and milk.

The flow of urea into the rumen is highly variable and its regulation is not well understood (Abdoun et al., 2007). Some of the factors associated with the amount of urea recycled back to the rumen can be summarized from reviews by Abdoun et al. (2007), Reynolds and Kristensen (2008), and Recktenwald (2010) as follows: (1) rumen-blood concentration gradient, (2) activity of ruminal microbes as stimulated by increased availability of rapidly fermentable carbohydrates, (3) increased concentration of VFA that may modify epithelial surface area and/or permeability, (4) intra-ruminal $NH_3$-N concentration (negatively associated with urea entry), (5) expression of urea transporters in rumen epithelium, though impact of this route of urea recycling is still inconclusive.

## 2.2. NITROGEN DIGESTION IN THE LOWER DIGESTIVE TRACT

Undegraded feed and endogenous proteins, as well as microbial protein in the form of microbial cells, some non-hydrolyzed urea, and some $NH_3$-N leave the reticulo-rumen by passage through the lower tract. Proteins are not affected during their passage through the omasum, although urea and $NH_3$-N can be absorbed through the omasal wall (van der Walt and Meyer, 1988).

Once in the abomasum, digestion of the aforementioned proteins begins by the action of gastric enzymes which are secreted by specific cells of the abomasal epithelium. Due to the secretion of hydrochloric acid by the parietal cells (Barrett et al., 2010), abomasal content presents a pH of 2.0, which is responsible for the death and disintegration of microbial cells, along with the activation of pro-enzymes or zymogens (Harfoot, 1978). Because they are proteins, gastric and pancreatic proteases are secreted as zymogens to avoid self-digestion and also tissue damage before they reach their targeted substrates. Pepsin is secreted as the inactive pepsinogen by the chief cells and is activated when it comes into contact with hydrochloric acid (Barrett et al., 2010). Pepsin cleaves peptide bonds adjacent to aromatic AA, which results in the release of peptides of different length (Barrett et al., 2010). Chief cells of the pre-ruminant abomasum secrete chymosinogen or renninogen, also known as pro-chymosin or pro-rennin (Roy and Stobo, 1975). Once activated into chymosin or rennin by acidic conditions, this enzyme cleaves the k-casein phenylalanine-methionine bond, resulting in protein coagulation and consequently permitting for a greater residence time and enhanced proteolysis (Foltmann, 1969; Guilloteau et al., 2009).

Protein digestion continues in the small intestine by the action of pancreatic and intestinal enzymes. Pancreatic juice and bile, the latter synthesized by the liver and stored in the gallbladder, enter the small intestine at the duodenal level. Sodium bicarbonate content of pancreatic juice, bile, and intestinal secretions raises the intestinal pH from about 3.0 in the duodenal bulb (next to the pylorus) to 6.5 in the rest of the duodenum,

7.1 in the jejunum, and 8.0 at the ileum (Ben-Ghedalia et al., 1974; Harfoot, 1978; Barrett et al., 2010). Because pepsin has an optimum pH of 1.6–3.2, its digestive action might be somewhat extended to the proximal portion of the duodenum but terminated further on (Armstrong and Hutton, 1975; van der Walt and Meyer, 1988; Barret et al., 2010). On the other hand, pancreatic proteases and peptidases are activated from their zymogen forms when pH conditions are above 5 and they display maximum activity at pH 7.5 to 8.0 (Ben-Ghedalia et al., 1974; Armstrong and Hutton, 1975). Consequently, their activity is delayed to the distal duodenum, jejenum and mid-ileum (Ben-Ghedalia et al., 1974).

Pancreatic enzymes include endo- and exo-peptidases which attack centrally located or terminal peptide bonds, respectively. The first group includes trypsin, chymotrypsins, and elastase, preferentially cleaving at the carboxyl end of arginine and lysine, aromatic AA, and valine, leucine, serine, and alanine, respectively (Armstrong and Hutton, 1975). The carboxyl ends of terminal lysine and arginine are not cleaved by the exo-peptidase carboxypeptidase A but are a preferential substrate of carboxypeptidase B (Armstrong and Hutton, 1975). When trypsin enters the duodenum, it is activated from its zymogen, trypsinogen, by the brush border enterokinase, whose high polysaccharide content prevents it from being self-digested before it can exert its effect (Barrett et al., 2010). Once activated, trypsin activates itself in an auto-catalytic chain reaction, along with chymotrypsinogens and pro-carboxipeptidases (Harfoot, 1978; Barrett et al., 2010).

Similarly to pancreatic carboxypeptidases, the intestinal aminopeptidases cleave terminal AA; however, the latter target the amino instead of the carboxyl end (McDonald,

1985; Bergmann, 2006). Intestinal juice also includes other exo-peptidases such as dipeptidases (Holmes and Lobley, 1989). Harfoot (1978) reported that, based on available information, intestinal enzymes were probably derived from exfoliated intestinal epithelial cells rather than being true secretions. More recently, it has been demonstrated that rather than being free in the intestinal lumen, intestinal enzymes are actually localized on the external surface of the microvillus membrane, anchored to it by a segment that is embedded in the lipid bilayer (Holmes and Lobley; 1989). This arrangement allows for terminal digestion directly on the surface of the brush border and in the immediate vicinity of the transporters involved in the absorption of nutrients into the epithelial cells (Holmes and Lobley; 1989).

In a review, Chen and Ørskov (2004) reported that because nucleic acids from feed are completely fermented in the rumen, nucleic acids entering the small intestine are essentially of microbial origin. Desquamations of intestinal epithelium may also contribute to the nucleic acid pool. Nucleic acids are split into nucleotides by pancreatic nucleases, which are then split into nucleoside and phosphoric acid. Nucleoside is further cleaved into a 5-carbon sugar and a nitrogenous base (purine or pyrimidine bases) by enzymes located on the luminal surface of the mucosal cells (Barrett et al., 2010).

The contribution of intestinal microbes to protein and other nutrient digestion in the small intestine is assumed to be small due to a much smaller microbial population and shorter retention time compared to those in the rumen (Harfoot, 1978). On the other hand, high microbial proteolytic activity has been observed in the caecum and proximal colon;

the fermentation process and N cycle in this site is similar to those described in the rumen (van der Walt and Meyer, 1988). Origin of N-containing compounds entering the large intestine is similar to that of N-containing compounds entering the small intestine. Due to the location of the large intestine in the digestive tract, distal to absorption sites, N that is incorporated into microbial proteins in the large intestine is lost in feces. The NRC (2000) suggests a hind gastrointestinal tract digestibility of 80% for UIP and true MCP.

## 2.3. NITROGEN ABSORPTION FROM THE LOWER DIGESTIVE TRACT

Based on results from several studies, Webb (1990) reported that the jejenum and ileum are the main sites for AA absorption into the enterocyte, which occurs by simple and facilitated diffusion (sodium-independent), and active transport (sodium-dependent). Because AA concentration is greater in the intestinal lumen than in the enterocyte, absorption of AA occurs against a concentration gradient and might be driven by the energy potential of a sodium gradient (Webb, 1990). Amino acids are co-transported with sodium from the lumen into the cell across the brush border membrane, which means that sodium is transported along a concentration gradient (high extracellular and low intracellular sodium; Armstrong and Hutton, 1975; Webb, 1990). The sodium gradient is maintained by a sodium-potassium ATPase located in the basolateral membrane of the enterocyte (Webb, 1990). Part of absorbed AA is used by the enterocyte for its own metabolism and the rest is transported out through the basolateral membrane and incorporated into the hepatic portal blood.

Information accumulated during the last 50 years provides evidence that di- and tri-peptides are actually absorbed into the enterocyte by a system which relies on hydrogen instead of sodium (Webb, 1990; Barrett et al., 2010). Due to substantial presence of peptidases, it has been widely accepted that intact peptides may not leave the enterocyte via the basolateral membrane, though several studies have reported the presence of di- and tri-peptides in portal blood (Webb, 1990). As expressed by Webb (1990), the presence of peptide-AA in portal circulation does not provide conclusive evidence of transfer from the intestinal lumen because it may also represent degradation products from intestinal protein turnover.

Nucleosides, 5-carbon sugars, and nitrogenous bases (purines and pyrimidines) derived from nucleic acid digestion are absorbed by active transport via transporters located on the luminal surface of the enterocytes (Nolan, 1999; Tas and Susenbeth, 2007; Barrett et al., 2010) within which they are extensively catabolized. If nucleosides are absorbed as such, they are degraded by intestinal nucleoside phosphorylases (Nolan, 1999) to the corresponding sugar and nitrogenous base. Pyrimidines thymine, cytosine, and uracil are converted to beta-alanine, ammonia, $CO_2$, and other end-products also produced during AA catabolism; therefore, no distinctive nitrogenous products result (Newsholme and Leech, 1983; Tas and Susenbeth, 2007). The purines adenine and guanine are catabolized to their respective derivatives hypoxanthine and xanthine; the latter is also produced from hypoxanthine by the enzyme xanthine oxidase (Nolan, 1999). This enzyme is also responsible for the catabolism of xanthine to another major purine

derivative, uric acid. Due to high concentration of xanthine oxidase in cattle mucosal cells, absorbed purines are almost completely converted into uric acid before reaching the portal bloodstream to the liver (Nolan, 1999). In addition, high activity of xanthine oxidase in cattle plasma (Chen et al., 1990) and liver (Tas and Susenbeth, 2007) may result in extensive degradation of hypoxanthine and xanthine to uric acid before or once the molecules reach the liver, respectively.

Amino acids derived from microbial degradation of proteins in the large intestine can be absorbed from the lumen. However, the amount transferred to the portal blood is quantitatively unimportant (van der Walt and Meyer, 1988).

## 2.4. FATE OF ABSORBED NITROGEN-COMPOUNDS

Once distributed from the liver to the rest of the body, circulating peptides are transported inside tissue cells and further hydrolyzed in the cytosol providing free AA (Webb, 1990). These AA, along with free AA absorbed from the small intestine as such, can be used by tissue cells for their own metabolism. Free AA are transported from the interstitial space across the cell membrane by carrier systems mainly involving active transport (Newsholme and Leech, 1983). Absorbed AA are used as such or transformed to various non-essential AA in amounts needed to serve as protein building blocks and neurotransmitters. In addition, utilization of absorbed AA involves their catabolism to $NH_3$-N and a keto-acid, the former being usually eliminated from the body and the latter serving as ATP and/or glucose sources and, under certain circumstances, also as a carbon

source for fatty acid synthesis.

Synthesis of glutamate, aspartate and alanine involves the amination of the keto-acids ketoglutarate, oxaloacetate, and pyruvate, respectively, which are derived from glucose metabolism (Newsholme and Leech, 1983). Syntheses of other AA are more complex and described in detail by Newsholme and Leech (1983).

Protein biosynthesis takes place in the cytosol, specifically in the ribosomes, where transfer RNA carries the corresponding AA indicated by the sequence specified in the messenger RNA. In ruminants, the ATP required for protein synthesis is mainly provided by acetate oxidation.

On the other hand, for AA to be used as carbon sources, the amino-group has to be removed from the carbon backbone. Only the gut releases the amino-group in the form of $NH_3$-N directly into the portal vein from where it is transported to the liver. Due to the toxic nature of $NH_3$-N, cells of other tissues release $NH_3$-N into blood in the form of AA. This transaction is achieved by a transamination of the amino-group from the original AA to α-ketoglutarate, glutamate, or pyruvate, which yields glutamate, glutamine, or alanine, respectively, plus the carbon backbone (α-ketoacid) corresponding to the original AA (Newshome and Leech, 1983). Once in the liver, the amino-group from glutamate, glutamine, or alanine is removed by deamination, yielding $NH_3$-N, $NADH^+$, and the corresponding α-ketoacid. Urea is synthesized from $NH_3$-N, while 2.5 molecules of ATP are produced from $NADH^+$, which added to the 2.5 molecules of ATP produced from

fumarate derived from the urea cycle, results in the net production of 1 ATP per molecule of urea synthesized (for more details on energy cost of urea synthesis refer to Section 2.1.3).

As described in Section 2.1.3 and the present one, urea is the compound through which ruminally absorbed $NH_3$-N as well as $NH_3$-N from AA catabolism is recycled to the rumen or excreted from the body of ruminants. In comparison with fresh-water organisms, mammals are denied large volumes of water and consequently cannot excrete a highly diluted solution of $NH_3$-N directly; therefore, they convert $NH_3$-N into the less toxic compound urea (Newsholme ad Leech 1983). Animals and insects that have an egg stage during which water availability is severely restricted, excrete $NH_3$-N as the nearly insoluble uric acid (Newsholme ad Leech 1983). Uric acid is also produced by mammals; however, as it was explained in Section 2.3, it is produced as a result of intestinal absorption and metabolism of purines.

The biochemical basis of $NH_3$-N toxicity is not yet conclusive (Newsholme and Leech, 1983). However, one of the most accepted mechanisms of $NH_3$-N poisoning is depleting the mitochondrial pool of α-ketoglutarate, a key intermediate in the tricarboxylic acid (TCA) cycle, by the reversible reaction to glutamate. With increased $NH_3$-N concentrations, the reaction proceeds in the direction of formation of glutamate from amination of α-ketoglutarate, which in turn results in less α-ketoglutarate available for TCA cycle. Consequently, less $NADH^+$ and $FADH^+$ are produced, which decreases

the transfer of electrons in the electron transport chain, and finally ATP synthesis by oxidative phosphorylation, resulting in neurological symptoms. Other hypotheses for the basis of $NH_3$-N toxicity have been described by Newsholme and Leech (1983). Rumen pH is positively related to risk of $NH_3$-N toxicity because it increases the proportion of $NH_3$ to $NH_4^+$, the former being easily and rapidly absorbed through the rumen wall to the blood (Abdoun et al., 2007).

As mentioned previously, deamination (in the gut) or transamination (in other tissues) of AA takes place to permit the resulting keto-acid to be used as a carbon source. Depending on the AA that are being catabolized in each tissue, the resulting carbon backbones are acetyl-CoA, pyruvate, ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate. Mitochondrial acetyl-CoA can enter TCA for $NADH^+$ and $FADH^+$ synthesis, which will lead to ATP production through oxidative phosphorylation. The other intermediates of AA catabolism undergo further conversion to acetyl-CoA before being oxidized in TCA cycle for ATP production (Newsholme and Leech, 1983).

Unlike monogastrics, ruminants are limited or impaired to use mitochondrial acetyl-CoA, such as the one produced from glycolysis or AA catabolism, for cytosolic fatty acid synthesis due to low expressions or negligible activities of the enzymes ATP-citrate lyase (ATP-CL) and NADP-malate dehydrogenase (NADP-MDH; Ingle et al., 1972; Bauman, 1976). However, the expression of NADP-MDH in adipose tissue increases with increased concentrations of blood insulin when feeding diets rich in non-structural carbohydrate (glucose synthesis-promoting) to ruminants (Laliotis et al., 2010).

Schoonmaker et al. (2004) observed increased activity of ATP-CL in intramuscular fat with increasing intake of concentrates. Similarly, activity of ATP-CL and NADP-MDH was increased in subcutaneous adipose tissue when animals were fed more concentrated diets (Smith and Crouse, 1984; Schoonmaker et al., 2004). Bauman (1976) reported a significantly increased utilization of glucose for lipogenesis, along with increased activities of ATP-CL and NADP-MDH, when glucose was infused intravenously to lambs. Therefore, in situations when high-concentrate diets are fed to ruminants, greater glucose and insulin concentrations result. These responses lead to increased activity of key enzymes which permit mitochondrial acetyl-CoA from AA catabolism to also be used as a carbon source for fatty acid synthesis in the cytosol, in a manner similar to that observed in monogastric animals.

Results from several studies reviewed by Markantonatos (2006) indicated that even if all the propionate absorbed from the rumen could be dedicated to glucose synthesis, its contribution would represent only 25 to 60% of total glucose produced in ruminants. Therefore, catabolism of AA provides a major source of carbon for glucose synthesis in ruminants (Armstrong and Hutton, 1975). Pyruvate, ketoglutarate, succinyl-CoA, fumarate, and oxaloacetate derived from the catabolism of AA such as alanine, glutamate, valine, tyrosine, and asparate can effectively serve as glucose precursors via gluconeogenesis (Newsholme and Leech, 1983). On the other hand, because 2 carbons are lost as $CO_2$ during TCA cycle, the 2-carbon molecule acetyl-CoA from AA catabolism cannot be utilized as a glucose precursor. Therefore, no glucose can be

derived from leucine and lysine catabolism since their catabolism renders only acetyl-CoA.

Uric acid derived from intestinal catabolism of purines is transported to the liver where most of it is catabolized to the water-soluble purine derivative allantoin by the action of hepatic uricase (Chen and Ørskov, 2004; Tas and Susenbeth, 2007). Because allantoin and uric acid cannot be used by body cells (Chen et al., 1990), they are excreted mainly in urine, but also secreted in saliva and milk (Tas and Susenbeth, 2007). Based on the origin and metabolism of purine bases, determination of urinary purine derivatives is used as a method to estimate microbial protein synthesis in the rumen.

As mammals, humans and ruminants excrete uric acid as a product of purine metabolism; however, the proportion excreted is smaller in humans. The explanation for this difference may reside in differences in the capacity to synthesize antioxidants other than uric acid (Álvarez-Lario and Macarrón-Vicente, 2010) and differences in accessibility to water between species. In their natural environment, ruminant diets contain a large amount of water from grass or fresh forages, allowing them to excrete the water-soluble allantoin instead of the water-insoluble uric acid. In addition, conservation of the capacity to synthesize the important antioxidant vitamin C in ruminants, may have transformed them to be less dependent on other antioxidants like uric acid. Conversely, loss of uricase activity in humans during the Miocene epoch could be associated with the previous loss of capacity to synthesize vitamin C in a period during which vitamin C ingestion was high (Álvarez-Lario and Macarrón-Vicente, 2010). In human evolution,

loss of uricase activity may have resulted from a strategy of retaining a beneficial product (uric acid) rather than from one where a harmful waste has to be disposed (Álvarez-Lario and Macarrón-Vicente, 2010).

## 3. PROTEIN REQUIREMENTS OF BEEF CATTLE

In 1996, the National Research Council (NRC) released the most current version of the Nutrient Requirements of Beef Cattle, which was then updated in 2000. One of the most important differences between these latest versions and the previous ones is the form in which cattle protein requirements are expressed. Previously, protein requirements were expressed on a Crude Protein (CP) basis while in the latest versions protein requirements are expressed on a Metabolizable Protein (MP) basis. However, it must be clarified that ruminants have requirements of AA from MP, and MP *per se* is not the required nutrient.

### 3.1. METABOLIZABLE AND NET PROTEIN

***Metabolizable protein*** represents the protein fraction that has been already digested and absorbed in the small intestine and is therefore available for the animal to use in various metabolic functions. ***Net protein*** (NP) is the MP fraction that is finally retained in the body after undergoing various biochemical transformations including transaminations and protein synthesis described in Section 2.4; its value is estimated as MP multiplied by its efficiency of use.

## 3.2. EFFICIENCY OF USE OF METABOLIZABLE PROTEIN

The ***efficiency of use of a protein***, such as MP, depends on (1) the biological value (BV) of that protein and (2) the efficiency of use of an ideal protein or ideal mixture of AA (Oldham, 1987) for maintenance, growth, gestation or lactation. The BV of MP depends on the biological value of dietary UIP and that of ruminally synthesized true MCP. The BV of the former depends upon (a) the quality of feed protein and (b) the metabolic process for which the protein is intended. For example, UIP from corn grain is deficient in arginine while that from corn gluten meal is considered adequate when compared to the arginine concentration in milk. However, UIP from corn gluten meal is considered deficient in arginine when compared to the arginine concentration in muscle (Van Soest, 1994). On the other hand, the BV of true MCP is considered constant and deemed to be high, though deficient in methionine, histidine and tryptophan but adequate in lysine when compared to muscle AA composition (Van Soest, 1994).

The fact that the BV of a protein is different for growth than for lactation implies that the efficiency of use of MP varies depending on the metabolic process (maintenance, growth, gestation, lactation). In addition, efficiency for growth is likely not constant across animal weight/age and rate of gain. Based on data from Ainslie et al. (1993) and Wilkerson et al. (1993), the NRC (2000) suggests an efficiency of use of MP of 49.2% for equivalent empty body weight (EQEBW) above 300 kg (662 lb), and greater MP efficiency for EQEBW below 300 kg (Efficiency = 83.4 – 0.114 x EQ Shrunk BW).

Changes in MP efficiency across BW/age might be related to a larger amount of actively proliferating and differentiating satellite cells in younger than older animals. The role of satellite cells is to fuse with the mature muscle fiber and provide the nuclei -DNA- necessary to control an additional portion of cytoplasm; thus, permitting increased protein synthesis, accretion, and muscle hypertrophy. However, satellite cells fuse faster than they proliferate. Consequently, the number of satellite cells progressively decreases as the animal becomes older, and those that remain become quiescent, reducing protein accretion (M. White and W. Dayton, University of Minnesota, personal communication). Therefore, AA from MP intended for protein synthesis and final accretion (NP for growth) may decrease as maturity increases, thus decreasing efficiency of use of MP for growth. In other words, as the animal becomes older, relatively more AA from MP might be used for functions not directly related to protein accretion, such as gluconeogenesis or ATP synthesis described in Section 2.4. The effect of increased rate of gain on physiological age may also explain a decreased efficiency of MP use with increasing rate of gain.

### 3.3. METABOLIZABLE PROTEIN BALANCE

Metabolizable protein balance results from the difference between ***MP supply*** and ***MP requirements***. Requirements of MP are estimated based on the factorial method; factors included are maintenance (metabolic fecal and urinary losses, and scurf losses), growth, fetal growth, and lactation. Estimations of MP requirements for maintenance and growth based on the NRC model are summarized in Table 1. To estimate true metabolic

fecal losses (intestinal desquamations and digestive enzymes), indigested dietary N as well as microbial N (complete microbial cells or cell walls) have to be subtracted from total fecal N. True metabolic urinary losses account for protein turnover in body tissues and include creatinine, purine derivatives, and urea. The NRC (2000) suggests a maintenance requirement of 3.8 g of MP/kg of metabolic shrunk body weight ($SBW^{0.75}$). Net protein requirements for growth are estimated considering body weight, rate of gain, and targeted end point based on degree of intramuscular fat achieved (marbling; Table 1).

Based on the NRC model (2000), MP supply can be estimated as the summation of true dietary UIP and true ruminally synthesized MCP digested and absorbed in the small intestine. Consequently, MP supply is estimated as *dietary UIP x proportion of true protein in dietary UIP x dietary true UIP intestinal digestibility + MCP x proportion of true protein in MCP x true MCP intestinal digestibility*. As described in Sections 1 and 2.2, UIP and MCP are considered to be 100 and 80% true protein, respectively, while their intestinal digestibility is assumed at 80% (Storm et al., 1983a,b; NRC, 2000). Therefore, *MP supply = dietary UIP x 1 x 0.8 + MCP x 0.8 x 0.8* or *UIP x 0.8 + MCP x 0.64*.

## 4. POTENTIAL DEGRADABLE INTAKE PROTEIN DEFICIT IN CONCENTRATE-BASED DIETS

As mentioned previously, MCP represents a high proportion of MP supply to the host animal. In addition, VFA produced as the result of ruminal fermentation of feed,

microbial growth and MCP synthesis, are essential to provide the animal with energy sources and fatty acid precursors (acetate, butyrate) as well as a glucose precursor (propionate). Consequently, adequate supply of nitrogen as well as carbohydrates and other growth factors to enhance ruminal microbial growth, is essential for an efficient beef production process from both protein and energy supply perspectives.

Based on the information presented in the previous sections, high dietary inclusion of rapidly-fermentable carbohydrates can result in increased DIP requirements. In addition, diets formulated using corn grain as the energy source may not supply adequate amounts of DIP because corn protein is considered to be approximately 60% UIP (Lardy et al., 1998; NRC, 2000). Compared with proteins from barley, oats, and wheat, corn protein presents a hydrophobic condition, low solubility, and slow rate of hydrolysis and degradation in the rumen. These characteristics of corn proteins are defined by the high concentration of ruminal fluid-insoluble prolamins (like zein) and glutelins relative to albumins and globulins, and the characteristic continuity and cross-linkages of the protein matrix (Delfino, 1986; Rooney and Pflugfelder, 1986; Herrera-Saldana et al., 1990b; Van Soest, 1994; Philippeau et al., 2000).

Concentration of DIP in high-moisture corn (HMC) is considered greater (45.1 to 48.8%) than that in dry-rolled corn (DRC; 34.3%) due to positive effects of early harvesting on the maturity of the corn kernel, increased moisture, and/or fermentation and solubilization of protein matrix that occurs during the ensiling period (Benton et al., 2005). Babnik and Verbič (2004) observed that the rapidly degradable protein fraction

increased, the slowly degradable protein fraction decreased, while the degradation rate of the latter fraction increased due to ensiling of corn grain. On the other hand, due to heat exposure, DIP concentration in steam-flaked corn (SFC) can be limited. Lykos and Varga (1995) reported a significantly decreased CP degradability for SFC (29.4%) compared with finely ground corn (53.3%) and a numerically decreased CP degradability compared with cracked corn (37.1%). Heat treatment can denature protein by altering its structure and biological functions (Neurath et al., 1944) and, if excessive, it can render protease-resistant products through the condensation of AA, mainly the ones with a free amino group, with reducing sugars, mainly furanoses like arabinose and fructose, rather than pyranoses like glucose and xylose (Van Soest, 1994). The occurrence of this condensation, named the Maillard reaction, is significantly increased by temperatures above 60 °C and water content around 30% (Van Soest, 1994). Due to the presence of arabinose, feeds rich in hemicellulose and pectin are much more reactive than those rich in starch or cellulose (Van Soest, 1994). Heat treatment of grains and forages can decrease solubility of protein (Tagari et al., 1962; Lykos and Varga, 1995) and extent of degradation of protein in the rumen and the small intestine; conversely, heat treatment of feeds can increase the flow of dietary AA to the small intestine, which in turn can increase the nutritive value of feed protein, depending on its biological value. Heating above the optimal temperature may overprotect the protein, significantly decreasing ruminal and intestinal digestion, thus increasing fecal losses of N (Van Soest, 1994). Broderick and Craig (1980) and Craig and Broderick (1981) reported decreased ruminal

degradation and true intestinal digestibility of protein from 78.8 to 25.1% and from 90.6 to 70.8%, respectively, when increasing heating time of cottonseed meal. However, due to a proportionally larger effect on ruminal degradability of protein and, consequently, on escape of dietary AA, intestinal availability of dietary AA was increased. Based on the concepts discussed in previous sections, effect of reduced N degradability on MCP synthesis may depend on the degree of reduction and on the availability of rumen fermentable carbohydrates. Stern et al. (1985) observed reduced *in situ* protein degradability while flow of bacterial N to the duodenum was not affected with increasing heat treatment of whole soybeans included in ground corn-corn silage-based diets fed to dairy cows.

