IMPACT OF FORAGE TYPE AND NITROGEN SUPPLEMENTATION ON FORAGE INTAKE AND NITROGEN METABOLISM IN BEEF STEERS

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirement for
the Degree of
DOCTOR OF PHILOSOFY
May, 2004

IMPACT OF FORAGE TYPE AND NITROGEN

SUPPLEMENTATION ON FORAGE INTAKE

AND NITROGEN METABOLISM IN

BEEF STEERS

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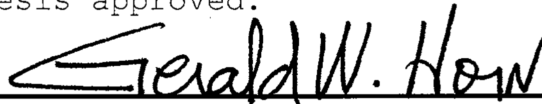

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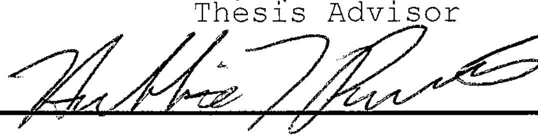

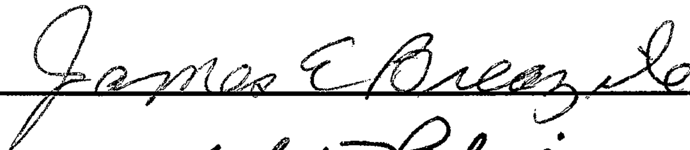

Dean of the Graduate College

ACKNOWLEGMENTS

I wish to express my deep appreciation to all who made possible the completion of this project. First of all, I want to thank to my major advisors Drs. Hebbie T. Purvis II and Gerald W. Horn for guidance, understanding, and friendship. To other members of my committee: Dr. James D. Breazile and Dr. Clint Krehbiel. Dr. Breazile for patience and time to discuss topics on nitrogen metabolism and digestive physiology.

My appreciation to the National Research Institute in Agriculture, Livestock and Forestry for the opportunity to come to Oklahoma State University and National Council of Science and Technology for the scholarship during the most of my time at OSU. Also, I extend my appreciation to the Professors of the Department of Animal Science and to the Department for financial support during this last year.

I want to thank Tim Bodine, Alejandro LaManna, Patrick, Jeff Weyers for help in laboratory and NP barn and friendship. All graduate students in the Department of Animal Science (Ivette, Celina, Calan, Chicho, Christy, Heather, etc). Steve Welty deserves a special thanks for

his invaluable help in the conducting the studies at the Nutrition and Physiology Research Center. Joan Summers, thanks for your help and for all material I took and never returned, Carolyn Lunsford thanks for your help and for fighting 'Leco' (LOCO) for me. Donna Perry, thanks for your help and your smiling face all the time.

I would like to say thanks to my parents, Ismael and Blanca who gave me the opportunity to study to get my degree in veterinary medicine. To my wife's parents and sisters and brothers for their support. Finally, to the most worth of my life, my wife, Soledad, who has always been cheering and supporting me everywhere, and my daughter, Lupita, from whom I enjoy every new thing that she learns step by step. Now, I enjoy learning as she does.

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CHAPTER I

INTRODUCTION

Nitrogen is the key element of amino acids, which are the basic units to build enzymes, hormones, proteins, and other immunological components. Additionally, amino acids are used in the synthesis of myriad of nitrogenous compounds such as purines, pyrimidines, glucosamines and neurotransmitters. Approximately, a human requires 75 g/d to meet his protein requirements.

Plants and grains are the main protein sources of protein for humans. Food Agriculture Organization (FAO, 2003) reported that plant products supplied two third of total protein consumption in 2001, and animal products the remainder. The demand for protein has been increasing due to rapid growth of the human population. To meet protein demand, cropland and plant productivity has increased greatly in the last 150 years. Fertilizer N has played a central role in the increase of the productivity of cropland. Yet in the 1800's, it was recognized that productivity of cropland would have to be increased to

supply food for the growing population. Although, it had been recognized that nitrogen increased grain crop yields (Lawess, 1847; cited Slim, 2001), it was recognized that there was not enough biologically available nitrogen to increase the plant productivity. Thus, began the global search for a biologically available N to increase food production. In spite of its immense availability ($3.9 x 10^{15}$ Mt), it was known that plants could not utilize atmospheric N_2. Guano and nitrate were the first deposits of N found and applied to grow food; however those deposits were very limited (100,000 Mt yr-1). Years later it was discovered that some plants and bacteria could utilize atmospheric N_2 (biological fixation), and some N_2 is made available during thunderstorms by the electric lightings. To be biologically active, atmospheric N_2 is reduced to ammonia by nitrogen-fixing bacteria. In nature, ammonia is oxidized to nitrate (NO_3) by nitrifying bacteria. In turn, NO_3 is absorbed and utilized by higher plants. It is estimated that about $300 x 10^6$ Mt (Slim, 2001) of N are made available each year by nitrifying bacteria. However this N is not available to be applied directly to crops. It was not until the discovery of the process to synthesize ammonia from atmospheric N_2 in the laboratory that the limiting factor in the plant growth was broken. Since then, the use

of nitrogen fertilization has increased substantially. Actually, it is estimated that human production of fertilizer nitrogen is greater than nitrification processes that occur in the nature inside of continental areas. World consumption of nitrogen fertilizer reached 8 x10^6 of Mt to in 2000 (FAO, 2003). Of the total world's dietary protein N (21-25 x 10^6 of Mt yr^{-1}), 40% comes from the Harber-Bosch process (Slim, 2001). It is unquestionable that fertilizers have played an important role in increasing protein production for mankind. In China, using traditional practices can produce about 60- 80 kg of food, utilizing fertilizer the productive increases to 240 kg. It is estimated that the utilization rate of fertilizers will have been doubled in 2050 (Slim, 2001;).

Unfortunately, plants do not efficiently assimilate the N applied directly, increasing the amount of nitrogen that is lost to the environment. An average N-fertilizer uptake efficiency or N-fertilizer recovery achieves only 31% and 37 % of applied N for rice and maize crops, respectively (Cassman et al., 2002), so more than the 60% of N is lost to the environment. Galloway and Cowling (2002) estimated that of each 100 molecules of ammonia N synthesized by the Haber-Bosch process, 14 ammonia molecules reached the mouth of a vegetarian, and only four

molecules reached the mouth under a carnivore diet. The situation is worse in animal food production because of low efficiencies of animals to assimilate additional N (Slim, 2001; Table 1). The lowest recovery efficiency is for beef cattle (5%). This contrasts with the higher efficiency of dairy cow (Table 1). Although it seems a wastage to feed nitrogenous compounds to beef cattle, we have to remember that ruminants are the only livestock species that can utilize efficiently residue crops or by products to transform them into a highly quality protein food for humans.

Fertilizers have been the key for increasing the world production of food, and it is estimated that more fertilizer will be utilized to produce more food. However, its use alters the nitrogen cycle. The amount of N fixed in nature and in the Haber-Bosch process exceeds the capacity of biological denitrification, resulting in N accumulation in environment. Nitrogen promotes the accelerated growth of microorganisms that exhaust oxygen in waters, producing the phenomenon called eutrophication. Thus, nutritionist's challenge is to find the best way to increase the protein conversion efficiency, without affecting animal productivity.