The characteristics of corn proteins previously described plus the proportion of crystalline (vitreous or waxy, greater amylopectin concentration) to amorphous (floury, greater amylose concentration) endosperm, also determine reduced accessibility to starch granules and degradation of corn starch by ruminal microbes (McAllister et al., 2007). Grain processing, such as grinding, rolling (DRC), ensiling (HMC), and steam-flaking (SFC), increases access to starch granules. This increased access is achieved either by physically reducing particle size and increasing exposed surface area, disrupting the starch structure, and/or disrupting, solubilizing, partially digesting or denaturing the protein matrix in which starch granules are embedded; the net result is an increase in ruminal, intestinal and total tract digestibility of starch (Owens et al., 1986; Nocek and Tamminga, 1991; Lykos and Varga, 1995; Owens and Soderlund, 2007). In this regard,

steam-flaking can be considered one of the most, if not the most intense processing method, because it increases the surface area that is available for microbial attachment and produces gelatinization of the starch, a process that disrupts the intermolecular hydrogen bonds and causes loss of crystallinity by a combination of moisture, heat, pressure, and mechanical shear force (Nocek and Tamminga, 1991; Lykos and Varga, 1995). In addition, steam-flaking disrupts the protein matrix by shear forces on hot grain during flaking, thus enhancing accessibility by ruminal microbes to starch granules (Zinn et al., 2002). Based on starch ruminal digestibility, corn processing methods which result in least to greatest starch availability are: whole, DRC, ground corn, HMC and SFC. Starch availability of HMC or SFC may be the greatest depending on moisture content of HMC and degree of processing of each (Galyean et al., 1976; Owens et al., 1986; Theurer, 1986; Nocek and Tamminga, 1991; Sniffen et al., 1992; Huntington, 1997; Cooper et al., 2002b; Erickson et al., 2002).

Finally, even though effects of grain processing on other factors that would in turn affect DIP balance have to considered as well, given both starch and protein ruminal degradabilities, the potential risk of producing ruminal DIP deficits (DIP requirements > DIP supply) is greater for SFC compared with HMC and DRC. Therefore, need for DIP supplementation to optimize animal performance might be greater for SFC-based diets. For example, Cooper et al. (2002a) individually fed 90 steers a DRC-, HMC-, or SFC-based diet supplemented with increasing DIP concentrations. Within each type of grain processing, greatest feed efficiency was achieved with diets containing 6.3, 10.1 or 9.5%

DIP for DRC-, HMC-, or SFC-based diets, respectively. Consequently, in order to optimize animal performance and considering that DIP concentration in non-supplemented diets was 4.8, 6.7, or 4.7%, dietary DIP should be increased 1.5, 3.4, or 4.8 percentage units or 31, 51, or 102% through DIP supplementation in DRC-, HMC-, or SFC-based diets, respectively.

Partial replacement of corn by distillers grains (DG) has become common in many feedlot diets. Distillers grains are a by-product of the ethanol production process, during which the starch in corn grain is fermented to ethanol by yeast. Since corn is nearly two-thirds starch (70% starch), the other components in the remaining one-third of the kernel become three-times concentrated in the resulting DG. For example, CP and NDF concentrations in corn grain are about 9 and 10%, respectively, while those in DG are near 30 and 30 to 40%, respectively (NRC, 2000; Beef Magazine, 2012; Paulus Compart et al., 2013). Therefore, the replacement of corn by DG in the diet basically implies replacing part of dietary starch by fiber and protein.

Rate of fermentation of NDF from DG was reported greater (6.3%/h) compared with NDF from other industry co-products incubated *in vitro* and unground (about 3.8%/h; Bhatti and Firkins, 1995). Additionally, and possibly partially related to reduced particle size (Bhatti and Firkins, 1995; Corrigan et al., 2009), rate of fermentation of carbohydrate-containing fractions (Fraction A: sugars, Fraction B1: starch, Fraction B2: digestible cell wall) of DG were found similar to those of HMC, and SFC but greater than those of DRC (Sniffen et al., 1992). However, considering both rate of fermentation and

proportion of these fractions (NRC, 2000), overall carbohydrate rate of fermentation might be slower for DG (10%/h) than for DRC (26%/h), HMC (35%/h), and SFC (37%/h). Regarding extent of ruminal digestion, Corrigan et al. (2009) reported that the predominant carbohydrate-containing fraction (NDF or starch) and *in situ* digestibility of DM after a 22-h incubation period were 43.5% and 41.7%, 51.2% and 70.3%, 89.1% and 77.4%, and 86.6% and 80.2%, for DRC, wet DG, HMC, and SFC, respectively.

As part of the ethanol production process, the kernel is mixed with water and cooked at high temperatures before the fermentation phase (Berger and Singh, 2010). These two conditions may favor the formation of Maillard products (Van Soest, 1994), thus decreasing protein degradability of resulting DG. In addition, increased concentration of hemicellulose in DG (≈ 17 to 23%; Beef Magazine, 2012; Dairy One, 2014), estimated as the difference between NDF and acid detergent fiber, may further favor the formation of protease-resistant products. As reported by Waldo and Goering (1979) and summarized by Sniffen et al. (1992), DG is one of the five common feeds that may contain important amounts of protein in the bound or indigestible form. Due to additional heat applied during drying, protein degradability of dried DG may be even smaller when compared with that of wet DG. Firkins et al. (1984) observed that protein apparently escaping rumen fermentation was greater for steers fed dried (54%) than wet DG (47%), even though differences were not statistically significant ($P > 0.05$). Similarly, Cao et al. (2009) reported that protein rumen degradability as well as intestinal digestibility of rumen-undegraded protein was greater for wet (53.1 and 69.7%)

compared with dried DG (38.0 and 64.0%). On average, reported DIP concentrations rank, from highest to lowest, as dry-rolled or cracked corn, wet DG, and dried DG (NRC, 2000; Beef Magazine, 2012).

Regarding the effect of various DIP concentration across DG products on animal performance, Ham et al. (1994) compared dried DG products differing in their proportion of acid detergent insoluble N (ADIN), which is considered to be undegradable by ruminal microbes (Sniffen et al., 1992). Therefore, increased ADIN implied decreased proportion of DIP and increased proportion of dietary UIP reaching the small intestine. A group of growing calves was fed a diet containing 32% sorghum silage, 50% corncobs, 15% dried DG, 0.98% urea, and 0.13% ammonium sulfate. Rate of gain was not affected by concentration of ADIN. This result may suggest that, due to moderate rate of fermentation of the growing diet, DIP supply was sufficient to meet DIP requirements and/or, if present, DIP deficit was overcome by sufficient urea recycled to the rumen in time from excess UIP absorbed at intestinal level.

Considering how amount ingested and rate and extent of ruminal fermentation of carbohydrates and proteins affect DIP balance, it would be reasonable to expect DIP balance being affected by level of inclusion of DG. In this regard, Ponce (2010) studied the potential occurrence of DIP deficit with SFC-based diets containing 15 or 30% of wet DG (90:10 corn:milo) fed to finishing steers. Based on average daily gain (ADG) and feed efficiency response to DIP supplementation (0, 1.5, or 3.0% DIP supplementation), the un-supplemented 15%-DG diet, but not the 30%-DG diet limited animal performance

due to a DIP deficit. Greater replacement of SFC by wet DG increased basal dietary DIP concentration due to high concentration of CP in the latter and might have further reduced diet rate of fermentation, two factors that would increase DIP balance, thus reducing need of DIP supplementation.

## 5. UREA INCLUSION IN FEEDLOT DIETS

Efforts have been made towards determining DIP requirements of ruminal microbes in feedlot cattle under various feeding conditions. Because urea is an inexpensive source of NPN, which is rapidly and completely degraded in the rumen, scientists have carried out studies to investigate the effect of adding rumen-degradable N in the form of urea, to feedlot diets for which DIP deficits were expected. A summarized description of diets evaluated in these studies is presented in Table 2. Studies included a basal, control-type diet, to which smaller amount or no urea was added, and several diets with increased DIP concentration achieved by urea supplementation. Consequently, dietary CP concentration increased while UIP remained fairly constant across treatments within study. Diets were based on DRC, SFC, HMC, dry-rolled barley (DRB), or steam-flaked barley (SFB), and some of them included various concentrations of DG (Table 2). Surprisingly, HMC was evaluated in only 2 studies (Cooper et al., 2002a), representing 9 different diets. Dietary DIP concentration was calculated based on reported diet composition and book-referenced values (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012) for CP and DIP (DRC: 9 and 40%; SFC: 9 and 38%; HMC: 9 and 60%; DRB: 11.5 and 67%; SFB: 12 and 61% for CP and DIP expressed on CP basis, respectively).

Results from most of the studies summarized demonstrated positive effects ($P \leq 0.10$) of urea inclusion on cattle ADG (Figure 1). Only in one study (reference 1-1, Table 2) there was a report of a decrease in ADG from 1.35 to 1.24 kg when increasing DIP concentration from 6.6 to 8.4% in DRC-based diets. Besides the full symbol that represents that study, other full symbols below the $ADG_{Ux}=ADG_{U0}$ line (Figure 1) correspond to the greatest DIP concentrations in a positive significant quadratic or cubic-type response (polynomial contrasts; references 2-2 and 4-4, Table 2). In other words, the aforementioned full symbols below the $ADG_{Ux}=ADG_{U0}$ line do not represent a particular contrast between the control-type and the greatest DIP-containing diets. Similar explanation is valid for full symbols right on the $ADG_{Ux}=ADG_{U0}$ equation (Figure 1). These results may suggest that effects of urea supplementation on ADG may depend, among others, on level of supplementation. Additionally, the non-significant (NS, $P > 0.10$) effects of increasing dietary DIP concentrations were found when $ADG_{U0}$ was equal or greater than 1.52 kg (Figure 1). Similarly, NS effects on ADG were mostly observed when dietary DIP concentration for the control-type diet was greater (Figure 2). This may imply that chances of improving ADG by urea supplementation would be greater when ADG is limited, at least partially, by reduced dietary DIP. However, several positive effects on ADG were also found with increased basal dietary DIP concentrations (Figure 2), which may suggest that additional factors, such as grain processing evaluated by Cooper et al. (2002a), may determine the dietary DIP concentration below which urea supplementation may positively affect animal performance.

As displayed in Figure 3, effect of urea supplementation on DMI is variable, which resulted in varying effects on feed efficiency (Figure 4), depending on the magnitude of the change in ADG and DMI. Results from several studies demonstrated increased DMI with increased dietary urea concentration (Milton et al., 1997b; Cooper et al., 2002a; Zinn et al., 2003; Gleghorn et al., 2004; Cole et al., 2006; Crawford et al., 2007; Kennington et al., 2009; Wagner et al., 2010; Ponce, 2010); yet, the basis for this response is not clear. Theoretically, increased OM digestibility due to increased microbial activity resulting from urea supplementation could explain an increase in DMI in diets whose intake is mostly regulated by gut fill or physical factors, which may likely not be the case for diets evaluated in the present studies. On the other hand, urea has ruminal alkalizing effect which may attenuate ruminal pH drops when highly fermentable diets are consumed, which in turn may positively affect DMI. However, this effect on ruminal pH is usually transitory (Zinn et al., 2003).

Based on information discussed in previous sections, beneficial effects of urea supplementation to DIP-deficient diets on animal performance might be explained by increased microbial activity, which may result in increased VFA production and MCP synthesis, the latter positively impacting animal performance under a negative MP balance generated by the un-supplemented diet. However, increased MCP synthesis may enhance animal performance even when MP balance for the basal, un-supplemented diet is positive, because AA supplied above requirements might be used as glucose precursors and/or energy sources. In theory, microbial activity may increase until DIP requirements

are fully met by DIP supply. Therefore, greatest animal performance derived from increased microbial growth should be achieved at that point or at least, it should not increase with increasing DIP balance beyond zero.

In an attempt to evaluate the relationship between DIP and MP balances and animal performance based on ADG and feed efficiency and, if possible, to determine DIP and MP balance that would maximize animal response, results from studies summarized in Table 2 were statistically analyzed in a meta-analysis. Additionally, dietary DIP, DMI, and type of grain that would predispose to ruminal DIP deficits were identified as well. Balances in terms of DIP and MP were calculated for each diet based on some modifications to Level 1 of the NRC model (2000), reported diet composition, ADG, DMI, initial BW, and final BW (when not reported, it was estimated as *initial BW + ADG x days on feed*), and book-referenced values (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012) for CP, DIP, and TDN concentration of ingredients (DRC: 88%; SFC: 93%; HMC: 91%; DRB: 84%; SFB: 90% TDN). One of the modifications introduced to the model was related to DIP requirement calculations, for which TDN from dietary supplemental fat sources was disregarded. Considering that efficiency of $NH_3$-N capture by ruminal microbes is below 100% due to direct absorption of $NH_3$-N across the rumen wall and passage of fluid from the rumen (NRC, 1985), other modifications were included. Balance in terms of DIP was estimated as *DIP supply – DIP requirements x 1.18*; where the 1.18 coefficient resulted from dividing 100 by 85%, the latter representing the efficiency of $NH_3$-N capture by ruminal microbes. When DIP balance

resulted positive, contribution of MCP to MP supply was calculated *as DIP requirements x 0.64*; when negative, the contribution was estimated as *DIP supply x 0.85 x 0.64* (NRC, 2001).

Data were analyzed using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC); models tested were weighted by number of treatment replications within study. Effects of DIP and MP balance on ADG were evaluated simultaneously, as well as their interaction; a term for the effect of type of grain was also included in the model. As expressed before, within studies that reported positive effects of adding urea on ADG, some of them demonstrated a concurrent increase in DMI (Milton et al., 1997b; Cooper et al., 2002a; Zinn et al., 2003; Gleghorn et al., 2004; Cole et al., 2006; Crawford et al., 2007; Kennington et al., 2009; Wagner et al., 2010; Ponce, 2010). Based on the hypothesis that effect of urea supplementation, thus DIP balance, on ADG could possibly be explained by its association with DMI, a term for DMI was included in the model as well. Therefore, the full model tested was $ADG = DIP\ balance + DIP\ balance^2 + MP\ balance + MP\ balance^2 + DIP\ balance*MP\ balance + Grain\ Type + DMI$. Factors were retained in the model when $P < 0.10$ (Type III Tests). Similar analysis was performed for feed efficiency, which was analyzed as kg of gain obtained per kg of feed consumed (G:F). Grain used in study 5-1 was analyzed as if it was SFC while that used in study 9-2 was analyzed as if it was DRC (Table 2).

## 5.1. RELATIONSHIP BETWEEN DEGRADABLE INTAKE PROTEIN AND METABOLIZABLE PROTEIN BALANCES AND RATE OF GAIN

Metabolizable protein balance (first and second order terms) and its interaction with DIP balance were non-significant (NS; $P > 0.26$). Rate of gain followed a quadratic response with increasing DIP balance for each of the 3 grain types ($P = 0.001$ for grain type; Figure 5). Within the range evaluated, the quadratic response demonstrated that ADG was more responsive to increased DIP balance when the latter was negative, that is, when there was an unmet demand of ruminal DIP. The fact that *P*-value from Type III Tests for DIP balance was significant ($P < 0.012$ for first order and quadratic terms) in a model in which MP balance was included as a factor, suggests that positive effects of restoring DIP balance in the rumen on ADG might be related not only to its potential effects on increased MP supply through increased MCP synthesis (Crawford et al., 2007) but also to its potential effects on feed ruminal digestibility and VFA production. Ruminal digestibility of starch was increased from 47.1 to 64.4% (Milton et al., 1997b), from 88.0 to 93.1% (Zinn et al., 2003), and from 83.4 to 89.8% (Crawford, 2007) when increasing urea supplementation to DRC-, SFB-, or SFC-based diets, respectively. Similarly, Milton et al. (1997b) reported a significant increase while Milton et al. (1997a), Zinn et al. (2003) and Crawford (2007) observed a numerical improvement in OM ruminal digestibility with increased dietary urea. Numerical increases in VFA concentration were reported by the aforementioned authors except Crawford (2007).

Similarly, DIP balance remained as a significant factor in the ADG-prediction

equation even after including the effects of DMI ($P < 0.001$). Again, this may indicate that effects of DIP balance on ADG are not only related to potential effects of urea on increased DMI but also possibly to enhanced ruminal fermentation and feed digestibility, and energy value of the feed. This result is consistent with those observed for DIP balance on feed efficiency (see next Section).

Based on equation from Figure 5, DIP balance that would result in maximum ADG is 78.9 g/d, corresponding to an ADG of 1.63, 1.64, and 1.66 kg/d for DRC, HMC, and SFC, respectively. This result suggests that optimum DIP balance is about 79 g greater than that estimated by the NRC model (2000) to meet DIP requirements. In other words, this result does not challenge the assumption that DIP balance beyond zero should not enhance animal performance but that DIP requirements based on ADG response might be greater than estimated by the model. As mentioned in previous paragraphs, the NRC model determines DIP requirements as TDN intake x microbial efficiency, which is estimated based on eNDF and its association with ruminal pH. Overestimation of the negative impact of reduced eNDF on ruminal pH and consequently on microbial efficiency may result in decreased estimated DIP requirements, which in turn may partially explain an actual optimum DIP balance being greater than expected. For example, measured ruminal pH in the study by Milton et al. (1997a) was greater (5.82) than that predicted by the model (5.65). Ruminal pH-affecting factors other than eNDF, like supplemental urea, along with additional factors that may affect microbial efficiency, such as great DMI and associated increased rate of passage, which are not considered by

Level 1 of the NRC model, may help explain the increased optimum DIP balance. In that regard, selection in favor of improved animal performance and greater intake capacity of modern compared with older cattle may relate to greater DIP requirements.

Potentially, a DIP deficit can be partially or totally reversed by a positive intestinal MP balance (MP supply greater than MP requirements), through urea being recycled back to the rumen (NRC, 2000). Due to the fact that the term for the interaction between DIP and MP balances was non-significant ($P = 0.79$), there is no evidence to support the hypothesis that positive effects of increasing DIP balance on ADG might be dependent on MP balance. This result demonstrates that reducing a negative DIP balance through the addition of urea resulted in increased ADG even when urea could potentially be recycled from excess MP. This result does not indicate that urea was not recycled from extra MP to supply additional N to the rumen; it merely suggests that either the amount of recycled urea was not enough to reverse a DIP deficit or that urea from excess MP supply was not recycled to the rumen synchronous to the time of ruminal digestion of rapidly-fermentable diets. This may explain why urea supplementation was effective to reduce a DIP deficit, irrespective of MP balance.

As expressed before, MP balance was not a significant ($P > 0.26$) variable when modeling ADG. This result does not neglect the importance of meeting MP requirements or the role of MP-derived AA as glucose precursors. As summarized by Markantonatos (2006), at most 60% of the glucose can be derived from propionate in ruminants. Therefore, the role of MP as a significant contributor to gluconeogenesis may be

underestimated. However, probably related to the significant association between DMI and MP balance (r = 0.51; $P < 0.001$) which was also reported by DiCostanzo (2007) for a different set of data, beneficial effects of MP balance on animal performance might be explained in terms of increased DMI, which would in turn explain why MP balance was a non-significant factor when simultaneously included with DMI in the ADG model.

## 5.2. RELATIONSHIP BETWEEN DEGRADABLE INTAKE PROTEIN AND METABOLIZABLE PROTEIN BALANCES AND FEED EFFICIENCY

Feed efficiency was estimated by a model that included the effect of DIP balance (first order and quadratic terms; $P < 0.01$) and grain type ($P < 0.001$; Figure 6). Terms for DMI, MP balance (first order and quadratic terms), and interaction between DIP and MP balances were not significant ($P > 0.12$).

As expected, G:F was greatest for SFC (0.176 ± 0.003), intermediate for HMC (0.155 ± 0.005), and lowest for DRC (0.152 ± 0.003; Figure 6) and improved when DIP balance increased from negative to 50.0 g/d. This result is consistent with the previously described significance of DIP balance as a factor explaining the variation in ADG, even after considering potential effects of the former on DMI. It may also emphasize the potential beneficial effects of increased DIP supply to DIP-deficient diets on OM and starch digestion, and VFA production, and consequently on feed value.

## 5.3. *CRITICAL* DIETARY DEGRADABLE INTAKE PROTEIN AND ITS INTERACTION WITH GRAIN TYPE

It was reported in the previous section that optimum DIP balance was 50 g/d, which corresponded to the greatest feed efficiency (0.156, 0.159, or 0.180 for DRC-, HMC- or SFC-based finishing diets; Figure 6). To identify which dietary DIP concentration would correspond to optimum DIP balance and consequently maximum G:F, the latter was modeled in terms of dietary DIP, grain type, and their interaction. Based on the hypothesis that replacement of corn by DG may result in decreased dietary rate of fermentation, mainly in SFC-based diets, and therefore in reduced optimum dietary DIP, terms for DG inclusion, and its interaction with dietary DIP and grain type were included in the model as well. Only dietary DIP (first order and quadratic terms, $P < 0.01$) and its interaction with grain type ($P = 0.02$) were retained in the model (Figure 7).

The fact that the effect of dietary DIP on feed efficiency was the same for diets with or without DG ($P = 0.60$ and $P = 0.86$ for DG inclusion and its interaction with dietary DIP, respectively), indicates that factors that potentially affect DIP requirements may compensate between those diets. For example, based on the hypothesis that the replacement of corn by DG basically implies replacing starch by NDF and protein, rate of fermentation was speculated to be reduced, thus reducing microbial efficiency and DIP requirements. However, this proposed effect could be compensated by positive effects resulting from reduced particle size of DG on fermentation rate, as well as possible increased intake, and therefore increased DIP requirements, when replacing 10 to 30%

corn with DG. In a meta-analysis, Klopfenstein et al. (2008) reported a quadratic increase in DMI with increasing DG inclusion in DRC- or HMC-based diets, with DMI being maximized at about 30% DG inclusion. Similarly, Luebbe et al. (2012) observed highest DMI with 15 to 30% DG inclusion in SFC-based finishing diets. Additionally, potential beneficial effects of protein from DG on ruminal pH may help compensate expected differences in terms of DIP requirements between diets. In that regard, literature reviewed by Dijkstra et al. (2012) demonstrates that charged molecules, such as proteins, are able to exchange cations (K, Ca, Mg) for protons, thus contributing to ruminal buffering capacity. Finally, limited number of studies involving urea inclusion in DG-containing diets may have restricted the ability to detect DG inclusion as a significant factor that could potentially affect the relationship between feed efficiency and dietary DIP.

Figure 7 depicts that maximum feed efficiency is achieved when dietary DIP is 7.6, 8.0, or 9.3% for DRC, HMC, or SFC, respectively. Therefore, *critical* dietary DIP, below which a DIP deficit and consequently need for DIP supplementation should be projected, varies depending on the degree of processing of the grain. Based on differences in rate of fermentation between diets, the observed difference in critical dietary DIP was expected. Potential MCP synthesis increases with increasing starch degradability (Spicer et al., 1986; Brake et al., 1989; Barajas and Zinn, 1998); therefore, it makes sense that DIP required for maximal animal performance would increase with more extensively processed grains. Opposed to what was observed in the present analysis, Cooper et al. (2002a) reported smaller optimum dietary DIP for SFC- (8.3%) compared with HMC-

based diets (10.0%). This result might be partially related to the greater energy value of SFC, which results in reduced DMI. It may also be related to the possible negative effects of high fermentability of SFC on ruminal pH, which in turn can further affect DMI (Britton and Stock, 1987; Zinn, 1990; Owens et al., 1997; Cooper et al., 2002a,b; Erickson et al., 2002; Zinn et al., 2002; Owens, 2005) as well as microbial efficiency. Together, these effects may result in reduced DIP requirements and consequently reduced need of dietary DIP.

Based on estimated critical dietary DIP concentration and assuming a basal DIP concentration of 3.8, 5.3, or 3.6% DIP in 85%-DRC-, -HMC-, or -SFC-based diets, respectively, need for urea supplementation would be 1.35, 0.96, or 2.03% for each type of diet, respectively. This need would be reduced for DG-containing diets due to increased basal dietary DIP.

### 5.4. *CRITICAL* FEED INTAKE AND ITS INTERACTION WITH GRAIN TYPE

Feed intake can greatly affect DIP requirements and consequently, the possibility of encountering a DIP deficit if diets are not supplemented with degradable N. As previously mentioned, the basis for increased DIP requirements with increasing intake of highly-digestible diets is related to increased supply of rapidly-fermentable carbohydrate to the rumen and increased rate of passage (increased microbial efficiency). This relationship suggests that a diet which would likely provide enough DIP to the rumen may generate a DIP deficit if DMI is greater than expected. For example, cattle that enter

the feedlot after a feed-restriction period may likely have increased risk of a DIP deficiency due to compensatory intake when they are offered *ad libitum* feed. Similarly, decreased initial BW may increase likelihood of a negative DIP balance, as lighter animals usually express greater relative intake (% of BW).

Based on data from studies summarized in Table 2, a model was tested to determine the relative intake beyond which ADG would positively respond to urea supplementation. Within study, the difference between ADG for the control-type diet and each of the urea-supplemented treatments was calculated. This variable was regressed against relative intake for each treatment and the interaction between relative intake and grain type. As in the previous analyses, the model was evaluated using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) and weighted by number of treatment replications per study. Both relative intake and its interaction with grain type were significant ($P < 0.01$). As guidance, relative intake beyond which urea supplementation may have a positive effect on ADG was 2.17 and 1.63% of BW (BW = (initial BW + final BW)/2) for DRC- and SFC-based diets, respectively. Due to limited data, critical relative intake could not be determined for HMC-based diets.

## 6. SLOW-RELEASE UREA INCLUSION IN FEEDLOT DIETS

Based on the information presented in the previous sections, balanced supply of N and energy is essential to enhance microbial growth, which may result in increased supply of energy and protein to the host animal and potentially in improved animal

performance. Additionally, it is logical to expect that asynchronous supply of N and energy may result in fermentation occurring largely without microbial growth, thus reducing efficiency of ruminal fermentation and increasing $NH_3$-N losses to the environment (Dijkstra et al., 1998; Cabrita et al., 2006; Reynolds and Kristensen, 2008). In this regard, synchronized supply does not necessary imply similar rates of fermentation between carbohydrate and N sources because, as emphasized by Valkeners et al. (2004), Cabrita et al. (2006), and Reynolds and Kristensen (2008), recycling of N via hepatic synthesis of urea may enable compensation for short-term effects of asynchrony. That might be the reason why there were variable results of synchronizing N and carbohydrate fermentation rates on ruminal fermentation parameters and performance of dairy (Cabrita et al., 2006) and beef cattle (Valkeners et al., 2004). However, scenarios should be recognized when the possibility of recycled N to overcome the effects of asynchrony exists. Situations in which N sources are fermented faster than carbohydrates (e.g. NPN, proteins, and NDF in young fresh forages), thus increasing proportion of $NH_3$-N absorbed through the rumen wall, or situations in which moderate fermentation rate of carbohydrates and excess MP absorbed at intestinal level are expected, may be prerequisites for recycled urea to compensate for DIP deficits. The latter might have been the case in the study by Jenkins et al. (2011) in which they evaluated the effect of adding urea to a DRC-based diet containing 10% dried DG on finishing cattle performance. Balances of DIP and MP for the no-urea diet were estimated by the authors at -92 and 268 g/d, respectively. Since MP requirements were met, it can

be assumed that N from the excess 268 g MP would be transformed to urea in the liver and that a high proportion of the synthesized urea would be recycled back to the rumen (Reynolds and Kristensen, 2008) to overcome an unmet demand of 92 g DIP. Lack of effect of urea supplementation on ADG and feed efficiency may indicate that excess MP absorbed at intestinal level might have been sufficient to supply recycled N in time when carbohydrates from DRC were fermented in the rumen. Similar results were observed by Stalker et al. (2004) when supplementing urea to forage-based diets containing 25 or 30% DG for which the need of DIP supplementation (negative DIP balance) and excess MP (positive MP balance) were calculated to be similar. Conversely, based on the positive response of animal performance to urea supplementation in the study by Cooper et al. (2002a), the DIP deficit (-19 g/d) generated by an 82%-HMC diet might not have been overcome by N recycled from excess MP (78 g/d). This result can be interpreted in terms of inadequacy of excess MP to meet, in time, the DIP deficit generated by a fast fermenting HMC-based diet.

About 25 and 80% of total $NH_3$-N produced in ruminal fluid by urea hydrolysis is released within the first 0.5 and 6 h post-incubation, respectively (DiLorenzo and DiCostanzo, 2007a, 2007b). Based on values reported by NRC (2000) and Sniffen et al. (1992), rate of starch fermentation ranged from 10 to 40 %/h, depending on degree of grain processing while that of NDF from DG ranged from 6 to 8 %/h; the rate of urea hydrolysis was 400 %/h. Therefore, even though supplementation of conventional urea in fast-fermenting diets improved ruminal fermentation (Milton et al., 1997b; Zinn et al.,

2003; Crawford et al., 2007) and animal performance (Figures 1, 5, 6, and 7), further beneficial effects may arise from the use of urea sources with reduced rate of $NH_3$-N release.

Slow-release N sources are designed to provide a sustained ruminal release of N as opposed to the rapid ruminal N release from conventional urea, yet faster than MP-derived N recycling. However, there is a significant variation in the pattern of $NH_3$-N release among different sources of slow-release N products (DiLorenzo and DiCostanzo, 2007a, 2007b). Therefore, depending on this pattern of hydrolysis, the use of slow-release urea (SRU) may permit a timely match between NSC fermentation and $NH_3$-N release, which in turn may result in improved N utilization by ruminal microbes, feed digestibility, and animal performance. Taylor-Edwards et al. (2009) noted that degradation rate of a polymer-coated SRU source (6.28%/h) may match rates of grain starch degradation (3.1 to 23.5%/h) reported by Herrera-Saldana et al. (1990b).

Biuret, an SRU product available in the market since the mid 1970's, results from the condensation of two molecules of urea; it can be synthesized *de novo* from $NH_3$ and $CO_2$ or by urea pyrolysis (Fonnesbeck et al., 1975). Due to reduced solubility in water, biuret is hydrolyzed to $NH_3$ and $CO_2$ in the rumen more slowly than conventional urea, thus reducing risk of $NH_3$ toxicity (Fonnesbeck et al., 1975). Starea is produced by passing starch sources, like finely ground grains, and urea through a cooker-extruder under conditions causing starch gelatinization, resulting in a product that degrades more slowly (Deyoe et al., 1968).

More recently, other SRU products like calcium-bound urea (Huntington et al., 2006; Álvarez Almora et al., 2012), polymer-encapsulated urea (Tedeschi et al., 2002; Taylor-Edwards et al., 2009; Ribeiro et al., 2011; Álvarez Almora et al., 2012; Bourg et al., 2012; Holder, 2012; López-Soto et al., 2014), or lipid-encapsulated urea (Owens et al., 1980), have been evaluated in beef cattle diets, with variable results (Table 3). Similar inconsistent results have been observed by Galo et al. (2003), Golombeski et al. (2006), and Xin et al. (2010) when including polymer-encapsulated, calcium-bound, or polyurethane-coated SRU, respectively, in diets for lactating dairy cows. Most of the studies involved forage-based diets (Table 3) and only the study by López-Soto et al. (2014) utilized low DG-containing diets. The high variability in results obtained by the use of SRU might be related to differences in basal diet composition and pattern of N release of various SRU products, as well as level of SRU supplementation, among others. Further, the possibility of increased N recycling to the rumen from conventional urea may attenuate differences between NPN sources. In addition, the fact that many studies lack negative controls (Table 3) prevents from evaluating if similar effects between conventional urea and SRU supplementation are due to no beneficial effects of the latter over the former, or because there was no need for any type of N supplementation. The most consistent results of SRU compared with conventional urea supplementation are a decrease in $NH_3$-N ruminal concentration and lack of effect on OM digestibility (Table 3).

Ribeiro et al. (2011) observed a decrease in ruminal pH (Table 3) with a polymer-

encapsulated SRU source compared with conventional urea, which might be associated with reduced $NH_3$-N ruminal concentration, the latter contributing to ruminal buffering capacity. Similarly, Álvarez-Almora et al. (2012) observed a decrease in ruminal pH and a concomitant decrease in the acetate-to-propionate ratio (A:P) with polymer-encapsulated SRU compared with conventional urea (Table 3), which may reflect the negative impact of reduced pH on the growth of fiber-fermenting bacteria. Conversely, increased A:P ratio with polymer-encapsulated SRU compared with conventional urea was reported by Holder (2012). It was suggested by the author that a slower steadier supply of $NH_3$-N may favor slow-growing fiber-digesting bacteria.

Interestingly, Taylor-Edwards et al. (2009) reported decreased ADG and G:F with low (0.4%) dietary inclusions of polymer-coated SRU compared with conventional urea, but no differences in animal performance between sources when each of them was included at intermediate rates (0.8 or 1.2%). Similarly, Tedeschi et al. (2002) reported reduced ADG and G:F with polymer-encapsulated SRU compared with conventional urea when each supplement was included in a DIP-deficient forage-based diet at 0.4% to meet 50% of DIP deficit, but similar response between sources when included at 1.2% to meet 100% of DIP deficit (Table 3). As reported by Holder (2012), the same SRU product used in Tedeschi et al. (2002) experiment demonstrated not only a slower N release but also a smaller amount of N released than conventional urea even after a 24-h incubation period. Therefore, reduced animal performance with SRU compared with conventional urea at low N supplementation may result from limited N availability from SRU.

## 7. SUMMARY

As a consequence of ruminal fermentation of feed and microbial growth, the host animal is provided with a great proportion of its AA requirements as well as with VFA, essential as energy, glucose, and fatty acid precursors. Synthesis of MCP is dependent upon the balance between DIP supply and requirements; DIP requirements are affected by the amount of OM digested in the rumen and the amount of MCP produced per unit of OM fermented (efficiency of MCP synthesis). Therefore, several interrelated factors can affect the amount of MCP synthesized in the rumen, such as OM intake, availability of rumen fermentable carbohydrates and N, rate of fermentation of the diet, ruminal pH, and microbial outflow rate, among others.

Due to the increased inclusion of rapidly fermentable carbohydrates, high intake of feedlot diets can potentially generate a ruminal DIP deficit which, if not overcome, may result in limited cattle performance. The risk of a DIP deficit may be increased for corn-based diets since corn CP ruminal degradability is usually low.

Driven by a beneficial price relationship, partial replacement of corn grain by DG in feedlot diets has become a common practice in many regions of the United States. Particular characteristics of the product, such as low starch content, high CP and NDF content, reduced CP ruminal degradability, reduced particle size, along with its positive effect on whole diet DM intake when included at up to 30% of dietary DM, may create ruminal conditions that would affect the risk for DIP deficit.

Several studies have evaluated the effect of increasing dietary DIP through the inclusion of urea on feedlot cattle performance. A meta-analysis of the results from these studies revealed:

- Rate of gain and feed efficiency followed a quadratic response with increasing DIP balance through urea dietary inclusion; ADG and feed efficiency increased until DIP balanced reached 79 or 50 g/d, respectively.
    - This result suggests that optimum DIP balance is about 79 or 50 g greater than that estimated by the NRC model (2000) to meet DIP requirements.

- Positive effects of inclusion of urea on ADG were independent from MP balance and DMI. This result suggests:
    - Positive effects of restoring DIP balance on ADG might be related not only to its potential effects on increased MP supply through increased MCP synthesis, and to its potential effects on increased DMI, but also to its possible impact on ruminal fermentation and energy value of the feed.
    - Reducing a negative DIP balance through the addition of urea resulted in increased ADG even when urea could potentially be recycled from excess MP, suggesting that either the amount of recycled urea was not enough to reverse a DIP deficit or that urea from excess MP supply was not recycled to the rumen synchronous to the time of ruminal digestion of rapidly-fermentable diets.

- Maximum feed efficiency was achieved when dietary DIP was 7.6, 8.0, or 9.3% for DRC-, HMC-, or SFC-based diets, respectively.
    - The observed difference in critical dietary DIP is related to differences in rate of fermentation between diets.
    - Assuming a basal DIP concentration of 3.8, 5.3, or 3.6% DIP in a 85%-DRC-, -HMC-, or -SFC-based diets, respectively, need for urea supplementation would be 1.35, 0.96, or 2.03% for each type of diet, respectively. This need would be reduced for DG-containing diets due to increased basal dietary DIP.

- Relative intake beyond which urea supplementation may have a positive effect on ADG was 2.17 and 1.63 %BW for DRC and SFC, respectively.
    - The observed difference in critical relative intake is likely related to differences in rate of fermentation between diets and its impact on DIP requirements.

Even though the aforementioned studies have evaluated the effect of increasing dietary DIP through the inclusion of urea on feedlot cattle performance, as well as others have evaluated its effects on ruminal fermentation, studies involving DG-containing diets are warranted.

Synchronized supply of energy and N in the rumen may permit a more efficient N utilization by ruminal microbes, improved microbial growth, increased N retention, and reduced N excretion. Because rates of degradation of carbohydrates and urea do not

match, ruminal microbes and cattle may further benefit from the use of SRU sources over conventional urea when added to DIP-deficient diets. However, supplementation of SRU has resulted in inconsistent results across various studies, many of them involving forage-based diets and with no DG inclusion. Therefore, there is a need for information regarding potential beneficial effects of SRU compared with conventional urea inclusion in high energy-diets containing DG.

## 8. RESEARCH OBJECTIVES

The overall goal of this research was to provide information about the need of DIP supplementation through the inclusion of conventional urea or SRU sources differing in rate of N release to high-concentrate low-DG-containing diets. To attain this goal, the following objectives were proposed:

1. Evaluate the effects of adding conventional urea to a high-concentrate moderate-DG containing diet for which a DIP deficit is expected on finishing cattle performance, carcass characteristics, ruminal fermentation parameters, and total tract digestibility.

2. Evaluate the effects of adding one of two sources of SRU in comparison with conventional urea to a high-concentrate moderate-DG containing diet for which a DIP deficit is expected on ruminal fermentation parameters and total tract digestibility.

**Table 1.** Estimations of protein requirements for maintenance and growth suggested by NRC (2000)

| | Metabolizable protein (MP), g/d | Net protein (NP), g/d |
|---|---|---|
| Maintenance | 3.8 g/kg $SBW^{0.75}$ | MP x 0.67[†] |
| Growth | If,<br>• EQEBW < 300 kg, MP = NP / [(83.4 – 0.114 x EQSBW)/100][†]<br>• EQEBW ≥ 300 kg, MP = NP / 0.492[†] | SWG x [268 - 29.4 x (RE/SWG)] |

[†] Efficiency of use of MP
SBW (shrunk body weight) = 0.96 x full weight
EQEBW (equivalent empty body weight) = 0.891 x EQSBW
EQSBW (equivalent shrunk body weight) = SBW x (SRW/FSBW)
SRW (standard reference weight) = 478 kg for animals finished at small marbling (28% fat)
= 462 kg for animals finished at slight marbling (27% fat)
= 435 kg for animals finished at trace marbling (25% fat)
FSBW, actual final shrunk body weight at the body fat endpoint selected
SWG, shrunk weight gain
$RE = 0.0635 \times EQEBW^{0.75} \times EBG^{1.097}$
EBG = 0.956 x SWG

**Table 2**. Summarized description of diets (U0: smaller amount or no urea added, and Ux: increased urea inclusion) used in various research studies to evaluate the effect of increased degradable intake protein (DIP) through the inclusion of urea on feedlot cattle performance

| Reference [1] | Grain | | Distillers grains | | Urea inclusion, % | | Dietary DIP, % [3] | | Dietary crude protein, % [4] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Type [2] | Inclusion, % | Type | Inclusion, % | U0 | Ux | U0 | Ux | U0 | Ux |
| 1-1 | DRC | 85.3 | - | - | 0.91 | 1.55 | 6.6 | 8.4 | 12.1 | 14.0 |
| 2-1 | DRC | 84.2 | - | - | 0 | 0.50 to 1.50 | 3.7 | 5.1 to 8.0 | 8.5 | 9.9 to 12.7 |
| 2-2 | DRC | 83.1 | - | - | 0 | 0.35 to 1.40 | 4.7 | 5.7 to 8.8 | 9.6 | 10.6 to 13.1 |
| 3-1 | DRC | 79.5 | - | - | 0 | 0.88 to 1.96 | 4.5 | 6.6 to 9.7 | 8.9 | 11.1 to 14.1 |
| 4-1 | HMC | 82.0 | - | - | 0 | 0.40 to 1.20 | 7.0 | 8.1 to 10.2 | 10.6 | 11.8 to 14.1 |
| 4-2 | SFC | 82.0 | - | - | 0 | 0.40 to 2.00 | 4.6 | 5.7 to 10.1 | 9.5 | 10.6 to 15.3 |
| 4-3 | DRC | 82.0 | - | - | 0 | 0.50 to 2.00 | 4.9 | 6.3 to 10.4 | 9.5 | 10.9 to 15.3 |
| 4-4 | HMC | 82.0 | - | - | 0 | 0.50 to 2.00 | 6.4 | 7.8 to 11.9 | 9.5 | 10.9 to 15.3 |
| 4-5 | SFC | 82.0 | - | - | 0 | 0.50 to 2.00 | 4.8 | 6.1 to 10.2 | 9.5 | 10.9 to 15.3 |
| 5-1 | SFB | 76.3 | - | - | 0 | 0.40 to 1.20 | 7.1 | 8.3 to 10.5 | 10.5 | 11.5 to 13.5 |
| 6-1 | SFC | 79.5 | - | - | 0.49 | 1.02 & 1.56 | 6.2 | 7.7 & 9.3 | 12.0 | 13.1 & 14.5 |
| 7-1 | SFC | 80.0 | - | - | 0 | 0.55 | 4.8 | 6.4 | 9.6 | 11.1 |
| 8-1 | SFC | 85.0 | - | - | 0 | 1.50 | 3.6 | 8.0 | 9.6 | 13.7 |
| 9-1 | DRC | 83.5 | - | - | 1.0 | 1.20 & 1.50 | 7.1 | 8.1 & 9.4 | 11.6 | 12.7 & 14.1 |
| 9-2 | DRB | 84.4 | - | - | 0 | 0.30 & 0.50 | 8.0 | 9.2 & 10.1 | 11.6 | 12.9 & 13.9 |
| 10-1 | SFC | 79.5 | - | - | 0.01 | 0.38 to 1.42 | 4.7 | 5.9 to 9.0 | 10.5 | 11.5 to 14.5 |
| 11-1 | SFC | 70.4 | 32:68 corn:milo wet | 9.7 | 0.68 | 0.89 & 1.09 | 7.3 | 7.9 & 8.5 | 13.3 | 14.0 & 14.7 |

**Table 2 (cont.)**

| Reference [1] | Grain | | Distillers grains | | Urea inclusion, % | | Dietary DIP, % [3] | | Dietary crude protein, % [4] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Type [2] | Inclusion, % | Type | Inclusion, % | U0 | Ux | U0 | Ux | U0 | Ux |
| 12-1 | SFC | 66.5 | 90:10 corn:milo wet | 14.9 | 0 | 0.52 & 1.06 | 6.7 | 8.2 & 9.8 | 12.9 | 15.0 & 15.6 |
| 12-2 | SFC | 52.6 | 90:10 corn:milo wet | 29.7 | 0 | 0.52 & 1.06 | 8.6 | 10.2 & 11.7 | 16.6 | 18.1 & 18.9 |
| 12-3 | SFC | 65.8 | 76:24 corn:milo wet | 15.4 | 0.54 | 0.80 & 1.06 | 8.1 | 8.8 & 9.6 | 13.7 | 14.5 & 15.3 |
| 13-1 | DRC | 76.0 | corn dried | 10 | 0 | 0.80 | 5.0 | 7.3 | 11.2 | 13.4 |
| 13-2 | DRC | 66.0 | corn dried | 20 | 0 | 0.63 | 6.0 | 7.8 | 13.4 | 15.1 |
| 13-3 | DRC | 59.5 | corn wet | 25 | 0 | 0.50 & 1.00 | 6.6 | 7.9 & 9.2 | 14.2 | 15.3 & 16.4 |

[1] **1-1:** Milton et al., 1997a; **2-1 and 2-2:** Milton et al., 1997b; **3-1:** Shain et al., 1998; **4-1 to 4-5:** Cooper et al., 2002a; **5-1:** Zinn et al., 2003; **6-1:** Gleghorn et al., 2004; **7-1:** Cole et al., 2006; **8-1:** Crawford et al., 2007; **9-1 and 9-2:** Kennington et al., 2009; **10-1:** Wagner et al., 2010; **11-1:** Vasconcelos et al., 2007; **12-1 to 12-3:** Ponce, 2010; **13-1 to 13-3:** Jenkins et al., 2011.

[2] DRC: dry-rolled corn; SFC: steam-flaked corn; HMC: high-moisture corn; DRB: dry-rolled barley; SFB: steam-flaked barley.

[3] Estimated based on reported diet composition, and ingredients' crude protein (CP) and DIP book-referenced values (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012). DRC: 40% DIP and 9% CP; SFC: 38% DIP and 9% CP; HMC: 60% DIP and 9% CP; DRB: 67% DIP and 11.5 CP; SFB: 61% DIP and 12% CP; Corn dried distillers grains: 40% DIP, and 32.4 and 30.4% CP for Jenkins et al., 2011 (analyzed CP). Corn wet distillers grains: 49% DIP and 29.7% CP, except for Jenkins et al. (2011) where 30.1% CP was reported; 90:10 corn:milo wet distillers grains (Ponce, 2010): 49% DIP and 33.4% CP (analyzed CP); 76:24 corn:milo wet distillers grains (Ponce, 2010): 49% DIP and 32.5% CP (analyzed CP); 32:68 corn:milo wet distillers grains (Vasconcelos et al., 2007): 49% DIP and 31.9% CP. DIP expressed on CP basis.

[4] Reported values.

**Table 3**. Summarized effects of dietary inclusion of different slow-release urea (SRU) sources (Ca: calcium-bound; L: lipid-encapsulated; P: polymer-encapsulated; O: other) on ruminal pH, microbial crude protein synthesis (MCP), microbial efficiency (MicEf), ruminal concentration of volatile fatty acid (VFA) and ammonia nitrogen ($NH_3$-N); acetate-to-propionate ratio (A:P), organic matter digestibility (OMD), average daily gain (ADG), and gain-to-feed ratio (G:F)

| Ref. | Control diet [1] | Diet based on [2] | SRU | pH [3] | | MCP [3] | | MicEf [3] | | VFA [3] | | $NH_3$-N [3] | | A:P [3] | | OMD [3] | | ADG [3] | | G:F [3] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| 14 | - and + | C | O | | | | | | | | | | | | | | | > | > | > | > |
| 15 | - and + | C | L | | | | | | | | | > | < | | | > | = | | | | |
| 16 | - and + | F C | P | | | | | | | | | | | | | | | $>^{50}>^{100}$ $=^{50}=^{100}$ | $<^{50}=^{100}$ $=^{50}=^{100}$ | $>^{50}>^{100}$ $=^{50}=^{100}$ | $<^{50}=^{100}$ $=^{50}=^{100}$ |
| 17 | + | NA | L | | | | = | | > | | = | | | | | | = | | | | | |
| 18 | + | F | Ca | | = | | | | | | | | < | | | | | | | | | |
| 19 | - and + | F | P | | = | | | | | | = | | < | | = | | | $>^{L}>^{I}>^{H}$ | $<^{L}=^{I}<^{H}$ | $>^{L}>^{I}>^{H}$ | $<^{L}=^{I}<^{H}$ |
| 20 | - and + | F | P | | < | | | | | | | > | < | | | = | = | | | | |
| 21 | + | F | P | | < | | | | | | = | | = | | < | | = | | | | |
| | + | F | Ca | | = | | | | | | = | | = | | = | | = | | | | |
| 22 | + | C | P | | | | | | | | | | | | | | = | | = | | = |
| 23 | + | 1:1 | P | | | | $=^{L}=^{H}$ | | | | $>^{L}<^{H}$ | | $<^{L}<^{H}$ | | $>^{L}>^{H}$ | | $=^{L}=^{H}$ | | | | |
| | + | 1:1 | P | | | | = | | | | | | | | | | < | | | | |
| | + | F | P | | | | | | | | | | | | | | | = | = | = | = |
| | + | F | P | | | | | | | | | | | | | | | | = | | = |
| 24 | - | C | P | = | | > | | > | | | | | | | | = | | | | | |

[1] Negative: basal diet; Positive: basal diet plus conventional urea.
[2] F: forage; C: concentrate; NA: not available.
[3] Negative: SRU diet (basal diet plus SRU) versus negative control; Positive: SRU versus positive control.
**14:** Huston et al., 1974; **15:** Owens et al., 1980; OMD results are actually dry matter digestibility (DMD) results; **16:** Tedeschi et al., 2002; within concentrate- or

forage-based diets, conventional urea and SRU supplementation was expected to meet 50 or 100% of DIP deficit ; **17:** Garret et al., 2005; **18:** Huntington et al., 2006; **19:** Taylor-Edwards et al., 2009; low (L; 0.4%), intermediate (I; 0.8 and 1.2%), and high (H, 1.6%) dietary concentrations of conventional urea and SRU were evaluated; **20:** Ribeiro et al., 2011; **21:** Álvarez-Almora et al., 2012; OMD results are actually DMD results; **22:** Bourg et al., 2012; **23:** Holder, 2012; lower (L, 10.9%) and higher (H, 12.1%) dietary CP concentrations were evaluated; **24:** López-Soto et al., 2014.

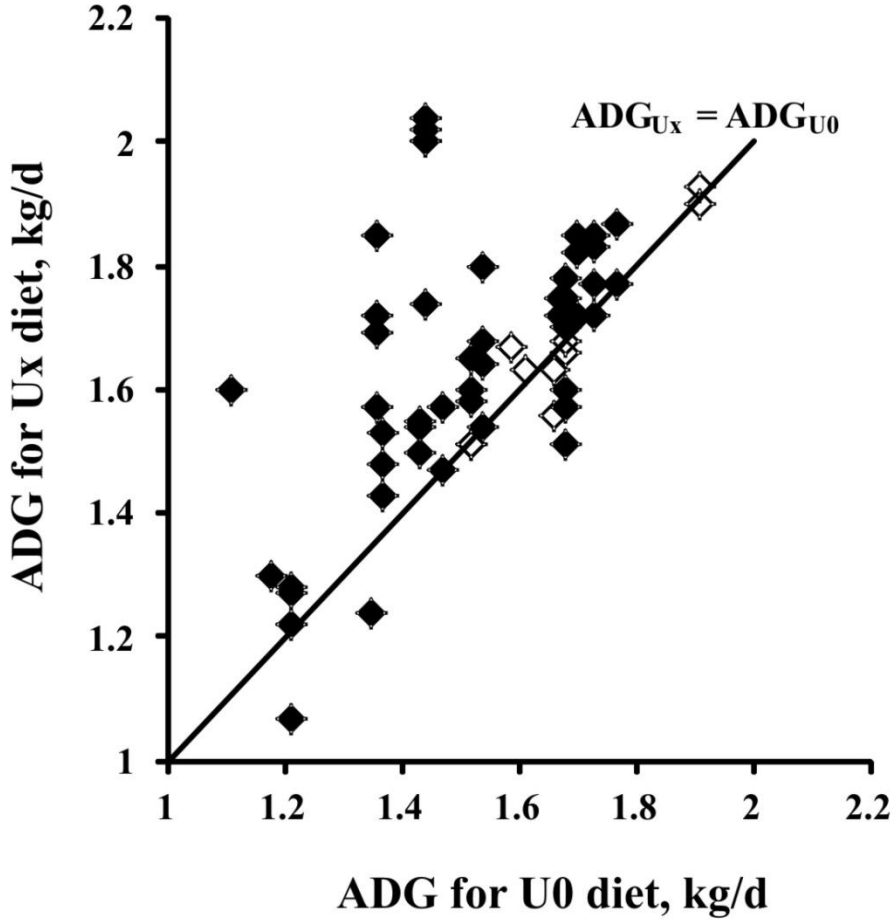


**Figure 1.** Effect of increased dietary degradable intake protein concentration through the addition of urea on feedlot cattle average daily gain (ADG). Data from various studies reported in Table 2. U0: smaller amount or no urea added; Ux: increased urea inclusion; ♦: significant effects ($P \leq 0.10$); ◊: non-significant effects ($P > 0.10$).

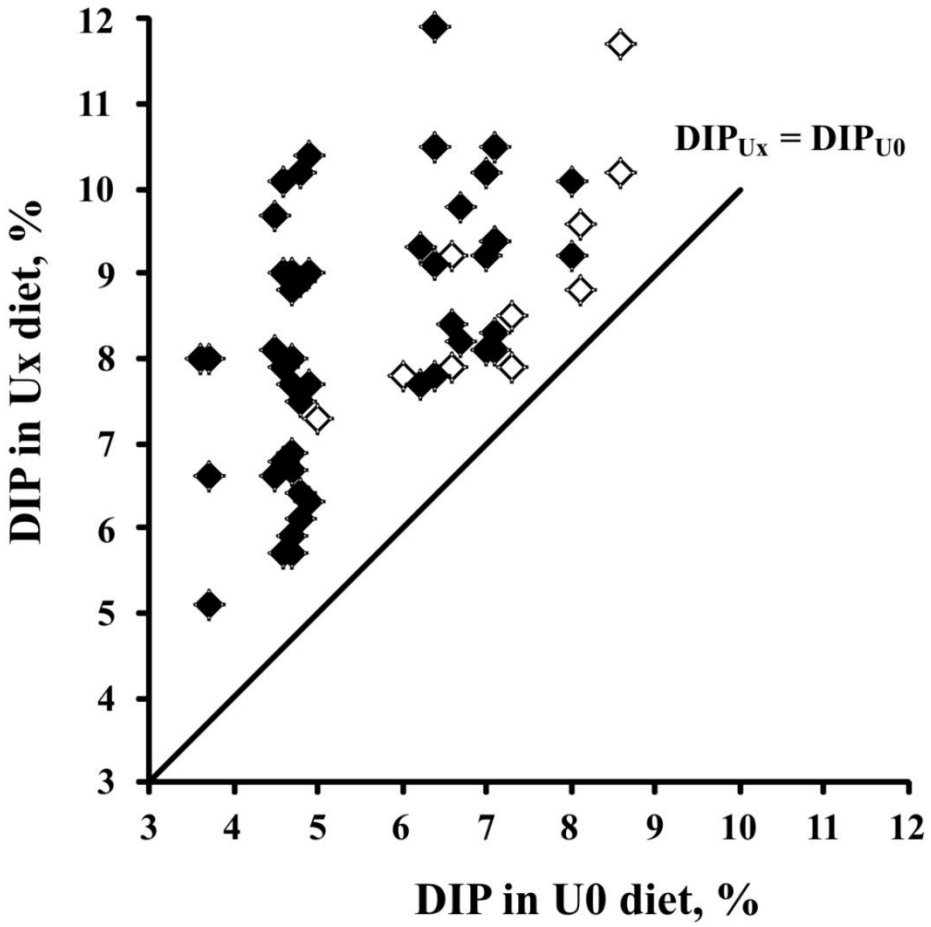


**Figure 2.** Effect of increased urea supplementation on dietary degradable intake protein (DIP) concentration. Data from various studies reported in Table 2. U0: smaller amount or no urea added; Ux: increased urea inclusion; ♦: significant effects on average daily gain ($P \leq 0.10$); ◊: non-significant effects ($P > 0.10$).

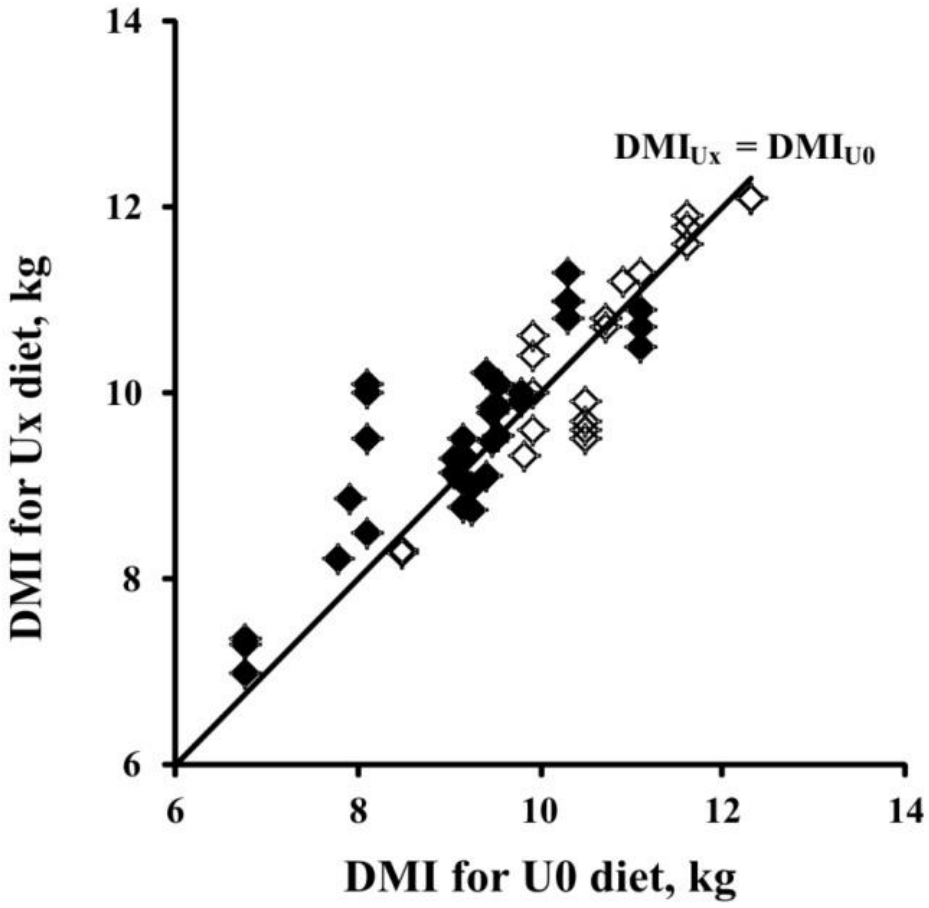


**Figure 3.** Effect of increased dietary degradable intake protein concentration through the addition of urea on feedlot cattle dry matter intake (DMI). Data from various studies reported in Table 2. U0: smaller amount or no urea added; Ux: increased urea inclusion; ♦: significant effects ($P \leq 0.10$); ◊: non-significant effects ($P > 0.10$).

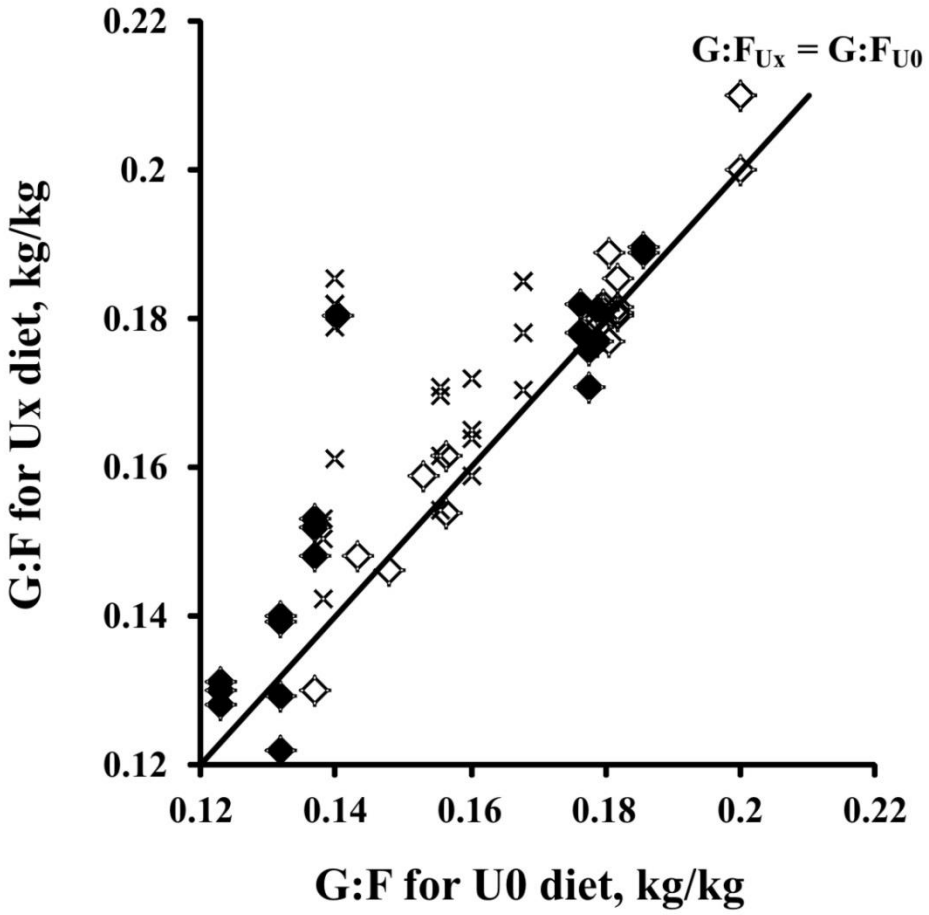


**Figure 4.** Effect of increased dietary degradable intake protein concentration through the addition of urea on feedlot cattle feed efficiency (G:F, expressed as kg of gain obtained per kg of feed consumed). Data from various studies reported in Table 2. U0: smaller amount or no urea added; Ux: increased urea inclusion; ♦: significant effects ($P \leq 0.10$); ◊: non-significant effects ($P > 0.10$); ×: not analyzed by the authors.

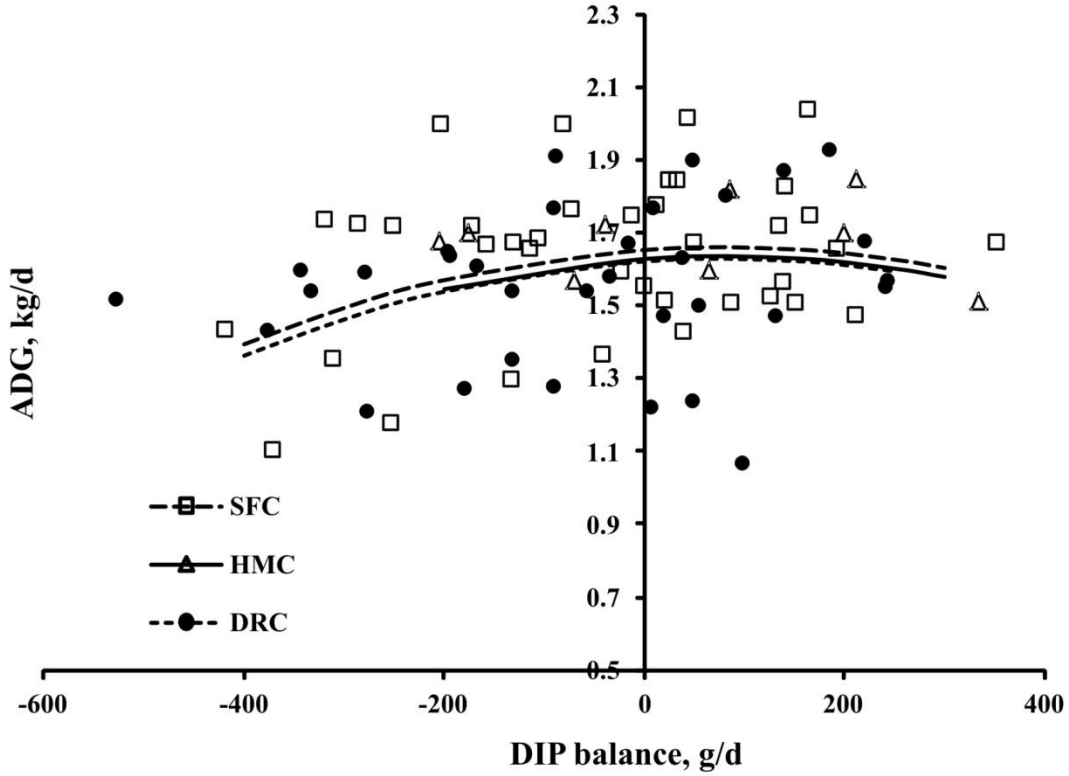


**Figure 5.** Average daily gain (ADG) as affected by degradable intake protein (DIP) balance in dry-rolled corn- (DRC), high-moisture corn- (HMC), or steam-flaked corn- (SFC) based finishing diets (various studies reported in Table 2).

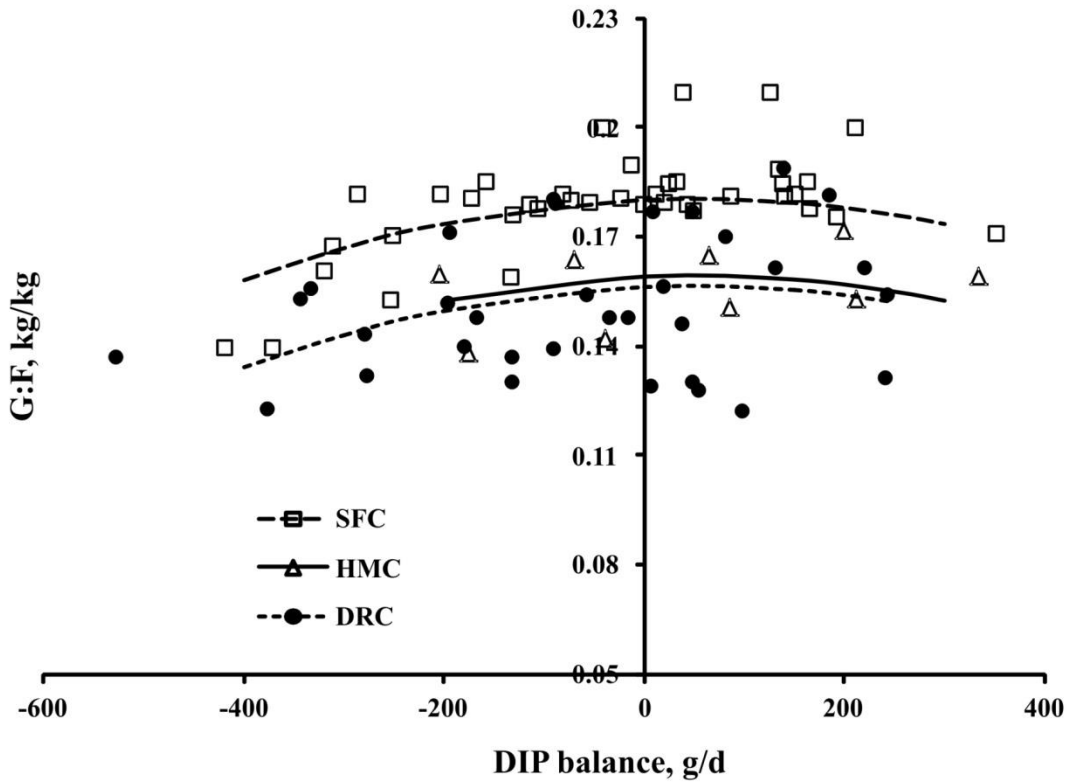


**Figure 6.** Feed efficiency (kg of gain:kg feed; G:F) as affected by degradable intake protein (DIP) balance in dry-rolled corn- (DRC), high-moisture corn- (HMC), or steam-flaked corn- (SFC) based finishing diets (various studies reported in Table 2).

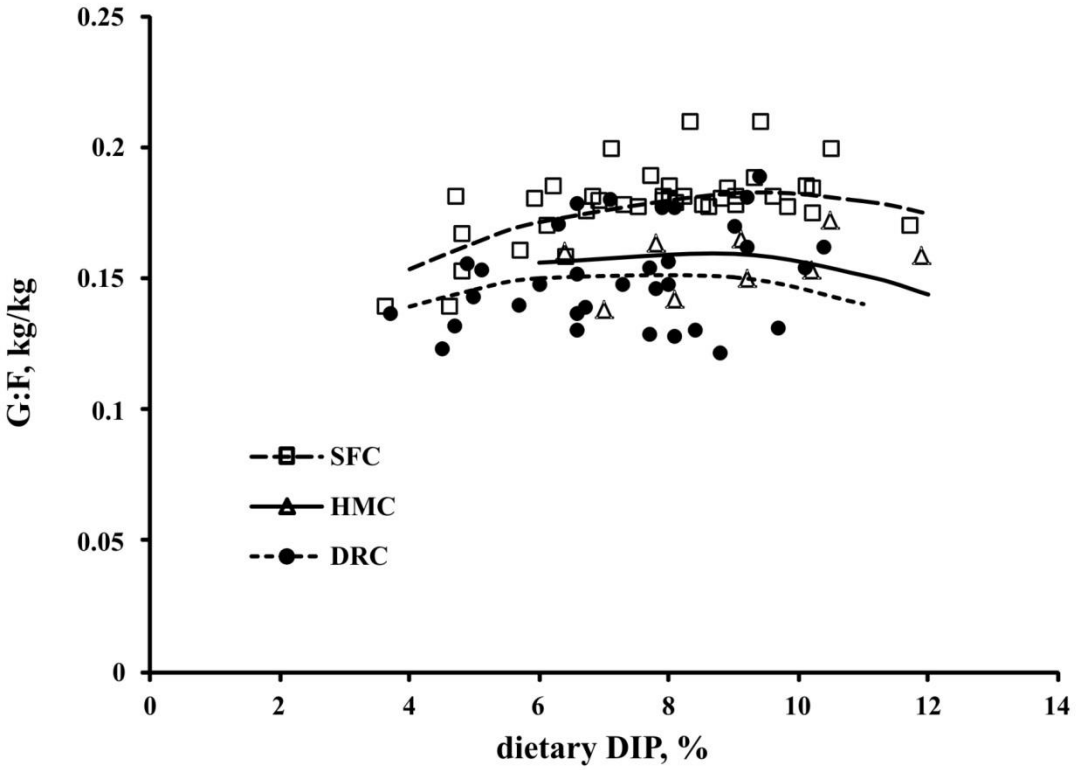


**Figure 7.** Feed efficiency (kg of gain:kg feed; G:F) as affected by degradable intake protein (DIP) concentration in dry-rolled corn- (DRC), high-moisture corn- (HMC), or steam-flaked corn- (SFC) based finishing diets (various studies reported in Table 2).

# CHAPTER 2

# EFFECT OF UREA INCLUSION IN DIETS CONTAINING DRIED CORN DISTILLERS GRAINS ON FEEDLOT CATTLE PERFORMANCE, CARCASS CHARACTERISTICS, RUMINAL FERMENTATION, TOTAL TRACT DIGESTIBILITY, AND PURINE DERIVATIVES-TO-CREATININE INDEX.

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## SUMMARY

Increased availability of rapidly fermentable carbohydrates and great proportion of corn-derived CP may result in degradable intake protein (DIP) deficit. Therefore, ruminal DIP deficit may result from high dietary inclusion of processed corn grain and small to moderate inclusion of corn distillers grains (DG). Two experiments were conducted to evaluate the effect of increasing dietary DIP concentration through the inclusion of urea on feedlot cattle performance, carcass characteristics, ruminal fermentation, total tract digestibility, and purine derivatives-to-creatinine (PDC) index. In Exp. 1, forty-two steers (428 ± 5 kg initial BW) were assigned randomly to one of three diets containing 0 (CON), 0.4 (LU), or 0.6% (HU) urea to provide 6.4, 7.5, or 8.0% DIP, respectively, and 52% dry-rolled corn (DRC), 12% high-moisture corn (HMC), 20% corn dried DG with solubles (DDGS), 10% ryegrass haylage, and dry supplement (DM basis). Steers were fed *ad libitum* once daily using a Calan gate system. Carcass-adjusted final BW and DMI

were similar among treatments ($P \geq 0.58$). Carcass-adjusted ADG was greater ($P \leq 0.04$) for HU compared with LU and CON, and was similar ($P = 0.73$) between LU and CON. Carcass-adjusted G:F was greater ($P = 0.03$) for HU compared with LU, tended ($P = 0.09$) to be greater compared with CON, and was similar ($P = 0.61$) between LU and CON. Carcass characteristics were similar ($P \geq 0.34$) among treatments. In Exp. 2, four ruminally cannulated steers (347 ± 18 kg initial BW) were assigned randomly to a replicated 2 x 2 Latin square design. Steers were fed the same CON or HU diets used in Exp. 1 *ad libitum* once daily. Differences in PDC index were used as indicators of differences in microbial CP synthesis. Ruminal pH, OM intake, and starch and CP digestibility were not affected by treatment ($P \geq 0.13$). Digestibility of OM and NDF, and ruminal concentration of ammonia-N and total VFA were greater ($P \leq 0.04$) for HU compared with CON. Purine derivatives-to-creatinine index was similar ($P = 0.81$) between treatments at 2 h before feeding, 4% lower and 14% greater for HU compared with CON at 4 and 10 h post-feeding, respectively ($P < 0.01$). These results suggest that, due to limited DIP supplied by a DRC and HMC-based feedlot diet containing 20% DDGS, urea supplementation resulted in improved ruminal fermentation and feed digestibility, which may explain the concurrently improved cattle performance.

**Keywords:** degradable intake protein, distillers grains, feedlot cattle, microbial protein, urea, volatile fatty acids

## INTRODUCTION

Degradable intake protein (DIP) is the proportion of dietary CP that is ruminally degradable (NRC, 2000). A daily supply of DIP is necessary to meet requirements for

microbial CP (MCP) synthesis. *De novo* synthesis of AA requires N, carbon skeletons, and ATP; the last two are mostly derived from fermentation of dietary carbohydrates while dietary N is the main N source (Allison, 1969; Leng and Nolan, 1984; Russell, 2002). Fermentation rate of feed is positively associated with microbial efficiency (MCP produced/TDN intake; Van Soest, 1994) as it permits faster microbial growth and thereby dilutes microbial maintenance requirements. Therefore, high dietary inclusion of extensively processed grains may result in increased DIP requirements. In addition, corn-based diets may not supply adequate amounts of DIP because corn CP is approximately 60% rumen-undegradable. Consequently, addition of a highly rumen-degradable N source, like urea, could result in improvements in ruminal fermentation and animal performance (Milton et al., 1997b; Shain et al., 1998: Cooper et al., 2002a; Zinn et al., 2003; Gleghorn et al., 2004; Cole et al., 2006; Crawford, 2007; Crawford et al., 2007; Kennington et al., 2009; Wagner et al., 2010).

Content of CP in corn distillers grains (DG) ranges between 24 and 32% (NRC, 2000). However, DG may contain a high proportion of CP in the indigestible form (Sniffen et al., 1992). Few researchers have investigated the need of DIP supplementation in DG-containing diets (Vasconcelos et al., 2007; Ponce, 2010; Jenkins et al., 2011) while none of them evaluated the impact of urea supplementation on ruminal fermentation and feed digestibility at moderate dietary DG inclusion.

The objective of these experiments was to evaluate the effect of increasing DIP concentration from urea in concentrate-based finishing diets with moderate corn DG inclusion on feedlot cattle performance, carcass characteristics, ruminal fermentation, and total tract feed digestibility.

## MATERIALS AND METHODS

Two experiments were conducted concurrently at the University of Minnesota North Central Research and Outreach Center (NCROC) in Grand Rapids, MN. Animal care and handling procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

### *Experimental diets and DIP and metabolizable protein balance estimations*

Three dietary treatments containing 0 (CON), 0.4 (LU), or 0.6% (HU) dietary urea and expected to generate a negative, zero, or positive DIP balance, respectively, were evaluated in Exp. 1. Only CON and HU were evaluated in Exp. 2. All diets contained 12% high-moisture corn (HMC), 20% dried DG with solubles (DDGS), 10% ryegrass haylage, 2.9% dry supplement, and dry-rolled corn (DRC; Table 1). Dietary TDN and CP concentrations measured 80.8, 81.6 and 79.2%, and 14.0, 15.1, and 15.6% for CON, LU, and HU, respectively. Dietary DIP concentrations were estimated at 6.4, 7.5, and 8.0% for CON, LU, and HU, respectively (Table 1), using measured CP and book-referenced DIP concentrations (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012) of each diet ingredient.

Balances of DIP and metabolizable protein (MP) were calculated for each treatment using Level 1 of the NRC model (2000). Nitrogen available for synthesis of MCP is represented by DIP supply, which is estimated by the model as a function of DMI and dietary DIP concentration. On the other hand, needs of rumen-degradable N for MCP synthesis are represented by DIP requirements; the latter are calculated as TDN intake adjusted by microbial efficiency. Microbial efficiency is defined as g of MCP produced

per 100 g of TDN. The model assumes that, when present, DIP deficiencies (DIP requirements > DIP supply) are overcome by degradable N supplementation. Therefore, the amount of MCP synthesized in the rumen per day is represented by daily DIP requirements. Additionally, DIP and MP balances were estimated based on some modifications to Level 1 of the NRC model (2000). Efficiency of $NH_3$-N capture by ruminal microbes is below 100% due to direct absorption of $NH_3$-N across the rumen wall and passage of fluid from the rumen (NRC, 1985). Therefore, DIP balance was estimated as *DIP supply – DIP requirements x 1.18*. When DIP balance resulted positive, contribution of MCP to MP supply was calculated as *DIP requirements x 0.64*; when negative, the contribution was estimated as *DIP supply x 0.85 x 0.64* (NRC, 2001). In Exp. 2, treatment ADG used for MP requirements calculations was estimated as $DMI_{experiment\ 2} \times G{:}F_{experiment\ 1}$.

### *Experiment 1*

#### *Cattle processing and feeding protocol*

Thirty-three purebred Angus steers originating from NCROC and nine Angus-crossbred steers originating from a commercial operation (428 ± 5 kg initial BW) were blocked by source and assigned randomly within each source to one of three dietary treatments. Steers were adapted to treatment diets for 14 d by increasing DM delivery of the same diet they received during the experimental period. Steers were fed *ad libitum* once daily at 0800 using a Calan gate individual feeding system (American Calan, Northwood, NH, USA). Before feeding, feed refusal was recorded and feed delivery adjusted. Amount of feed delivered was increased 0.5 kg/d when the bunk was found empty, kept the same when feed crumbs weighing up to 0.5 kg were found, or decreased

half the weight of the refusal when the latter was over 0.5 kg. Refusals were weighed and sampled weekly. Dry matter intake was estimated as the difference between weekly delivery and refusal. Diets and ingredients were sampled when a new batch of feed was prepared.

On d -27, steers were implanted with Synovex Choice (Zoetis, Florham Park, NJ, USA; 100 mg trenbolone acetate and 14 mg estradiol benzoate). Steers were weighed individually every 28 d before feeding. Shade and water were always available. On d 85, steers were humanely harvested at a commercial abattoir and carcass characteristics were measured. Final BW was adjusted using individual HCW and group dressing percentage (60.6%). Daily gain and G:F were adjusted using carcass-adjusted final BW.

***Experimental design and data analyses***

Experimental units (animal) were arranged in a generalized randomized complete block design, with 14 replications per treatment and source as the blocking factor. Source and its interaction with treatment were considered as random effects.

Feedlot cattle performance data, ribeye area, $12^{th}$ rib fat thickness, HCW, and marbling score were analyzed using the Mixed procedure while USDA yield and quality grade frequencies were analyzed using the Genmod procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Effects were considered significant when *P*-values were less than or equal to 0.05 and were considered trends when *P*-values were between 0.05 and 0.10. When *P*-value for treatment effect was equal or less than 0.10, treatment means were separated using a *t* test (*pdiff* option). The statistical significance of the source-by-treatment interaction was determined by a $Chi^2$ test between the full and reduced models

(Casler, 2006). When null hypothesis was not rejected (calculated $Chi^2$ value < critical $Chi^2$ value), data were analyzed based on the reduced model.

### *Experiment 2*

#### *Experimental design, cattle handling, and feeding protocol*

Four Holstein steers (347 ± 18 kg initial BW) fitted with flexible ruminal cannula (Bar Diamond, Inc., Parma, ID, USA) were assigned randomly to a duplicated 2 x 2 Latin square design to allow for 4 replications per treatment. The experiment consisted of two 21-d periods. Steers were weighed prior to feeding on d 1 and 2 of each period. Steers were group-housed by treatment in pens from d 1 to 15 and individually housed in metabolism stalls from d 16 to 21. During the first 13 d of each period, steers were adapted to their assigned diet which was offered at 0900 h. Bunks were monitored daily to adjust feed offering. From d 14, diets were offered *ad libitum* and refusals were recorded daily and kept in the bunk unless they represented more than 5% of daily feed delivery. In this case, the refusal was removed, weighed and sampled. Otherwise, refusals were removed, weighed and sampled at the end of each period.

#### *Sample and data collection, and laboratory analyses*

To determine digestibility of OM and its components, animals were intra-ruminally dosed at 0700 and 1900 h daily with 7.5 g of chromic oxide contained in porcine gelatin capsules from d 11 to 21. Fecal grab samples were collected at 0700, 1300, and 1900 h from d 17 to 21 and stored at -20 °C. After completion of the experiment, fecal samples were freeze-dried, ground, and composited by steer and period. Chromium concentration in feces was used to estimate fecal daily output and it was determined using an atomic absorption spectrophotometer (AAanlyst 200; Perkin Elmer, Walther, MA, USA) based

on procedure suggested by Williams et al. (1962). Feed and fecal OM, CP, starch, and NDF contents were determined by methods 942.05, 990.03, 920.40, and 2002.04 (AOAC, 2012), respectively. Digestibility of OM, starch, and NDF was estimated as *[(intake – excretion) / intake] x 100*. To avoid overestimation of CP (N x 6.25) digestibility, the latter was estimated as *{[(Total CP intake – CP intake derived from supplemental urea) - CP excretion] / (Total CP intake – CP intake derived from supplemental urea)} x 100*.

Ruminal pH was recorded by sensors programmed to measure and record ruminal pH every 5 minutes (Kahne Ltd., Auckland, New Zealand). Probes were inserted into the rumen of each steer on d 16 and removed at the end of each period. Data were downloaded from individual probes upon completion of each period and composited by steer and period on an hourly basis for a 24-h period.

Concentration of VFA and ammonia-N ($NH_3$-N) were measured in ruminal fluid samples collected on d 21 at -1, 2, 4, 8, 12, 16, and 24 h post-feeding. Ruminal fluid samples were collected by a manual suction strainer inserted through the ruminal cannula. Concentration of VFA was determined using a Hewlett Packard 6890 gas chromatographer (Agilent Tech., Santa Clara, CA, USA) based on the procedure suggested by Erwin et al. (1961). Concentration of $NH_3$-N was determined by steam distillation (Bremner and Keeney, 1965) with magnesium oxide using a 2300 Kjeltec analyzer unit (Foss Tecator AB, Höganäs, Sweden).

Differences in purine derivatives-to-creatinine (PDC) index between treatments were used as indicators of differences in MCP synthesis (Chen and Ørskov, 2004). The

PDC index was defined as $BW^{0.75} \times (PD / C)$, where PD (purine derivatives) and C (creatinine) were expressed in m*M* and BW in kg (Chen et al., 2004). Urine is the means through which PD from the metabolism of microbial nucleic acids (Chen and Ørskov, 2004) and C from muscle metabolism (Lofgreen and Garrett, 1954; Van Niekerk et al., 1963) are excreted. Therefore, PD (allantoin and uric acid) and C were simultaneously determined in urine samples (Shingfield and Offer, 1999) using a high-performance liquid chromatographer (Gilson, Middleton, WI, USA). Urine samples were collected following the same protocol used for fecal sample collection. After completion of the experiment, urine samples were composited by time, steer, and period.

***Data analyses***

Chen and Gomes (1995) proposed a formula to predict absolute MCP flow from urinary PD. Many of the factors included in this model are highly variable which may result in estimations inconsistent with MCP flow estimated from duodenal purines (Crawford, 2007). However, urinary PD are a reliable marker to estimate relative differences in MCP flow (Südekum et al., 2006). Therefore, the effect of urea supplementation on MCP flow and microbial efficiency was analyzed using absolute PD derived-MCP flow and microbial efficiency, though reported in relative terms. Absolute MCP flow was estimated as $[(PDa / 0.900) \times 70 \times 6.25] / (0.116 \times 0.830 \times 1000)$, where PDa represented PD originated from ruminal microbes (mmol/d) and was estimated as $(PDe - 0.385 \times BW^{0.75}) / 0.850$, being PDe the amount of PD excreted by the animal (mmol/d; Chen and Gomes, 1995). The latter was estimated based on urinary PD concentration and total urine output, assuming that the internal marker C was excreted at a rate of 28 mg/kg of BW (McCarthy et al., 1983; Whittet et al., 2004). Microbial

efficiency was estimated as *[MCP flow (g/d) / OM digested (g/d)] x 100*. Relative differences in MCP flow and microbial efficiency between treatments were estimated as $[(A_{HU} - A_{CON}) / A_{CON}] \times 100$, where $A_{CON}$ and $A_{HU}$ were defined as absolute MCP flow or microbial efficiency for CON and HU diets, respectively.

Data collected after the end of adaptation period (d 16) were analyzed using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). For pH, VFA, $NH_3$-N, PDC index, and MCP flow a repeated measure structure was considered. The model included dietary treatment, time, treatment-by-time interaction, and period as fixed effects and animal within square and square as random effects. Degrees of freedom were calculated requesting the Satterthwaite option. The best covariance structure was selected for each variable following procedure suggested by Casler (2006). Briefly, unstructured matrices of variances, covariances, and correlations were requested. Based on this information, inadequate structures were ruled out. When more than one structure was potentially suitable, final selection was made based on information criteria. To decide between models that differed in number of parameters, a $Chi^2$ test involving the *residual log likelihood* criteria was performed.

When significant and of interest, the time effect was evaluated by polynomial contrasts, for which coefficients for unequally spaced sampling times were obtained through the IML procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). Level of significance was set and treatment means separation was performed as described in Exp. 1.

## RESULTS AND DISCUSSION

Based on $Chi^2$ tests, the reduced model (without source-by-treatment interaction) was adjusted for all variables evaluated in Exp. 1. Initial BW, carcass-adjusted final BW, and DMI were similar among treatments ($P \geq 0.58$; Table 2); expressed as a proportion of BW, DMI averaged 2.45%. Carcass-adjusted ADG was greater for HU compared with LU ($P = 0.02$) and CON ($P = 0.04$), and was similar between LU and CON ($P = 0.73$). Carcass-adjusted G:F tended to be different between treatments ($P = 0.08$; Table 2), being greater ($P = 0.03$) or tending to be greater ($P = 0.09$) for HU compared with LU and CON, respectively, and being similar between LU and CON ($P = 0.61$). Hot carcass weight, $12^{th}$ rib fat thickness, LM area, marbling score, yield grade, and quality grade were similar among treatments ($P \geq 0.34$; Table 3).

Similar results on ADG and G:F were reported by Ponce (2010) when increasing dietary DIP from 6.8 to 8.3% through inclusion of urea in a steam-flaked corn (SFC)-based diet containing 15% DG. On the contrary, no beneficial effects on performance were observed by this author when increasing dietary DIP concentrations beyond 8.0% or when adding urea to a diet containing 30% DG; the latter resulted in dietary DIP increasing from 8.9 to 12.0%. Likewise, Jenkins et al. (2011) did not observe improvements in ADG or G:F when dietary DIP was increased from 5 to 9.2% (data not provided by the authors) by adding urea to DRC-based diets containing 10 to 25% DG. Requirements of DIP are a moving target, basically driven by energy available for microbial growth (intake of rumen-fermentable carbohydrates) and microbial efficiency. Therefore, contrasting results of adding urea to feedlot rations among research studies could be related to differences in rumen digestibility across carbohydrates and N sources,

DMI, and ruminal pH, among others. For example, based on ruminal fermentation of carbohydrates, Cooper et al. (2002a) reported that dietary DIP concentration beyond which no further improvements in animal performance were observed measured 10.0, 8.3, or 6.3% for HMC-, SFC-, or DRC-based finishing diets, respectively. In addition, because of great CP content and despite great rumen-undegradable CP concentration, greater inclusion of DG may increase DIP supply thus reducing need for urea supplementation. In this regard, Ponce (2010) reported beneficial effects on animal performance when adding urea to a 15% DG-containing diet; however, those effects were not observed when the basal diet contained 30% DG. Inclusion of a less fermentable grain like DRC and/or inclusion of 20 or 25% DG in the study by Jenkins et al. (2011) may partially explain lack of response to urea supplementation. Also, authors suggested that urea potentially recycled to the rumen from excess MP may have overridden need of urea supplementation. However, and despite great rumen fermentability of SFC and a small inclusion of DG (10% of dietary DM), Vasconcelos et al. (2007) did not observe beneficial effects of increasing dietary DIP from 7.2 to 8.4% through urea inclusion on animal performance. These apparent contradictory results may be partially explained by the effect of other factors that affect microbial efficiency, which are in turn modified when changing diet fermentability. For example, greater VFA and eventually lactate ruminal concentration resulting from increased rate of fermentation may reduce ruminal pH. By increasing proton and VFA anions concentration inside the microbial cells, decreased ruminal pH can increase the amount of ATP used to pump protons out of the cell and reduce the amount of ATP available for microbial growth (Strobel and Russell, 1986; Klemps et al., 1987; Russell and Wilson, 1996; Russell, 2007); this may result in

reduced microbial efficiency (Russell and Dombrowski, 1980; Strobel and Russell, 1986). Therefore, even though SFC-based diets may result in increased availability of rumen fermentable carbohydrates, potentially reduced ruminal pH may negatively impact microbial efficiency thus reducing DIP requirements and need of urea supplementation.

In agreement with Exp. 1, intake was not affected by treatment ($P \geq 0.24$, Table 4) in Exp. 2 and averaged 10.4 ± 0.13 and 9.9 ± 0.12 kg/d of DM and OM, respectively. Intake of DM represented 2.24% of BW. In contrast, NDF digestibility was improved by the addition of urea (43.8 and 52.3 ± 4.8% for CON and HU, respectively; $P = 0.04$; Table 4). This result, plus a numerical increase in starch digestibility (79.3 and 81.7 ± 3.8% for CON and HU, respectively; $P = 0.30$) might have explained greater OM digestibility for HU (72.3 ± 2.9%) compared with CON (69.1 ± 2.9%; $P = 0.04$). Despite $NH_3$-N being demonstrated as the main source of N for protein synthesis, $NH_3$-N plays a more important role in nutrition of the relatively slow-growing fiber-digesting bacteria compared with the fast-growing soluble sugar-fermenting or starch-digesting bacteria (Bryant and Robinson, 1963; Hungate, 1966). This may help explain urea supplementation significantly enhancing NDF digestibility. Digestibility of CP was similar between treatments ($P = 0.99$) and averaged 67.7 ± 2.9% (Table 4). Milton et al. (1997b), Zinn et al. (2003), and Crawford (2007) reported increased starch ruminal digestibility from 47.1 to 64.4%, from 88.0 to 93.1%, and from 83.4 to 89.8%, respectively, when increasing urea supplementation to DRC-, steam-flaked barley (SFB)-, or SFC-based diets. Increased total tract digestibility of OM was also observed in the study by Zinn et al. (2003).

Except one hour before feeding, when $NH_3$-N concentration was similar between treatments (Fig. 1), HU yielded a greater $NH_3$-N concentration than CON, mainly during the first hours post-feeding due to a rapid increase of $NH_3$-N concentration with the former (treatment-by-time, $P = 0.06$; Table 5). DiLorenzo and DiCostanzo (2007a,b) observed that about 25% of total $NH_3$-N produced in ruminal fluid by urea hydrolysis is released within the first 0.5 h post-incubation *in vitro*. Therefore, it is possible that the $NH_3$-N spike for HU treatment observed in the present experiment at 2 h post-feeding represents a point in the diminishing phase of a larger and earlier $NH_3$-N peak. Interestingly, $NH_3$-N concentration for CON was below 5 mg/dL for most of the day. Satter and Slyter (1974) reported beneficial effects of the addition of NPN supplements to ruminant rations on microbial protein yield when the prevailing concentration of ruminal $NH_3$-N was less than 5 mg/dL.

As part of protein ruminal degradation, AA are deaminated and $NH_3$-N is released, contributing to $NH_3$-N ruminal pool. Even though CP ruminal digestibility was not estimated, based on similar total tract CP digestibility between CON and HU, greater $NH_3$-N ruminal concentration with the latter could be mainly attributed to urea dietary inclusion.

Ruminal concentration of $NH_3$-N peaked 2 h after feeding, decreased from 2 to 8 h post-feeding, and then slightly increased until the next feeding event for HU treatment (Fig. 1). Except for the absence of a peak, a similar trend was observed for CON (treatment-by-time, $P = 0.06$).

In agreement with significantly greater OM digestibility, total VFA concentration was greater for HU (119.1 ± 7.0 m*M*) than for CON (86.4 ± 7.0 m*M*; $P < 0.01$), and this result was independent from time (treatment-by-time, $P = 0.23$; Table 5). Numerical increase in total VFA concentration was reported by Milton et al. (1997a,b) and Zinn et al. (2003) when adding urea to DRC- or SFB-based diets, respectively. Similarly, propionate and acetate concentrations were greater ($P \leq 0.03$) for HU compared with CON (Table 5), which may reflect the significantly greater NDF and the numerically greater starch digestibility (Table 4). Total VFA concentration was affected by time ($P < 0.01$, Table 5), increasing greatly from 2 to 12 h post-feeding and decreasing thereafter (Fig. 2). A concurrent and opposite response to time was observed for ruminal pH ($P < 0.01$, Table 5 and Fig. 2). As reported by Zinn et al. (2003), 86.6% of ruminal pH variation can be explained by VFA ruminal concentration. Since urea has ruminal alkalizing effect which may attenuate ruminal pH drops when highly fermentable diets are consumed (Zinn et al., 2003), potential negative effects of greater total VFA concentration on ruminal pH in HU compared with CON could have been attenuated by the inclusion of urea, thus resulting in similar pH between treatments ($P = 0.31$, Table 5). Similar to what was observed for total VFA and ruminal pH, total VFA and $NH_3$-N concentrations showed an opposed response to time (Fig. 1 and 2), likely reflecting grater $NH_3$-N microbial use during times of increased microbial activity and VFA production.

Acetate and propionate concentrations were affected by time ($P \leq 0.05$), increasing from -1 to 8 h post-feeding and decreasing thereafter for HU, while varying slightly from -1 to 8, peaking at 12 h post-feeding, and decreasing thereafter for CON (treatment-by-time, $P \leq 0.02$; Table 5 and Fig. 3). This can be interpreted as microbial activity being

stimulated after feeding. Interestingly, and except for a concurrent increase at 8 or 12 h post-feeding for HU or CON, respectively, response to time of branched-chain VFA (BCVFA) concentration was almost opposite to that observed for acetate and propionate concentrations (Fig. 3, $P < 0.01$, Table 5). As opposed to other VFA, BCVFA result solely from fermentation of branched-chain AA and represent carbon backbone sources for the synthesis of microbial branched-chain AA (Allison and Bryant, 1963; Allison, 1969; Russell, 2002), in particular for cellulolytic bacteria. The latter cannot synthesize branched-chain AA *de novo* and they do not seem to have AA-fermenting activity; therefore, they rely on AA-fermenting bacteria to provide them with BCVFA as precursors for branched-chain AA synthesis (Allison and Bryant, 1963; Russell, 2002). Consequently, the response of acetate and propionate to time in comparison with that of BCVFA could be interpreted as a consequence of microbial activity, which in turn affects the balance between carbohydrate- and AA-derived VFA production and utilization.

Considering similar dietary non-urea CP concentration, DMI, and CP digestibility between treatments and a possibly increased microbial activity associated with greater OM digestibility and carbohydrate-derived VFA for HU compared with CON, a smaller BCVFA concentration for HU compared with CON was expected. However, such difference (Fig. 3) was not significant ($P = 0.65$, Table 5) at any sampling time.

Based on similar dietary starch and NDF concentrations, DMI, and starch digestibility between treatments, and greater NDF digestibility for HU compared with CON, it would have been reasonable to observe greater acetate-to-propionate ratio and acetate molar proportion, and smaller propionate molar proportion for HU compared with CON. However, none of these three variables was affected by treatment ($P \geq 0.52$, Table

5). The gram-negative ruminal bacteria *Fibrobacter succinogenes* plays an important role in fiber digestion, producing not only acetate, but important amounts of succinate, which can be used by saccharolytic bacteria to produce propionate (Hungate, 1966; Harfoot, 1978; Baldwin and Allison, 1983, Stewart and Flint, 1989). In addition, it is known that *F. succinogenes* hydrolyzes hemicellulose to gain access to cellulose, because it is unable to use the released pentoses (Hungate, 1966; Stewart and Flint, 1989; Russell, 2002; Suen et al., 2011), which are then available for saccharolytic bacteria. Compared with *F. succinogenes*, fiber-digesting, lesser-succinate-producing bacteria like *Ruminococcus albus* (Hungate 1966; Baldwin and Allison, 1983, Russell, 2002) may present smaller competitive advantages in an ionophore-containing ruminal environment due to the presence of a gram-positive cell membrane structure (Callaway et al., 2003). A possible more prevailing presence of *F. succinogenes* in the monensin-containing ruminal environment (Stewart and Flint, 1989) generated in the present experiment may relate to lack of effect of dietary treatment on acetate-to-propionate ratio and acetate and propionate molar proportions, being partially explained by a compensation between greater NDF digestibility and an increased proportion of NDF-derived glucose and pentoses not being fermented to acetate.

Propionate molar proportion increased quadratically ($P = 0.04$, Fig. 4) throughout the day, regardless of treatment (treatment-by-time, $P = 0.27$, Table 5). Molar proportions of acetate and butyrate decreased and increased quadratically ($P \leq 0.03$, Fig. 4), respectively, only for CON (treatment-by-time, $P \leq 0.07$, Table 5) and this opposed response to time might reflect the fact that for the synthesis of each mole of butyrate two moles of acetate are removed from the pool. This resulted in a trend ($P = 0.06$) for a

quadratic decrease ($P = 0.09$, Fig. 4) in the acetate-to-propionate ratio from -1 to 16 h post-feeding, irrespective of treatment (treatment-by-time, $P = 0.58$). This decrease in acetate-to-propionate ratio with time might be associated with a concurrent decrease in ruminal pH (Fig. 1). Due to differential susceptibility, ruminal pH can differentially affect ruminal bacteria populations (Russell and Wilson, 1996), which in turn may alter VFA molar proportions depending on prevailing VFA-production pathways present in each bacteria group. Russell (1998) reported that 25% of the reduction in acetate-to-propionate ratio could be explained by the reduction of pH alone. This author and Van Kessel and Russell (1996b) indicated that increased propionate proportion with decreasing pH represents a means of hydrogen disposal in an environment unfavorable for methanogens. In addition, studies reviewed by Dijkstra et al. (2012) reported that bacteria have the ability to shift VFA-producing pathways in response to changes in pH while fermenting the same substrate.

A significant treatment-by-time interaction was observed for PDC index ($P < 0.01$, Table 5) which was similar ($P = 0.81$) between treatments at 2 h before feeding, 4% lower and 14% greater for HU compared with CON at 4 and 10 h post-feeding, respectively ($P < 0.01$). This resulted in a quadratic or first-order increase with time in PDC index for CON or HU, respectively ($P < 0.01$, Fig. 5), which suggests that greater differences between treatments could potentially be observed at later hours post-feeding. Likely reflecting changes in microbial activity and MCP synthesis, the effect of time after feeding on PDC index was consistent with that on VFA and $NH_3$-N concentrations and ruminal pH. Given gastrointestinal transit time, metabolic processes, and excretion, urinary PD provides a delayed reflection of ruminal microbial activity. Therefore, it

would have been useful to determine urinary PD concentration beyond 10 h post-feeding. In other words, a better understanding of the relationship between urinary PD and VFA and $NH_3$-N concentrations could have been established if urinary PD had been determined throughout a 24-h cycle representative of VFA and $NH_3$-N collection protocol. An effect of time and urea supplementation on PD-to-C ratio (PD:C) was reported in the study by Crawford (2007), where PD:C was greater at 9 compared with -1 h post-feeding and for animals fed a SFC-based diet containing 1.5 compared with 0% dietary urea. Chen and Ørskov (2004) reported that intestinal flow of microbial purines and DMI correlated to PDC index. Because DMI was not affected by treatments, differences in PDC index between treatments can be attributed to differences in MCP synthesis. Results in PDC index indicated no differences ($P = 0.84$) in MCP flow between treatments at 2 h before feeding, and a 5% decrease and a 20% increase for HU compared with CON at 4 and 10 h post-feeding, respectively ($P < 0.01$). As a result of similar OM intake and greater OM digestibility, the amount of OM digested tended ($P = 0.08$) to be greater for HU compared with CON. Greater amount of OM digested and MCP flow for HU compared with CON resulted in similar microbial efficiency between treatments ($P = 0.97$).

Based on potential negative effects of decreased ruminal pH on microbial efficiency, Level 1 of NRC model (2000) estimates microbial efficiency as maximum microbial efficiency (13%) affected by a coefficient called the “effective NDF adjusted” (eNDFadj) which depends on dietary effective NDF (eNDF) concentration. The latter refers to the proportion of the NDF that is sufficiently large (> 1.18 mm; NRC, 2000) to be effective at stimulating chewing, rumination, and salivation, and consequently at

mitigating ruminal pH drop. Therefore, dietary concentration of eNDF is used by the model to predict ruminal pH and estimate eNDFadj coefficient. The model assumes that at 20% eNDF, microbial efficiency is maximized. Therefore, for diets containing as much or more than 20% eNDF the coefficient value is 1, while a reduction of 0.025 units per each percentage unit of dietary eNDF below 20% is integrated in the model. Based on similar dietary eNDF among treatments (6.6%, Table 1), model-estimated pH for any of the three diets was 5.71 and the associated estimated microbial efficiency was 8.71%. In addition, similar dietary TDN (Table 1) and DMI (Table 2) resulted in similar DIP requirements across diets (894 g/d, Table 6). Conversely, due to differences in dietary DIP concentration, DIP supply was smallest for CON (807 g/d, Table 6), intermediate for LU (948 g/d), and greatest for HU (1,019 g/d). Consequently, estimated DIP balances were -87, 54, and 125 g/d for CON, LU, and HU, respectively (Table 6). Based on these results, a question to address would be why ADG and G:F were improved with HU compared with LU if DIP requirements were already met and even exceeded with the latter. As expressed before, model-estimated pH was 5.71. However, ruminal pH measured in Exp. 2, which was similar for CON and HU (Table 5), averaged 5.86. When measured pH was integrated in the NRC model, microbial efficiency increased from 8.71 to 9.88%. Dijkstra et al. (2012) demonstrated that charged molecules, such as proteins, are able to exchange cations (K, Ca, Mg) for protons, thus contributing to ruminal buffering capacity. Therefore, partial substitution of DRC by DG in the diets evaluated in both experiments might have resulted in ruminal pH being greater than that estimated by the model. Consequently, measured pH-based DIP balances were -169 and 4 g/d for CON and HU, respectively (Table 6). Possibly, feeding the LU diet would have resulted in a

negative and intermediate DIP balance between CON and HU. Therefore, actual pH being greater than that predicted by the model may have accounted for increased microbial efficiency and DIP requirements. This observation would explain why supplementing urea at 0.6% resulted in an almost complete balance between DIP requirements and supply, which in turn resulted in enhanced feed digestibility, ruminal fermentation, and animal performance compared with including 0 or 0.4% dietary urea. Potential positive effect of high DMI observed in both studies (2.45 and 2.24% of BW for Exp. 1 and 2, respectively), likely permitted by greater ruminal pH, on microbial efficiency is not considered in the NRC model (2000) and may also help explain DIP requirements being greater than projected. The positive association between microbial efficiency and DMI arises from the positive effect of DMI on particle, liquid, and microbial outflow rates. Various studies reported an increase in both fractional rate of passage and dilution rate in cattle with increasing DMI (Sniffen and Robinson, 1987) or DMI expressed as a percentage of BW (Evans, 1981a,b). Faster outflow rates result in increased microbial efficiency (Isaacson et al., 1975; Russell and Baldwin, 1979; Meng et al., 1999) because mean age of microbial population is decreased, a younger population is subject to lower death and predation rates (Van Soest, 1994; Meng et al., 1999; Russell, 2002), and because faster outflow rates select in favor of fast-growing microbes (Meng et al., 1999), reducing the proportion of energy intended for microbial maintenance.

The fact that LU did not enhance animal performance compared with CON may suggest that the alleviation of the DIP deficit produced by including 0.4% urea was not sufficient to improve ruminal fermentation in a magnitude that would produce a noticeable impact on animal response.

When considering inefficiencies in $NH_3$-N capture by ruminal microbes (NRC, 2001) estimated DIP balances were -318 and -145 g/d for CON and HU, respectively (Table 6). This result may suggest that further improvements in ruminal fermentation and animal performance could have been observed with a better balance between DIP supply and requirements resulting from urea included at a rate above 0.6% of dietary DM.

Interestingly, ruminal fermentation and animal performance were improved with HU compared with CON even when MP requirements were met with both diets (Table 6). In other words, animal performance improved with increasing positive MP balance (231 or 274 g/d for CON or HU, respectively; Table 6). DiCostanzo (2007) reported a quadratic increase in ADG with increasing MP balance up to approximately 200 g/d. Because DMI affects MP balance directly by affecting at least the amount of undegradable protein ingested, positive associations between MP balance and animal performance might be explained by a positive association between the former and DMI, which was also reported by DiCostanzo (2007). However, this was not the case in the present experiment, because DMI was not affected by treatment, that is, variations in MP balance across treatments were not related with variations in DMI. Based on calculations performed considering inefficiencies in $NH_3$-N capture and MCP contribution to MP balance under negative DIP balance (DIP supply x 0.85 x 0.64, Table 6), increased MP balance with HU (274 g/d) compared with CON (231 g/d) was due to increased MCP synthesis as a result of increased DIP supply through the inclusion of urea and despite increased MP requirements driven by increased ADG. Estimation of increased MCP synthesis by the addition of urea, and therefore its contribution to MP balance, was in agreement with the 14% increment in PDC index measured for HU compared with CON

at 10 h post-feeding. Amino acids supplied above requirements might be used as glucose precursors and/or energy sources. As summarized by Markantonatos (2006), at most, 60% of the glucose can be derived from propionate in ruminants. Therefore, MP may have an important role as a contributor to gluconeogenesis, which, together with increased feed digestibility and VFA concentrations, may explain improved animal performance when including 0.6% urea in the diet.

Potentially, a DIP deficit can be partially or totally reversed by a positive intestinal MP balance, through urea being recycled back to the rumen (NRC, 2000). The fact that urea supplementation was effective to enhance ruminal fermentation irrespective of a positive MP balance does not indicate that urea was not recycled from extra MP to supply additional N to the rumen; it merely suggests that either the amount of recycled urea was not enough to reverse a DIP deficit or that urea from extra MP was not recycled to the rumen in time to be effectively utilized during ruminal digestion of a rapidly-fermentable diet.

Microbial N accounts for 50 to 85% of total AA-N entering the small intestine of ruminants (Storm et al., 1983b; van der Walt and Meyer, 1988). In addition, VFA and MCP resulting from ruminal fermentation and microbial growth are essential to provide the animal with energy sources and fatty acid precursors (acetate, butyrate) as well as a glucose precursor (propionate). Consequently, adequate supply of N as well as carbohydrates and other growth factors to enhance ruminal microbial growth is essential for an efficient beef production process. In the present experiments, a combination of high DMI (2.24 to 2.45% BW), moderate DG inclusion (20%), small replacement of DRC by a highly-fermentable grain like HMC (12%), and ruminal pH within a

physiological range (5.86) may help explain increased DIP requirements and consequently beneficial effects of 0.6% urea supplementation on ruminal fermentation and feed digestibility, which in turn may explain the concurrently improved animal performance. The fact that DMI was similar while ADG, G:F, feed digestibility, and VFA concentrations were greater when adding 0.6% urea compared with no urea supplementation demonstrates the beneficial effects of increasing DIP supply to a DIP-deficient diet on feed value.

**Table 1.** Composition (DM basis) of diets used in Exp. 1 (CON, LU, and HU) and Exp. 2 (CON and HU)

| | Treatment[1] | | |
|---|---|---|---|
| | CON | LU | HU |
| Dry-rolled corn, % | 55.1 | 54.7 | 54.5 |
| High-moisture corn, % | 12.0 | 12.0 | 12.0 |
| Dried distillers grains w/ solubles, % | 20.0 | 20.0 | 20.0 |
| Ryegrass haylage, % | 10.0 | 10.0 | 10.0 |
| Mineral-vitamin supplement, %[2] | 2.9 | 2.9 | 2.9 |
| Urea, % | 0.0 | 0.4 | 0.6 |
| NEg, Mcal/kg | 1.33 | 1.37 | 1.31 |
| TDN, % | 80.8 | 81.6 | 79.2 |
| CP, % | 14.0 | 15.1 | 15.6 |
| Basal DIP, %[3] | 6.4 | 6.4 | 6.4 |
| Supplemental DIP, %[3] | 0.0 | 1.1 | 1.6 |
| Total DIP, %[3] | 6.4 | 7.5 | 8.0 |
| eNDF, %[3] | 6.6 | 6.6 | 6.6 |

[1] CON: Control; LU: Low urea; HU: High urea diet.
[2] Contained 1,028 g of Rumensin 90 (Elanco Animal Health, Greenfield, IN, USA) per ton.
[3] Based on book-referenced degradable intake protein (DIP) and effective NDF (eNDF) concentrations for each ingredient (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012).

**Table 2.** Effect of urea inclusion in a finishing diet on feedlot cattle performance (Exp. 1)

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| | CON | LU | HU | SEM | *P*-value |
| *No. steers* | *14* | *14* | *14* | - | - |
| Initial BW, kg | 437 | 434 | 432 | 16 | 0.93 |
| Final BW[2], kg | 600 | 594 | 613 | 20 | 0.58 |
| DMI, kg/d | 12.6 | 12.6 | 12.9 | 0.8 | 0.73 |
| ADG[3], kg | 1.91[b] | 1.88[b] | 2.12[a] | 0.07 | 0.04 |
| G:F[3] | 0.154[ab, y] | 0.151[b, y] | 0.167[a, x] | 0.008 | 0.08 |

[1] CON: Control; LU: Low urea; HU: High urea diets containing 0, 0.4 or 0.6% urea of dietary DM, respectively.
[2] Adjusted using individual HCW and group dressing percentage (60.6%).
[3] Based on carcass-adjusted final BW.
[ab] Means with uncommon superscripts differ ($P < 0.05$).
[xy] Means with uncommon superscripts differ ($P < 0.10$).

**Table 3.** Effect of urea inclusion in a finishing diet on carcass characteristics (Exp. 1)

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| | CON | LU | HU | SEM | *P*-value |
| *No. steers* | *14* | *14* | *14* | - | - |
| HCW, kg | 364 | 360 | 372 | 12 | 0.58 |
| Marbling score[2] | 444 | 413 | 434 | 19 | 0.49 |
| 12th rib fat thickness, cm | 1.09 | 1.19 | 1.22 | 0.09 | 0.56 |
| LM area, sq. cm | 81.9 | 81.3 | 84.5 | 2.3 | 0.39 |
| *USDA Quality grade, %* | | | | | |
| Prime | 6.3 | 0.0 | 0.0 | 4.3 | 0.35 |
| Choice | 65.7 | 72.8 | 58.5 | 12.7 | 0.69 |
| Select | 28.0 | 27.2 | 41.5 | 12.2 | 0.59 |
| *USDA Yield grade, %* | | | | | |
| 2 | 37.0 | 16.0 | 37.0 | 12.7 | 0.34 |
| 3 | 63.0 | 77.7 | 63.0 | 13.2 | 0.64 |
| 4 | 0.0 | 6.3 | 0.0 | 4.3 | 0.35 |

[1] CON: Control; LU: Low urea; HU: High urea diets containing 0, 0.4 or 0.6% urea of dietary DM, respectively.
[2] 300 = Slight00; 400 = Small00; 500 = Modest00

**Table 4.** Effect of urea inclusion in a finishing diet on intake and total tract digestibility (Exp. 2)

| | Treatment[1] | | | |
|---|---|---|---|---|
| | CON | HU | SEM | *P*-value |
| *No. steers* | *4* | *4* | - | - |
| *Intake, kg/d* | | | | |
| DM | 10.4 | 10.3 | 0.1 | 0.27 |
| OM | 9.9 | 9.9 | 0.1 | 0.24 |
| *Digestibility, %* | | | | |
| OM | 69.1 | 72.3 | 2.9 | 0.04 |
| NDF | 43.8 | 52.3 | 4.8 | 0.04 |
| CP[2] | 67.7 | 67.6 | 2.9 | 0.99 |
| Starch | 79.3 | 81.7 | 3.8 | 0.30 |

[1] CON: Control; HU: High urea diets containing 0 or 0.6% urea of dietary DM, respectively.
[2] Estimated based on non-urea nitrogen intake.

**Table 5.** Effect of urea inclusion in a finishing diet on ammonia-N ($NH_3$-N) and VFA concentrations, VFA molar proportions, and pH in ruminal fluid, and purine derivatives-to-creatinine (PDC) index at different times post-feeding (Exp. 2)

| | Treatment[1] | | | *P*-value | | |
|---|---|---|---|---|---|---|
| | CON | HU | SEM | Treatment | Time | Treatment x Time |
| *No. steers* | *4* | *4* | - | - | - | - |
| $NH_3$-N, mg/dL | 3.85 | 8.51 | 1.08 | <0.01 | 0.07 | 0.06 |
| pH | 5.94 | 5.85 | 0.17 | 0.31 | <0.01 | 0.28 |
| *VFA, mM* | | | | | | |
| Total | 86.4 | 119.1 | 7.0 | <0.01 | <0.01 | 0.23 |
| Acetate | 44.4 | 56.0 | 3.9 | 0.03 | 0.05 | 0.02 |
| Propionate | 31.0 | 47.8 | 5.6 | <0.01 | <0.01 | <0.01 |
| Branched-chain[2] | 2.51 | 2.22 | 0.65 | 0.65 | <0.01 | <0.01 |
| Acetate-to-Propionate | 1.69 | 1.30 | 0.54 | 0.61 | 0.08 | 0.58 |
| *VFA, mol/100 mol* | | | | | | |
| Acetate | 51.2 | 47.1 | 4.2 | 0.52 | 0.06 | 0.07 |
| Propionate | 35.4 | 40.1 | 9.1 | 0.71 | 0.04 | 0.27 |
| Butyrate | 9.2 | 9.6 | 1.8 | 0.84 | 0.02 | <0.01 |
| PDC index | 102.0 | 105.1 | 4.9 | 0.25 | <0.01 | <0.01 |

[1] CON: Control; HU: High urea diets containing 0 or 0.6% urea of dietary DM, respectively.
[2] Summation of isobutyric, isovaleric, and two-methyl butyric acids.

**Table 6.** Estimated degradable intake protein (DIP) supply, requirements, and balance, and metabolizable protein (MP) balance for cattle fed finishing diets containing urea at 0 (CON), 0.4 (LU), or 0.6% (HU) of dietary DM

| | Experiment 1[a] | | | Experiment 2[b] | | Experiment 2[c] | |
|---|---|---|---|---|---|---|---|
| | CON | LU | HU | CON | HU | CON | HU |
| DIP supply | 807 | 948 | 1,019 | 658 | 831 | 658 | 831 |
| DIP requirements | 894 | 894 | 894 | 827 | 827 | 827 | 827 |
| DIP balance | -87 | 54 | 125 | -169 | 4 | -318 | -145 |
| MP balance | 489 | 489 | 436 | 402 | 352 | 231 | 274 |

[a] Estimated using Level 1 of NRC model (2000) which assumes that, when present, DIP deficiencies (DIP requirements > DIP supply) are overcome by degradable N supplementation. Therefore, the amount of microbial protein synthesized in the rumen per day is represented by daily DIP requirements.
[b] Estimated using Level 1 of NRC model (2000) and measured pH.
[c] Estimated using measured pH, and modifications to the Level 1 of the NRC model (2000): *DIP balance = DIP supply – DIP requirements x 1.18*; when DIP balance resulted positive, contribution of MCP to MP supply was calculated as *DIP requirements x 0.64*; when negative, the contribution was estimated as *DIP supply x 0.85 x 0.64* (NRC, 2001).

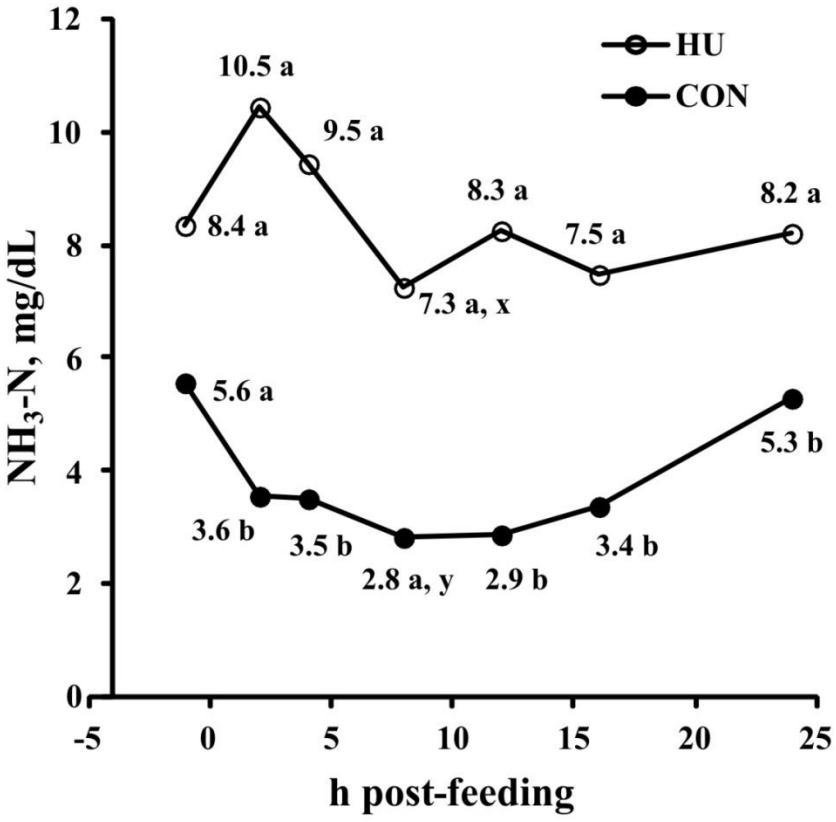


**Figure 1.** Ammonia-N ($NH_3$-N) as affected by hours post-feeding in animals fed a control (CON) or urea (HU) diet with no urea added or added at 0.6% of dietary DM, respectively (Exp. 2). [ab, xy] Means with uncommon superscripts differ within hour post-feeding at $\alpha = 0.05$ or 0.10, respectively.

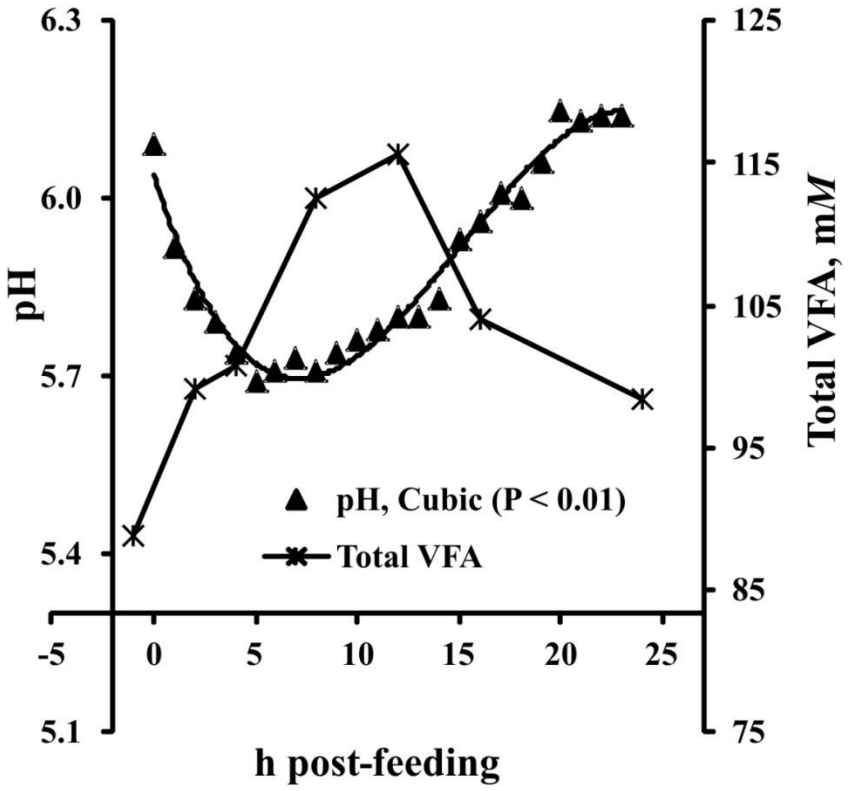


**Figure 2.** Ruminal pH and total VFA concentration as affected by hours post-feeding (Exp. 2).

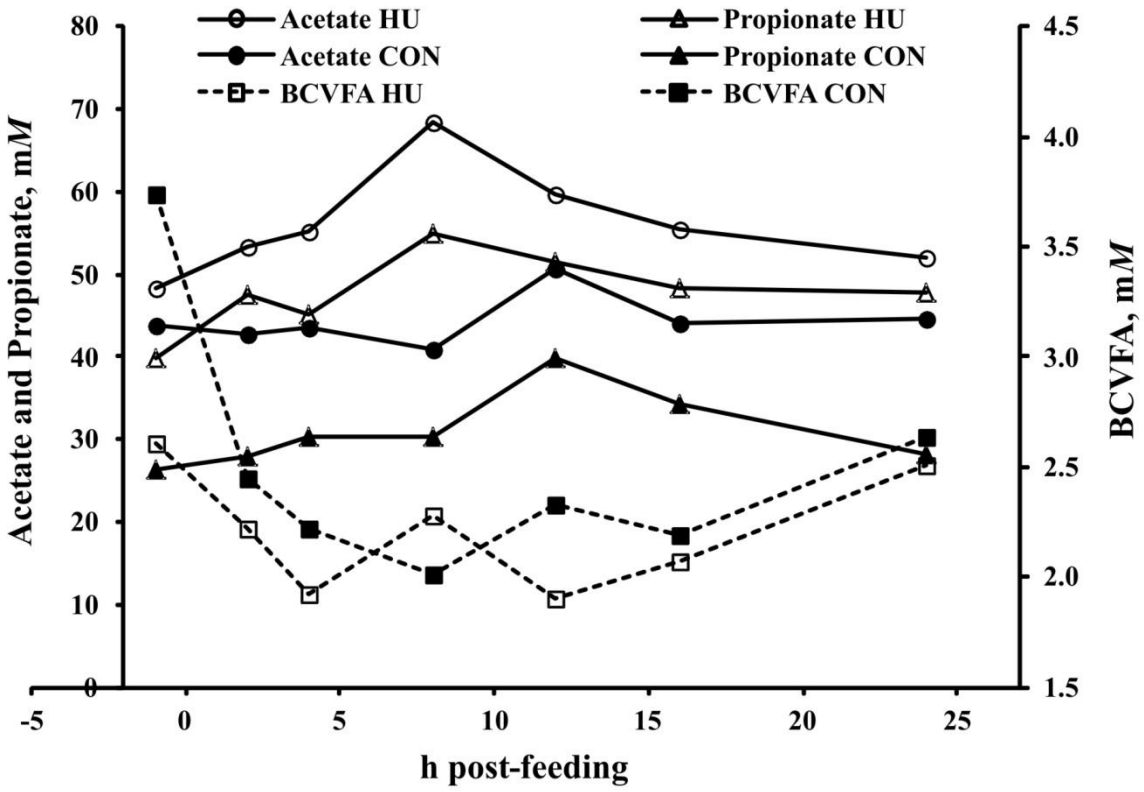


**Figure 3.** Acetate, propionate, and branched-chain VFA (BCVFA) ruminal concentrations as affected by hours post-feeding in animals fed a control (CON) or urea (HU) diet with no urea added or added at 0.6% of dietary DM, respectively (Exp. 2).

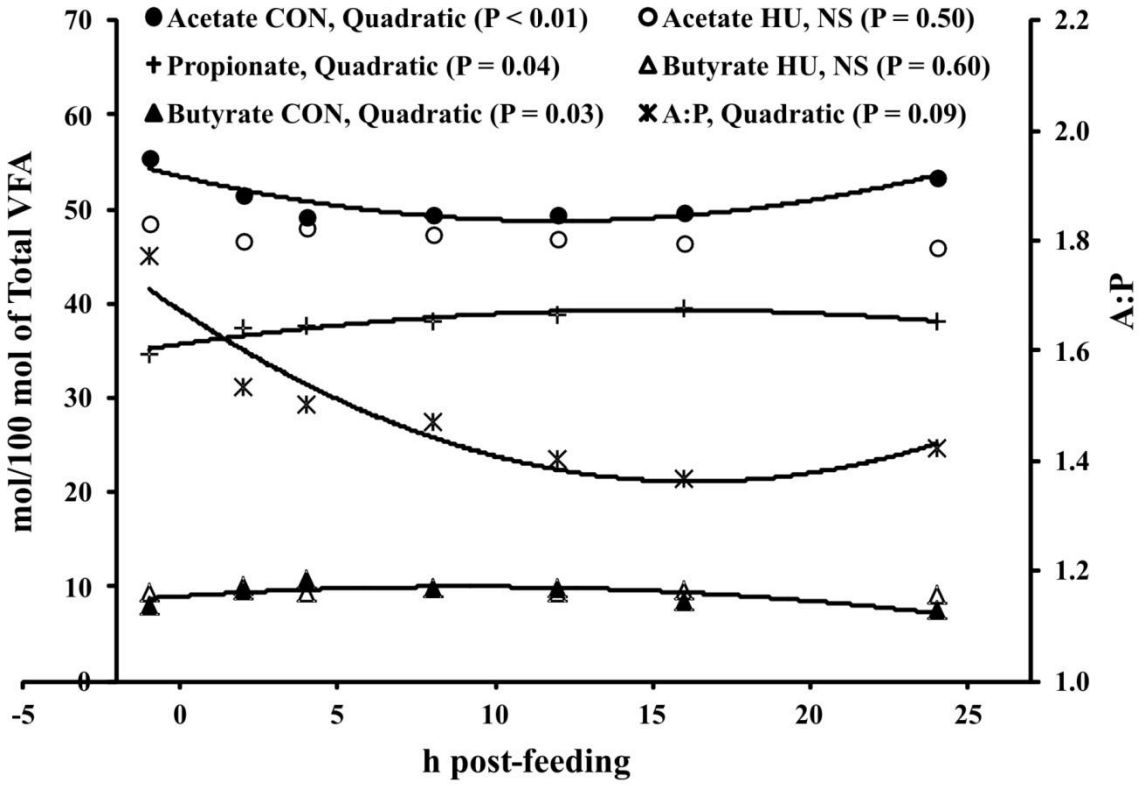


**Figure 4.** Acetate, propionate, and butyrate molar proportions and acetate-to-propionate ratio (A:P) as affected by hours post-feeding in animals fed a control (CON) or urea (HU) diet with no urea added or added at 0.6% of dietary DM, respectively (Exp. 2).

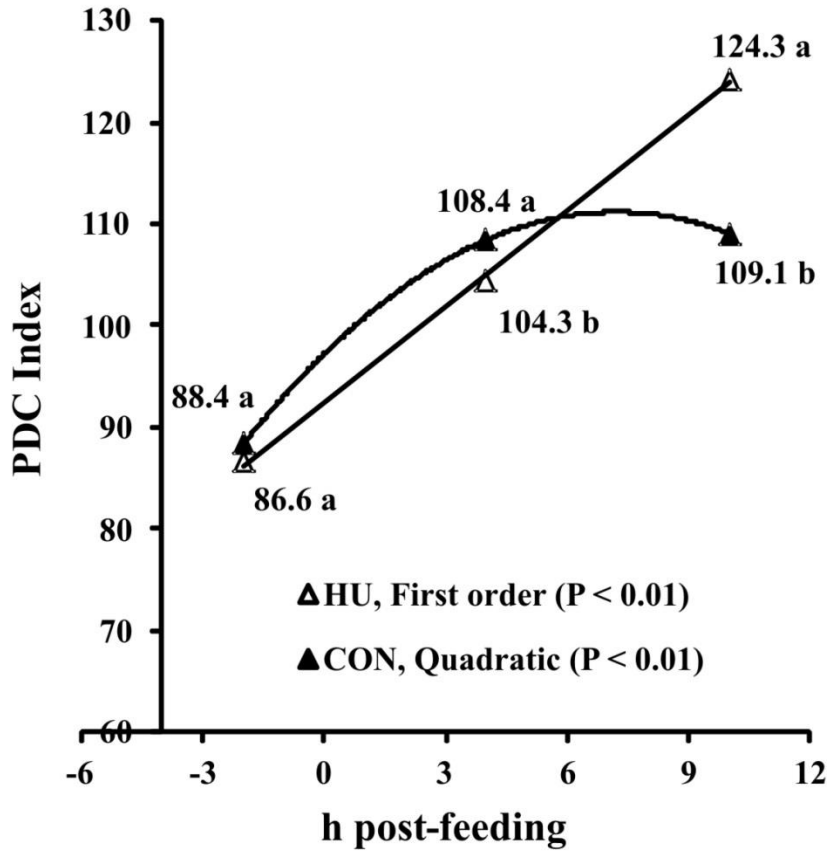


**Figure 5.** Purine derivatives-to-creatinine (PDC) index as affected by hours post-feeding in animals fed a control (CON) or urea (HU) diet with no urea added or added at 0.6% of dietary DM, respectively (Exp. 2). [ab] Means with uncommon superscripts differ within hour post-feeding at $\alpha = 0.05$.

# CHAPTER 3

# EFFECT OF SLOW-RELEASE UREA INCLUSION IN DIETS CONTAINING MODIFIED CORN DISTILLERS GRAINS ON TOTAL TRACT DIGESTIBILITY, RUMINAL FERMENTATION, AND PURINE DERIVATIVES-TO-CREATININE INDEX IN FEEDLOT CATTLE.

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## SUMMARY

Ruminal degradable intake protein (DIP) deficit may result when cattle are fed diets containing great inclusion of processed corn grain and small to moderate inclusion of corn distillers grains (DG). This deficit may arise from increased availability of rapidly fermentable carbohydrates and high rumen-undegradable CP concentration of corn grain. In addition, because rates of degradation of carbohydrates and urea may not match, beneficial effects may result from the use of slow-release urea (SRU) sources over conventional urea when added to DIP-deficient diets. An experiment was conducted to evaluate the effect of increasing DIP concentration through inclusion of one of two SRU sources or conventional urea in DG-containing feedlot diets on ruminal fermentation, total tract digestibility, and purine derivatives-to-creatinine (PDC) index. In addition, an *in situ* experiment was conducted to characterize N disappearance of all urea sources used. Four ruminally cannulated steers (588 ± 8 kg initial BW) were arranged in a 4 x 4

Latin square design and assigned randomly to one of four dietary treatments containing 0 (CON) or 0.6% urea in the form of conventional urea (UREA) or SRU as Optigen II (polymer-encapsulated urea; OPTI) or NitroShure (lipid-encapsulated urea; NITRO), and 30% corn earlage, 20% modified corn DG with solubles, 7.8% corn silage, 4.3% dry supplement, and dry-rolled corn (DM basis). Dietary DIP was estimated at 6.6 and 8.3% for CON and urea-containing dietary treatments, respectively. Steers were fed *ad libitum* once daily. Differences in PDC index between treatments were used as indicators of differences in microbial CP synthesis. Intake of OM, digestibility of OM, NDF, CP, and starch, ruminal pH, total VFA ruminal concentration, and PDC index were not affected by treatment ($P \geq 0.51$). Ruminal concentration of ammonia-N was greater for UREA and NITRO than CON and OPTI ($P < 0.01$) which was in agreement with N *in situ* disappearance. Supplementing DIP through inclusion of conventional urea or SRU did not affect feed intake, digestibility, nor most of ruminal fermentation parameters evaluated. Several confluent factors are discussed that may explain lack of need of urea supplementation in the present experiment. More research is warranted to evaluate the use of SRU in DIP-deficient diets.

**Keywords:** degradable intake protein, distillers grains, feedlot cattle, microbial protein, slow-release urea, volatile fatty acids

## INTRODUCTION

Extensively processed corn-based diets containing moderate concentrations of corn distillers grains (DG) can result in degradable intake protein (DIP) deficit because of increased availability of rumen-fermentable carbohydrates and great proportion of

rumen-undegradable corn CP. Results from several studies indicated beneficial effects of adding urea to highly-fermentable diets without DG (Milton et al., 1997b; Shain et al., 1998; Gleghorn et al., 2004; Cole et al., 2006; Crawford et al., 2007; Kennington et al., 2009; Wagner et al., 2010) or with DG (Ponce, 2010) on ruminal fermentation or animal performance.

Degradation rates for corn starch and NDF from DG range from 10 to 40%/h, and 6 to 8%/h, respectively, while that for urea is estimated at 400%/h (Sniffen et al., 1992; NRC, 2000). Asynchronous supply of N and energy may result in fermentation occurring largely without microbial growth, thus reducing efficiency of ruminal fermentation and increasing ammonia-N ($NH_3$-N) losses to the environment (Dijkstra et al., 1998; Reynolds and Kristensen, 2008). Therefore, ruminal microbes and cattle may further benefit from the use of slow-release urea (SRU) sources over conventional urea when added to DIP-deficient diets. However, potentially increased N recycling to the rumen from conventional urea compared with SRU may attenuate differences between urea sources. The inclusion of SRU in beef cattle diets has been evaluated, with variable results (Owens et al., 1980; Huntington et al., 2006; Tedeschi et al., 2002; Taylor-Edwards et al., 2009; Bourg et al., 2012; López-Soto et al., 2014). Most of the studies involved forage-based diets and only one study utilized DG-containing diets.

The objective of the present experiments was to evaluate effects of adding SRU (polymer- or lipid-encapsulated urea) or conventional urea to a high-concentrate, moderate-DG containing diet for which a DIP deficit was expected on ruminal fermentation and total tract digestibility.

## MATERIALS AND METHODS

The experiments were conducted at the University of Minnesota Rosemount Research and Outreach Center in Rosemount, MN. Animal care and handling procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee.

### *Experiment 1*

#### *Cattle handling, experimental diets and design, and feeding protocol*

Four Holstein steers (588 ± 8 kg initial BW) fitted with flexible ruminal cannula were arranged in a 4 x 4 Latin square design and assigned randomly to one of four dietary treatments containing 0 (CON, negative control) or 0.6% urea in the form of conventional urea (UREA, positive control) or SRU in the form of polymer-encapsulated urea Optigen II (OPTI, 89% urea; Alltech Inc., Nicholasville, KY, USA) or lipid-encapsulated urea NitroShure (NITRO, 89% urea; Balchem, New Hampton, NY, USA), and 30% corn earlage, 20% modified DG with solubles (MDGS), 7.8% corn silage, 4.3% dry supplement, and dry-rolled corn (DRC; DM basis, Table 1). The negative control was included to determine the need for DIP supplementation. Dietary concentration of urea was decided based on results from previous studies (Ceconi et al., 2012, 2013). Dietary CP measured 13 and 14.7% for CON and the urea-containing diets, respectively (Table 1). Dietary DIP was estimated using measured CP and book-referenced DIP concentrations (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012) of each diet ingredient, resulting in 6.6 and 8.3% for CON and the urea-containing diets, respectively (Table 1).

The experiment consisted of four 21-d periods. Steers were weighed prior to feeding on d 1 and 2 of each period. Steers were individually housed in pens from d 1 to 15, and in metabolism stalls from d 16 to 21. During the first 13 d of each period, steers were adapted to their assigned diet, which was offered at 0900 h. Bunks were monitored daily to adjust feed offered. From d 14, diets were offered *ad libitum* and refusals were recorded daily and kept in the bunk unless they represented more than 5% of daily feed delivery. In this case, the refusal was removed, weighed and sampled. Otherwise, refusals were removed, weighed and sampled at the end of each period.

***Sample and data collection, and laboratory analyses***

To determine digestibility of OM and its components, animals were intra-ruminally dosed at 0700 and 1900 h daily with 7.5 g of chromic oxide contained in porcine gelatin capsules from d 11 to 21. Fecal grab samples were collected at 0700, 1300, and 1900 h from d 17 to 21 and stored at -20 °C. After completion of the experiment, fecal samples were freeze-dried, ground, and composited by steer and period. Chromium concentration in feces was used to estimate fecal daily output and it was determined using an atomic absorption spectrophotometer (AAanlyst 200; Perkin Elmer, Walther, MA, USA) based on a procedure suggested by Williams et al. (1962). Feed and fecal OM, CP, starch, and NDF contents were determined by methods 942.05, 990.03, 920.40, and 2002.04 (AOAC, 2012), respectively. Digestibility of OM, starch, and NDF was estimated as [*(intake – excretion) / intake] x 100*. To avoid overestimation of CP (N x 6.25) digestibility, the latter was estimated as {*[(Total CP intake – CP intake derived from supplemental urea) - CP excretion] / (Total CP intake – CP intake derived from supplemental urea)} x 100*.

Ruminal pH was recorded by sensors programmed to measure and record ruminal pH every 5 minutes (Omega Engineering Inc., Stamford, CT, USA). Probes were inserted into the rumen of each steer on d 16 and removed at the end of each period. Data were downloaded from individual probes upon completion of each period and composited by steer and period on an hourly basis for a 24-h period.

Concentration of VFA and $NH_3$-N were measured in ruminal fluid samples collected on d 21 at -1, 2, 4, 8, 12, 16, and 24 h post-feeding. Ruminal fluid samples were collected by a manual suction strainer inserted through the ruminal cannula. Concentration of VFA was determined using a Hewlett Packard 6890 gas chromatographer (Agilent Tech., Santa Clara, CA, USA) based on the procedure suggested by Erwin et al. (1961). Concentration of $NH_3$-N was determined by colorimetry based on the phenol- hypochlorite assay (Broderick and Kang, 1980) and using a spectrophotometer (Gilford Stasar II, Gilford Instrument Laboratories Inc., Oberlin, Ohio, USA).

Differences in PDC index between treatments were used as indicators of differences in microbial CP (MCP) synthesis (Chen and Ørskov, 2004). The PDC index was defined as $BW^{0.75} \times (PD / C)$, where PD (purine derivatives) and C (creatinine) were expressed in m*M* and BW in kg (Chen et al., 2004). Urine is the means through which PD from the metabolism of microbial nucleic acids (Chen and Ørskov, 2004) and C from muscle metabolism (Lofgreen and Garrett, 1954; Van Niekerk et al., 1963) are excreted from the body. Therefore, PD (allantoin and uric acid) and C were simultaneously determined in urine samples (Shingfield and Offer, 1999) using a high-performance liquid chromatographer (Gilson, Middleton, WI, USA). Urine samples were collected

following the same protocol used for fecal sample collection. After completion of the experiment, urine samples were composited by time, steer, and period.

***Calculations and data analyses***

Chen and Gomes (1995) proposed a formula to predict absolute MCP flow from urinary PD. Many of the factors included in this model are highly variable which may result in estimations inconsistent with MCP flow estimated from duodenal purines (Crawford, 2007). However, urinary PD are a reliable marker to estimate relative differences in MCP flow (Südekum et al., 2006). Therefore, the effect of urea supplementation on MCP flow and microbial efficiency was analyzed using absolute PD-derived MCP flow and microbial efficiency, though reported in relative terms (%). Absolute MCP flow was estimated as [*(PDa / 0.900) x 70 x 6.25] / (0.116 x 0.830 x 1000)*, where PDa represented PD originated from ruminal microbes (mmol/d) and was estimated as $(PDe - 0.385 \times BW^{0.75}) / 0.850$, being PDe the amount of PD excreted by the animal (mmol/d; Chen and Gomes, 1995). The latter was estimated based on urinary PD concentration and total urine output, assuming that the internal marker C was excreted at a rate of 28 mg/kg of BW (McCarthy et al., 1983; Whittet et al., 2004). Microbial efficiency was estimated as *[MCP (g/d) / OM digested (g/d)] x 100*. Relative differences in MCP flow and microbial efficiency between treatments were estimated as $[(A_U - A_{CON}) / A_{CON}] \times 100$, where $A_{CON}$ and $A_U$ were defined as absolute MCP flow or microbial efficiency for CON and any urea-containing diet, respectively.

Balances in terms of DIP and MP were calculated for each treatment using Level 1 of the NRC model (2000) which assumes that, when present, DIP deficiencies (DIP requirements > DIP supply) are overcome by degradable N supplementation. Therefore,

the amount of MCP synthesized in the rumen per day is represented by daily DIP requirements, which are estimated as total digestible nutrients (TDN) intake affected by microbial efficiency, the latter being the amount of bacterial CP produced per 100 g of TDN. On the other hand, DIP supply is estimated as a function of DMI and dietary DIP. Additionally, DIP and MP balances were estimated based on some modifications to Level 1 of the NRC model (2000). Efficiency of $NH_3$-N capture by ruminal microbes is below 100% due to direct absorption of $NH_3$-N across the rumen wall and passage of fluid from the rumen (NRC, 1985). Therefore, DIP balance was estimated as *DIP supply – DIP requirements x 1.18*. When DIP balance resulted positive, contribution of MCP to MP supply was calculated as *DIP requirements x 0.64*; when negative, the contribution was estimated as *DIP supply x 0.85 x 0.64* (NRC, 2001).

Data collected after the end of the adaptation period (d 16) were analyzed using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). For pH, VFA, $NH_3$-N, PDC index, and MCP flow a repeated measure structure was utilized. The model included dietary treatment, time, treatment-by-time interaction, and period as fixed effects and animal as random effect. Degrees of freedom were calculated with the Satterthwaite option. The best covariance structure was selected for each variable following procedure suggested by Casler (2006). Briefly, unstructured matrices of variances, covariances, and correlations were requested. Based on this information, inadequate structures were ruled out. When more than one structure was potentially suitable, final selection was made based on information criteria. To decide between models that differed in number of parameters, a $Chi^2$ test involving the *residual log likelihood* criteria was performed. When null hypothesis was not rejected (calculated $Chi^2$ value < critical $Chi^2$ value), data

were analyzed based on the reduced model. When transformation of variables was applied to stabilize variances and/or normalize data, statistical analyses were performed on the transformed and non-transformed variable to report *P*-values and treatment means and corresponding standard errors, respectively.

Effects were considered significant when *P*-values were less than or equal to 0.05 and were considered trends when *P*-values were between 0.05 and 0.10. When *P*-value for treatment effect was below 0.10, treatment means were separated using a *t* test (*pdiff* option). When significant and of interest, the time effect was evaluated by polynomial contrasts, for which coefficients for unequally spaced sampling times were obtained through the IML procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC).

***Experiment 2***

***Cattle and sample handling, experimental design, and feeding protocol***

Two Holstein steers (710 ± 20 kg initial BW) fitted with flexible ruminal cannula and individually housed in pens were arranged in a randomized complete block design. Steers were adapted to Exp. 1 CON dietary treatment from d 1 to 7 and were fed *ad libitum* from d 8 to 10. Steers were fed daily at 0900 h.

On d 10, N-free polyester bags (5 cm x 10 cm, 50 micron porosity; Ankom Tech., Macedon, NY, USA) containing 0 (blank) or 1 g of conventional urea, Optigen II, or NitroShure with 99.7% DM were introduced in the ventral sac of the rumen of each steer at 0900 h and removed at 1, 2, 4, 6, 8, 12, and 24 h post-incubation/feeding. The amount of sample contained within each bag was set considering a relationship of 10 mg of sample/$cm^2$ of bag (Vanzant et al., 1998). For each incubation time, polyester bags were

placed into a weighted mesh bag which was attached to a string whose end was left outside the rumen to facilitate removal. Prior to incubation in the rumen, bags were placed in a water bath at 39 °C for 15 min to simulate insalivation (Nocek and English, 1986; Nocek, 1988). Immediately after retrieval from the rumen, bags were rinsed with cold water until rinsing water was clear and then dried. Bags corresponding to 0 h post-feeding were not incubated in the rumen but rinsed and dried after removed from the warm bath. Bags were prepared in duplicate for each combination of blank or urea source, and sampling time.

***Laboratory analyses***

Content of DM was determined by drying bags and samples in a forced-air oven at 60 °C for 48 h. Content of N was determined on dried samples using a 2300 Kjeltec analyzer unit (Foss Tecator AB, Höganäs, Sweden).

***Data analyses***

Data were analyzed using the Mixed procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC), where animal (block) was considered a random effect. Level of significance was set and treatment means separation was performed as described in Exp. 1.

## RESULTS AND DISCUSSION

Dietary treatments did not affect DM or OM intake ($P \geq 0.53$, Table 2) which averaged 14.9 ± 0.37 (2.09% of BW) and 13.9 ± 0.34 kg/d, respectively. Similarly, neither digestibilities of NDF (34.1 ± 2.2%), CP (64.8 ± 1.2%), nor starch (88.6 ± 0.9%) were affected by urea inclusion regardless of source, which resulted in similar OM digestibility among treatments ($P \geq 0.51$, Table 2). Likewise, no beneficial effects on DM

or OM digestibility were observed by Garret et al. (2005), and Álvarez Almora et al. (2012) and Holder (2012) when comparing supplementation of conventional urea with NitroShure or with Optigen II, respectively, to diets containing at least 50% forage. However, neither of these studies included a negative control to which the effectiveness of any type of urea form could be compared. Ribeiro et al. (2011) investigated the effects of urea and Optigen 1200 supplementation to steers consuming *Brachiaria* hay on ruminal fermentation parameters and feed digestibility. The authors reported similar OM digestibility between the negative control, conventional urea-, and SRU-supplemented diets.

Ruminal concentration of $NH_3$-N was affected by treatment ($P < 0.01$); greater $NH_3$-N concentration was observed for UREA and NITRO compared with CON and OPTI (Table 3). This result was observed primarily from -1 to 12 h post-feeding (treatment-by-time, $P = 0.06$; Table 3 and Figure 1; *P*-value of contrast UREA+NITRO vs. CON+OPTI < 0.10 at any sampling time except 16 h). Concentration of $NH_3$-N noticeably peaked at 4 h post-feeding for UREA. Ribeiro et al. (2011) reported ruminal concentrations of $NH_3$-N peaking between 1 and 2 h post-feeding; ruminal concentrations of $NH_3$-N were greater for steers receiving urea, intermediate for those supplemented with Optigen 1200, and smallest for the unsupplemented steers during the first 4 h post-feeding. Similarly, García-González et al. (2007) reported greater ruminal concentration of $NH_3$-N when adding urea but not when adding Optigen to 60%-corn silage diets. On the contrary, no differences in $NH_3$-N concentration between urea- and Optigen II-supplemented steers were observed by Álvarez Almora et al. (2012). Authors attributed this to endogenous urea being recycled back to the rumen and masking potential

differences between treatments. Results for ruminal concentration of $NH_3$-N observed in Exp. 1 are consistent with those observed in Exp. 2, where N disappearance from polyester bags was complete after a 15-min-exposure to warm water (0 h incubation) for UREA and NITRO, while it rapidly increased from 27.8% at 0 h to 63.0% at 1 h post-incubation and increased almost at a constant pace afterwards, up to 93.2% at 24 h post-incubation for OPTI (Figure 2). Holder (2012) reported that urea disappearance from polyester bags was complete for conventional urea at 0 h post-incubation in the rumen while it increased from 0 to about 20% in 1 h and increased thereafter up to 60% at 24 h for Optigen II. The fact that polyester bags were not soaked in warm water before incubation in the study by Holder (2012) may explain a slower Optigen II disappearance compared with that in the present experiment.

Results from *in vivo* and *in situ* experiments indicate contrasting ruminal concentration of $NH_3$-N and N release between SRU sources, with NITRO resulting in similar N release than conventional urea, and OPTI resulting in similar $NH_3$-N concentration than the non-supplemented diet. DiLorenzo and DiCostanzo (2007a,b) reported that rate of $NH_3$-N release in batch culture was faster for conventional urea compared with NitroShure, while DiLorenzo (unpublished) observed rate of $NH_3$-N release being faster for conventional urea, intermediate for NitroShure, and slower for Optigen II.

In agreement with OM digestibility results, total VFA concentration was not affected by treatments ($P = 0.99$, Table 3), which was consistent with results reported by Garret et al. (2005) and Álvarez Almora et al. (2012). However, VFA concentration was affected by time ($P < 0.01$, Table 3), quadratically increasing from -1 to 8 h post-feeding

and decreasing thereafter (Figure 3). This can be interpreted as microbial activity being stimulated after feeding. A concurrent and almost opposite response to time was observed for ruminal pH ($P < 0.01$, Table 3; cubic contrast $P < 0.01$, Figure 3), which was not affected by treatment ($P = 0.91$, Table 3). Variation in total VFA concentration explained 84.9% of variation in ruminal pH ($P < 0.01$). This observation is similar to the 86.6% reported by Zinn et al. (2003). Similarly to what was observed for total VFA and ruminal pH, the period of increased total VFA concentration mostly related to the period of decreased $NH_3$-N concentration and vice versa (Figures 1 and 3). These observations may reflect concomitant $NH_3$-N microbial use during times of increased microbial activity and VFA production. Nitrogen release from OPTI during times of maximum microbial activity was about 75% of that from NITRO or conventional urea (Figure 2).

Branched-chain VFA (BCVFA) result solely from the fermentation of branched-chain AA and represent carbon backbone sources for the synthesis of microbial branched-chain AA (Allison and Bryant, 1963; Allison, 1969; Russell, 2002), in particular for cellulolytic bacteria. In agreement with similar dietary non-urea CP concentration, DMI, and CP digestibility, and a possibly similar microbial activity associated with similar OM digestibility and total VFA concentration among treatments, BCVFA concentration was not affected by treatments ($P = 0.31$, Table 3).

Acetate-to-propionate ratio tended to be lower for OPTI compared with any of the other treatments ($P = 0.09$), due to similar acetate ($P = 0.18$) but greater propionate molar proportions ($P < 0.05$, Table 3). Based on similar DMI, and starch and NDF dietary concentrations and digestibilities among treatments, this result was not expected. Similarly, Álvarez Almora et al. (2012) observed reduced acetate-to-propionate ratio with

Optigen II compared with conventional urea, when these urea sources were included in forage-based diets. However, those authors as well as Ribeiro et al. (2011) also reported decreased ruminal pH with SRU compared with conventional urea. Low pH may negatively impact growth of fiber-fermenting bacteria and might be the result of lower ruminal concentration of $NH_3$-N, as $NH_3$-N contributes to ruminal buffering capacity. As mentioned previously, ruminal pH was not affected by treatments in the present experiment (Table 3); consequently, no association between variations in acetate-to-propionate ratio and ruminal pH across treatments can be established. Conversely, increased acetate-to-propionate ratio with Optigen II compared with conventional urea was reported by Holder (2012). It was suggested that a slower, steadier supply of $NH_3$-N may favor slow-growing fiber-digesting bacteria. Somewhat opposed to this suggestion, and considering that $NH_3$-N plays a more important role in nutrition of the relatively slow-growing, fiber-digesting bacteria (Hungate, 1966; Bryant and Robinson, 1963), reduced $NH_3$-N concentration with OPTI compared with other urea sources could have negatively impacted growth of fiber-digesting bacteria, thus resulting in decreased acetate-to-propionate ratio. However, this proposed mechanism of action is debatable since decreased $NH_3$-N concentration might reduce growth of cellulolytic bacteria when the former results in an unmet $NH_3$-N demand. Based on similar feed digestibility and total VFA concentration across treatments, the existence of a DIP deficit in the current experiment is, at least, arguable.

Propionate molar proportion increased while acetate molar proportion decreased quadratically ($P < 0.01$, Figure 4) throughout the day, regardless of treatment (treatment-by-time, $P \geq 0.59$, Table 3). This resulted in a cubic decrease ($P = 0.03$, Figure 4) in the

acetate-to-propionate ratio, which could have been associated with a concurrent decrease in ruminal pH (Figure 1). Due to differential susceptibility, ruminal pH can differentially affect ruminal bacteria populations (Russell and Wilson, 1996) which in turn may alter VFA molar proportions depending on prevailing VFA-production pathways present in each bacteria group. In addition, studies reviewed by Dijkstra et al. (2012) reported that bacteria have the ability to shift VFA-producing pathways in response to changes in pH while fermenting the same substrate. Russell (1998) reported that 25% of the reduction in acetate-to-propionate ratio could be explained by the reduction of pH alone. This author and Van Kessel and Russell (1996b) indicated that increased propionate proportion with decreasing pH represents a means of hydrogen disposal in an environment unfavorable for methanogens. A similar relationship between acetate-to-propionate ratio and ruminal pH was observed in the present experiment ($P < 0.01$), though the $R^2$ was lower (18%).

In agreement with previously described variables, PDC index was not affected by treatment ($P = 0.63$, Table 3) and this result was independent of time (treatment-by-time, $P = 0.91$). However, PDC index was smallest at -2 (268.7 ± 15.0), intermediate at 4 (306.1 ± 15.2), and greatest at 10 h post-feeding (342.0 ± 19.1, $P < 0.01$), resulting in a first order response with time ($P < 0.01$). This result suggests that greater PDC index could potentially be observed at later hours post-feeding. The effect of time on PDC index was consistent with that on VFA and $NH_3$-N concentrations, and ruminal pH, likely indicating increased microbial activity and MCP synthesis after feeding. Similarly, Crawford (2007) reported PD-to-C ratio being greater at 9 compared with -1 h post-feeding. As it was observed for PDC index, estimated MCP flow was similar among treatments ($P = 0.63$) and 35% and 14% greater at 10 compared with -2 and 4 h post-

feeding, respectively ($P < 0.01$). As a result of similar OM intake and digestibility, the amount of OM digested was similar ($P = 0.82$) among treatments. Similar amount of OM digested and MCP flow resulted in similar microbial efficiency among treatments ($P = 0.55$).

Similar dietary TDN (Table 1), DMI (Table 2), and model-estimated microbial efficiency resulted in similar DIP requirements across diets (Table 4). Level 1 of the NRC model (2000) estimates microbial efficiency by estimating ruminal pH, and the basis for this relationship is as follows. By increasing proton and VFA anions concentration inside the microbial cells, decreased ruminal pH can increase the amount of ATP used to pump protons out of the cell and reduce the amount of ATP available for microbial growth (Strobel and Russell, 1986; Klemps et al., 1987; Russell and Wilson, 1996; Russell, 2007); this may result in reduced amount of MCP synthesized per unit of feed fermented (Russell and Dombrowski, 1980; Strobel and Russell, 1986). In turn, ruminal pH is estimated based on dietary effective NDF (eNDF) concentration, which refers to the proportion of the NDF that is sufficiently large (> 1.18 mm; NRC, 2000) to be effective at stimulating chewing, rumination, and salivation, and consequently at mitigating ruminal pH drop. Since dietary eNDF was similar across treatments (6.6%, Table 1), model-estimated pH for any of the four diets was 5.71 and the associated estimated microbial efficiency was 8.71%. Conversely, due to differences in dietary DIP concentration, DIP supply was smaller for CON (983 g/d, Table 4) than any of the urea-supplemented diets (1,239 g/d). Consequently, estimated DIP balances were -40, 223, 224, and 224 g/d for CON, UREA, OPTI, and NITRO, respectively (Table 4).

In spite of the fact that feeding CON diet resulted in a 40-g DIP deficit, or even greater if measured (5.84) instead of estimated (5.71) ruminal pH is integrated in the model and/or if inefficiencies in $NH_3$-N capture by ruminal microbes are considered (*DIP balance = DIP supply – DIP requirements x 1.18*; NRC, 2001), supplementation of any form of urea did not improve feed digestibility or ruminal fermentation parameters. These results provide evidence in favor of absence of a DIP deficit produced by the CON diet. In addition, Satter and Slyter (1974) reported beneficial effects of the addition of non-protein N supplements to ruminant rations on microbial protein yield when the prevailing concentration of ruminal $NH_3$-N was less than 5 mg/dL. Even though DIP requirements fluctuate depending on DMI, rate of fermentation of the diet, and ruminal pH, among others, the fact that $NH_3$-N concentration for CON was above 5 mg/dL all throughout the day (Figure 1) may provide additional evidence in support of a lack of DIP deficit.

Possibly, other microbial-affecting factors that are not considered in the NRC model (2000) might have offset the projected DIP deficit. Relative DMI (2.09% BW) was less than expected (2.24 to 2.45% BW) based on previous studies (Ceconi et al., 2012; 2013), which may have decreased microbial efficiency, thus resulting in DIP requirements being smaller than expected. The basis underlying the positive association between microbial efficiency and DMI is the positive effect of DMI on particle, liquid, and microbial outflow rates. Results from various studies demonstrated an increase in both fractional rate of passage and dilution rate in cattle with increasing DMI (Sniffen and Robinson, 1987) or DMI expressed as a percentage of BW (Evans, 1981a,b). Faster outflow rates result in increased microbial efficiency (Isaacson et al., 1975; Russell and Baldwin, 1979; Meng et al., 1999) because mean age of microbial population is

decreased, a younger population is subject to lower death and predation rates (Van Soest, 1994; Meng et al., 1999; Russell, 2002), and because faster outflow rates select in favor of fast-growing microbes (Meng et al., 1999), reducing the proportion of energy intended for microbial maintenance. In addition, reduced rate of passage and a concomitantly increased rumen retention time in the present experiment may have permitted greater dietary CP degradability than expected.

Fermentation rate is an inherent property of the feed (Russell et al., 1992) that determines the amount of feed energy per unit of time for microbial growth; thus, it is positively associated with microbial efficiency (Van Soest, 1994) because it permits faster microbial growth and reduces microbial maintenance requirements. For example, based on rumen fermentability of carbohydrates, Cooper et al. (2002a) reported that dietary DIP beyond which no further improvements in animal performance were observed measured 10.0, 8.3, and 6.3% for high-moisture corn (HMC)-, steam-flaked corn-, or DRC-based finishing diets, respectively. Even though corn earlage provided HMC to the diet, which is highly fermentable, it also contributed a considerable amount of fiber (22.4% NDF), which may have reduced diet fermentation rate and microbial efficiency.

The amount of ATP derived per mole of substrate fermented and thus available for microbial growth varies among types of substrate fermented in the rumen, which consequently affects the amount of MCP synthesized per unit of OM fermented (microbial efficiency). Yield of ATP is greatest for glucose derived from polysaccharides (fiber and starch), intermediate for soluble carbohydrates (sugars) and smallest for protein (Van Duinkerken et al., 2011). Due to their fast rate of degradation, hexoses derived from

sugars influences fermentation towards synthesis of lactic acid, which produces only 2 ATP/mole compared with 4 ATP/mole derived from acetate or propionate formation (Russell, 2002; Van Duinkerken et al., 2011). Low ATP yield from protein fermentation might be related to the fact that, unlike hexoses, fermentation of AA does not involve glycolysis but only keto-acid decarboxylation, and that about 16% of a mole of protein (that ascribed to N content) is not involved in ATP generation. Consequently, dietary inclusion of 20% MDGS containing 30.6% CP might have reduced microbial efficiency, thus reducing DIP requirements and need for DIP supplementation.

Finally, a DIP deficit can be partially or totally reversed by a positive intestinal MP balance, through urea being recycled back to the rumen (NRC, 2000). As emphasized by Valkeners et al. (2004), Cabrita et al. (2006), and Reynolds and Kristensen (2008), recycling of N via hepatic synthesis of urea may enable to overcome short-term effects of asynchrony between carbohydrates and N rumen availability. In that regard, slower diet fermentation rate may have allowed a positive MP balance (Table 4) to recycle urea to the rumen in time to alleviate a potential DIP deficit. This might have also been the case in the study by Jenkins et al. (2011), in which they evaluated the effect of adding conventional urea to a DRC-based diet containing 10% dried DG on finishing cattle performance. Balances of DIP and MP for the no-urea diet were estimated by the authors at -92 and 268 g/d, respectively. Since MP requirements were met, it can be assumed that N from excess 268 g of MP would be transformed to urea in the liver and that a high proportion of the urea synthesized would be recycled back to the rumen (Reynolds and Kristensen, 2008) to overcome an unmet demand of 92 g of DIP. Lack of effect of urea supplementation on ADG and feed efficiency may indicate that excess MP absorbed at

the intestinal level may have been able to supply recycled N in time when carbohydrates from DRC were fermented in the rumen. Similar results were observed by Stalker et al. (2004) when supplementing urea to forage-based diets containing 25 or 30% DG for which the need of DIP supplementation (negative DIP balance) and excess MP (positive MP balance) were calculated to be similar. Conversely, based on the positive response of animal performance, ruminal fermentation, and feed digestibility to urea supplementation in the studies by Cooper et al. (2002a) and Ceconi et al. (2012, 2013), N recycled from excess MP may not have been able to overcome a DIP deficit generated by a HMC- or DRC+HMC-, 0%-urea-containing diets, respectively. This result can be interpreted in terms of inadequacy of excess MP to meet, in time, the DIP deficit generated by fast-fermenting diets.

In conclusion, likely due to lack of DIP deficit with the unsupplemented control diet, results from these experiments did not identify beneficial effects of Optigen II or NitroShure in place of conventional urea. Because previous studies have demonstrated improved ruminal fermentation, feed digestibility, and animal performance when supplementing DIP through the inclusion of conventional urea to rapidly-fermentable, moderate-DG-containing diets, more research is warranted to evaluate the use of SRU in diets for which a DIP deficit is actually expressed.

**Table 1.** Diet composition (DM basis).

| | Treatment[1] | | | |
|---|---|---|---|---|
| | CON | UREA | OPTI | NITRO |
| Dry-rolled corn, % | 37.9 | 37.3 | 37.2 | 37.2 |
| Corn earlage, % | 30.0 | 30.0 | 30.0 | 30.0 |
| Modified DG w/ solubles, % | 20.0 | 20.0 | 20.0 | 20.0 |
| Corn silage, % | 7.8 | 7.8 | 7.8 | 7.8 |
| Mineral-vitamin supplement, %[2] | 4.3 | 4.3 | 4.3 | 4.3 |
| Urea, % | - | 0.6 | - | - |
| Optigen II, % | - | - | 0.67 | - |
| NitroShure, % | - | - | - | 0.67 |
| NEg, Mcal/kg | 1.28 | 1.31 | 1.30 | 1.31 |
| TDN, % | 79.1 | 79.6 | 79.2 | 79.5 |
| CP, % | 13.0 | 14.7 | 14.7 | 14.7 |
| Basal DIP, %[3] | 6.6 | 6.6 | 6.6 | 6.6 |
| Supplemental DIP, %[3] | 0.0 | 1.7 | 1.7 | 1.7 |
| Total DIP, %[3] | 6.6 | 8.3 | 8.3 | 8.3 |
| eNDF, %[3] | 6.6 | 6.6 | 6.6 | 6.6 |

[1] CON: no urea added; UREA: 0.6% conventional urea; OPTI: 0.67% Optigen II (89% urea; Alltech Inc., Nicholasville, KY, USA); NITRO: 0.67% NitroShure (89% urea; Balchem, New Hampton, NY, USA) of dietary DM.
[2] Contained 495 g of Rumensin 90 (Elanco Animal Health, Greenfield, IN, USA) per ton.
[3] Based on book-referenced degradable intake protein (DIP) and effective NDF (eNDF) concentrations for each ingredient (Lardy et al., 1998; NRC, 2000; Beef Magazine, 2012).

**Table 2.** Effect of conventional or slow-release urea inclusion in a finishing diet on intake and total tract digestibility.

| | Treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| | CON | UREA | OPTI | NITRO | SEM | *P*-value |
| *No. steers* | *4* | *4* | *4* | *4* | - | - |
| *Intake, kg/d* | | | | | | |
| DM | 14.4 | 15.4 | 15.4 | 14.4 | 0.7 | 0.53 |
| OM | 13.5 | 14.3 | 14.4 | 13.5 | 0.7 | 0.58 |
| *Digestibility, %* | | | | | | |
| OM | 67.4 | 71.0 | 67.9 | 70.1 | 2.6 | 0.67 |
| NDF | 33.4 | 35.5 | 32.3 | 35.4 | 4.7 | 0.94 |
| CP[2] | 63.1 | 66.4 | 62.4 | 67.3 | 2.6 | 0.51 |
| Starch | 87.7 | 88.3 | 87.3 | 89.5 | 2.0 | 0.56 |

[1] CON: no urea added; UREA: 0.6% conventional urea; OPTI: 0.67% Optigen II (89% urea; Alltech Inc., Nicholasville, KY, USA); NITRO: 0.67% NitroShure (89% urea; Balchem, New Hampton, NY, USA) of dietary DM.
[2] Estimated based on non-urea N intake.

**Table 3.** Effect of conventional or slow-release urea inclusion in a finishing diet on ammonia-N ($NH_3$-N) and VFA concentrations, VFA molar proportions, and pH in ruminal fluid, and purine derivatives-to-creatinine (PDC) index at different times post-feeding.

| | Treatment[1] | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | CON | UREA | OPTI | NITRO | SEM | Treat | Time | Treat x Time |
| *No. steers* | *4* | *4* | *4* | *4* | - | - | - | - |
| $NH_3$-N, mg/dL | 7.64$^{b}$ | 9.56$^{a}$ | 7.42$^{b}$ | 10.03$^{a}$ | 2.15 | <0.01 | <0.01 | 0.06 |
| pH | 5.77 | 5.83 | 5.77 | 5.75 | 0.08 | 0.91 | <0.01 | 0.74 |
| *VFA, mM* | | | | | | | | |
| Total | 108.3 | 105.4 | 108.7 | 107.8 | 12.4 | 0.99 | <0.01 | 0.62 |
| Branched-chain[2] | 3.39 | 3.19 | 2.30 | 2.89 | 0.41 | 0.31 | 0.43 | 0.85 |
| Acetate-to-Propionate | 1.50$^{a, x}$ | 1.36$^{ab, x}$ | 1.08$^{b, y}$ | 1.42$^{ab, x}$ | 0.16 | 0.09 | <0.01 | 0.80 |
| *VFA, mol/100 mol* | | | | | | | | |
| Acetate | 49.0 | 47.6 | 45.7 | 48.6 | 1.1 | 0.18 | <0.01 | 0.59 |
| Propionate | 34.5$^{b}$ | 35.8$^{b}$ | 42.6$^{a}$ | 37.2$^{ab}$ | 2.4 | 0.07 | <0.01 | 0.99 |
| Butyrate | 11.7 | 11.8 | 7.5 | 9.5 | 2.2 | 0.40 | 0.17 | 0.43 |
| PDC index | 291.4 | 304.6 | 309.9 | 316.5 | 19.2 | 0.63 | <0.01 | 0.91 |

[1] CON: no urea added; UREA: 0.6% conventional urea; OPTI: 0.67% Optigen II (89% urea; Alltech Inc., Nicholasville, KY, USA); NITRO: 0.67% NitroShure (89% urea; Balchem, New Hampton, NY, USA) of dietary DM.
[2] Summation of isobutyric, isovaleric, and two-methyl butyric acids.
[ab] Means with uncommon superscripts differ ($P < 0.05$).
[xy] Means with uncommon superscripts differ ($P < 0.10$).

**Table 4.** Effect of conventional or slow-release urea inclusion in a finishing diet on degradable intake protein (DIP) supply, requirements, and balance.

| | Treatment[1] | | | |
|---|---|---|---|---|
| | CON | UREA | OPTI | NITRO |
| DIP supply[2] | 983 | 1,239 | 1,239 | 1,239 |
| DIP requirements[2] | 1,023 | 1,016 | 1,015 | 1,015 |
| DIP balance[2] | -40 | 223 | 224 | 224 |
| MP balance[2] | 681 | 673 | 672 | 672 |
| MP balance[3] | 561 | 673 | 672 | 672 |

[1] CON: no urea added; UREA: 0.6% conventional urea; OPTI: 0.67% Optigen II (89% urea; Alltech Inc., Nicholasville, KY, USA); NITRO: 0.67% NitroShure (89% urea; Balchem, New Hampton, NY, USA) of dietary DM.

[2] Estimated using Level 1 of NRC model (2000) which assumes that, when present, DIP deficiencies (DIP requirements > DIP supply) are overcome by degradable N supplementation. Therefore, the amount of microbial protein synthesized in the rumen per day is represented by daily DIP requirements.

[3] Estimated using modifications to the Level 1 of the NRC model (2000): *DIP balance = DIP supply – DIP requirements x 1.18*; when DIP balance resulted positive, contribution of MCP to MP supply was calculated as *DIP requirements x 0.64*; when negative, the contribution was estimated as *DIP supply x 0.85 x 0.64* (NRC, 2001).

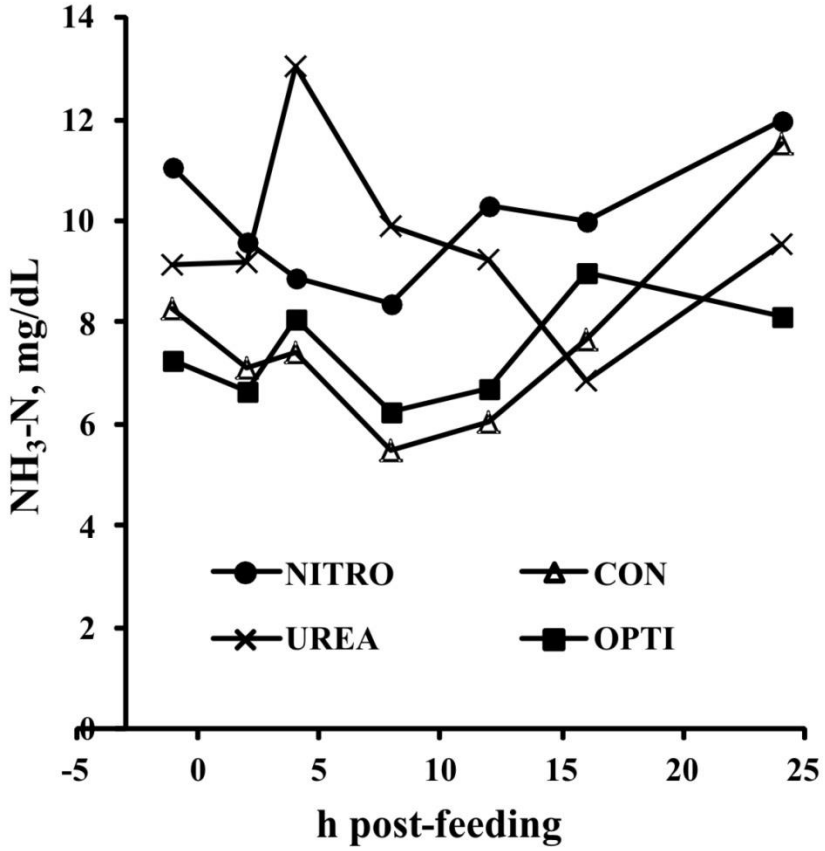


**Figure 1.** Ammonia-N ($NH_3$-N) as affected by hours post-feeding in animals fed diets containing 0 (CON) or 0.6% (DM basis) urea in the form of conventional urea (UREA) or one of two slow-release urea sources: Optigen II (OPTI; 89% urea; Alltech Inc., Nicholasville, KY, USA) or NitroShure (NITRO; 89% urea; Balchem, New Hampton, NY, USA).

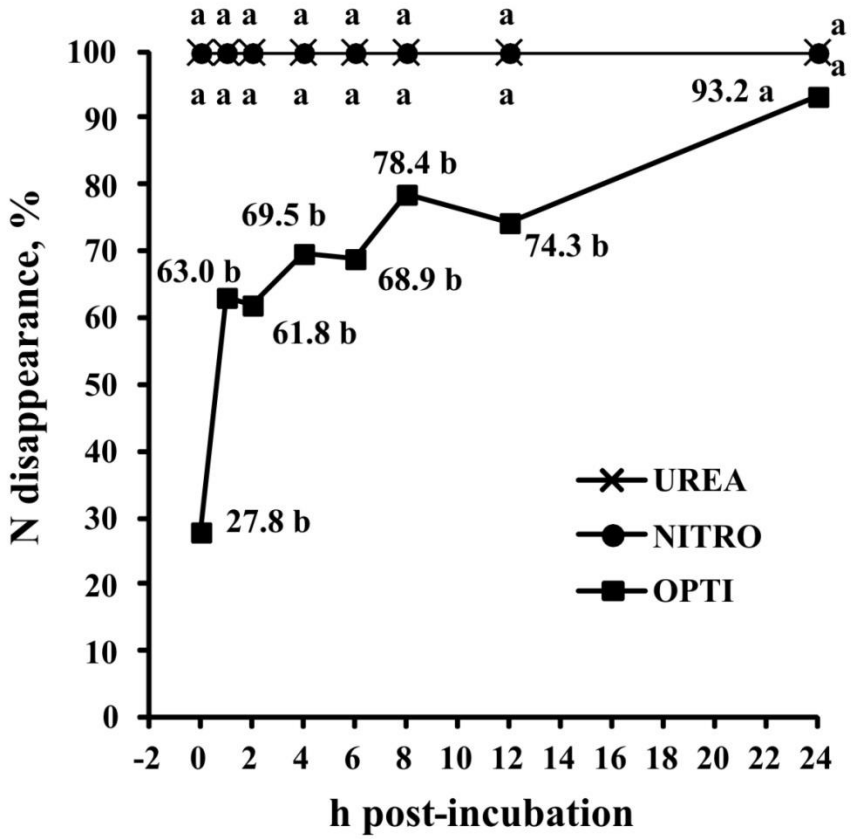


**Figure 2.** Nitrogen (N) disappearance from polyester bags for conventional urea (UREA) and two slow-release urea sources, Optigen II (OPTI; Alltech Inc., Nicholasville, KY, USA) or NitroShure (NITRO; Balchem, New Hampton, NY, USA), as affected by hours post-incubation in the rumen (Exp. 2). [ab] Means with uncommon superscripts differ within hour post-incubation ($P < 0.05$).

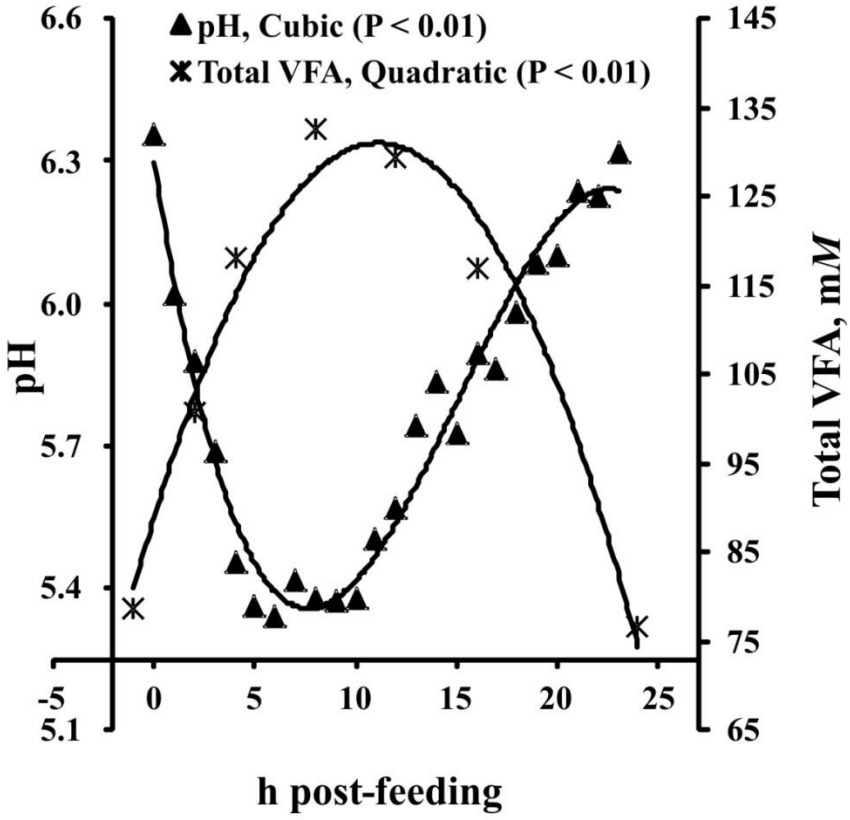


**Figure 3.** Ruminal pH and total VFA concentration as affected by hours post-feeding.

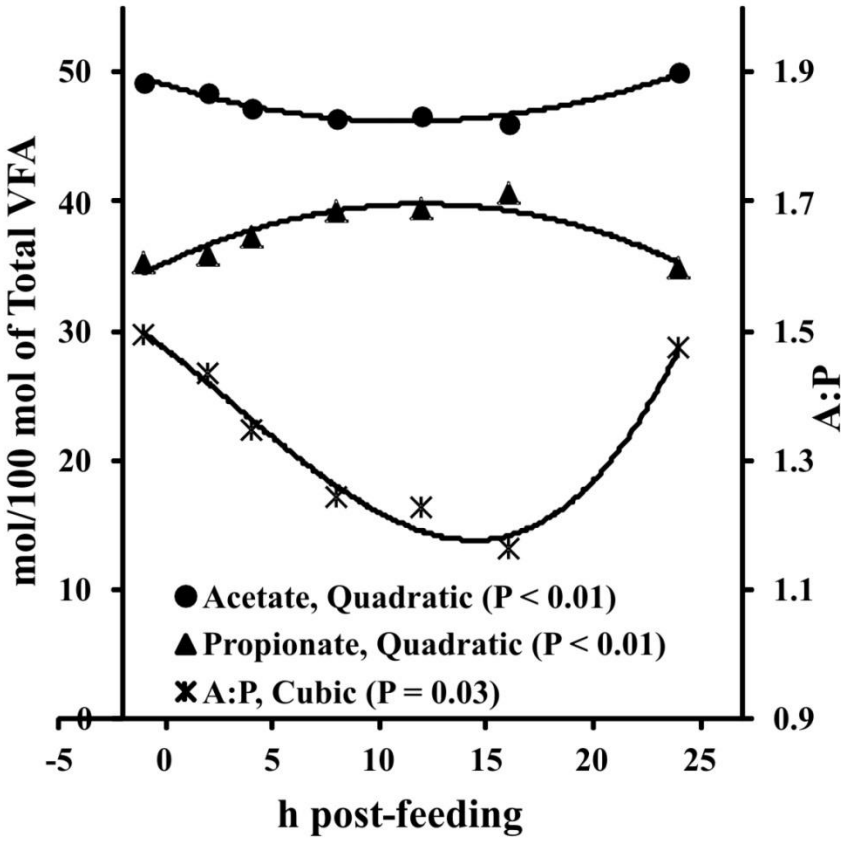


**Figure 4.** Acetate and propionate molar proportions and acetate-to-propionate ratio (A:P) as affected by hours post-feeding.

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