Table 1. Protein conversion efficiencies in animal food production[1]

Item	Product					
	Milk	Carp	Eggs	Chicken	Pork	Beef
Feed (kg /kg of edible weight)	1.1	2.3	2.8	4.5	7.3	20
Protein content (% of edible weight)	3.5	18	13	20	14	15
Protein conversion efficiency (%)	30	20	30	20	10	5

[1] Slim (2001)

CHAPTER II

REVIEW OF LITERATURE

Seasonal changes in nutritional composition of pastures

Solar energy and photosynthesis

Almost all life on Earth depends on energy emitted by the sun. However only higher plants and algae have the capacity to capture the solar energy, and are known as photosynthetic organisms because they capture energy through a photochemical process called photosynthesis. In photosynthesis, solar energy (free energy) is trapped in chemical bonds during the reduction of carbon. About 10^{11} ton of CO_2 are fixed each year in the world. Then, the trapped energy is used to synthesize carbohydrates, amino acids or lipids by plants. For animals, solar energy contained in chemical bonds becomes useful after they ingest plants, algae or other animals. The solar energy contained on chemical bonds becomes useful during the oxidation process of carbohydrates, amino acids or lipids. An over-simplification of the photosynthesis process is:

$$CO_2 + H_2O \xrightarrow{\text{Light}} O_2 + C_6H_{12}O_6 \quad \Delta G=2840 \text{ kj/mol (free energy)}$$

The factors are: sunlight, water, and carbon fixation. The greater the carbon fixation, the more biomass is synthesized. In most cases, we do not have any control of these factors. However, we need to understand how they can affect plant growth, natural distribution and how plants react to the changes in their availability.

Solar energy and climate

If sunlight intensity, air temperature, rainfall, and soil composition were the same everywhere, natural vegetation and its biomass production would be the same. The plant community would consist of just a few species. Fortunately, there is a great diversity of plant communities each one adapted to diverse conditions.

Although the solar radiation that reaches the earth is relatively constant (1400 kwh / m^2), the earth's surface does not receive uniform amounts of this energy year around. Such variation in solar radiation is mainly due to the movement earth around of the sun and the tilt of earth's axis with respect to the sun and is perceived as the succession of seasons through the year (Aguado and Burt, 2001). Because of differences in solar radiation, gradients of air temperature are formed among regions, resulting in movement of atmospheric air. We perceive this

movement as the wind or changes in atmospheric pressure. The most important consequence of air movement is that the humidity generated mainly in the oceans can be taken into continental areas. When two air masses of different temperature meet, humidity condenses producing the meteorological phenomenon called rain.

Climate and grasslands

Temperature, rainfall, and distribution pattern of rainfall are the main factors that determine climate. In addition, it is known that natural distribution of vegetation is well correlated to climate (McCloud and Bula, 1985). One of the most important plant communities in the world is the grassland, which is about 25% of the ice-free land surface (Pearson, 1997). The grasslands are found in regions characterized by an annual rainfall between 250 and 750 mm, monthly temperature over 22°C, a high rate of evaporation, and seasonal and annual droughts. A grassland community is defined as a land where grasses (family *Gramineae*) are the predominant species in community and predominant plant growth or life form. Grasses are herbaceous plants that don't form woody tissue. Grasses have hollow, round stems and small, nondescript flowers.

Grasslands of Oklahoma

Four types of ranges can be recognized in Oklahoma (Harlan et al., 1940): 1) Eastern Prairie Tall grass region, 2) Savanna, 3) Mixed Prairie and 4) Steppe.

In eastern prairie, dominant species in tall grass region are: Big bluestem (*Andropogon gerardii*), sand bluestem (*Andropogon hallii*), Indian grass, switch grass, and little bluestem (*Schizachyrium scorpium*).

Savanna and Mixed prairie mixed regions (shortgrass region) are located west of the tall grass region and include blue grama (*Bouteloua gracilis*), buffalo grass (*Buchloë dactyloides*) and buffelgrass (*Cenchrus Ciliaris*), blue grama and wheatgrass.

Steppe grasslands are located in west area of Oklahoma and Panhandle area (Desert-Grassland Region). The dominant species are: black grama *(Boutelona eriopoda*) and sideoats grama (*Bouteloua curtipendula*).

Cool- and warm-season grasses

Based on blooming period or growth season, grasses have been divided in cool- and warm-season growth grasses.

Cool-season grasses (CSG) make their maximum growth and are the most important producers of forages in the spring and early summer (Massengale, 2000). Cool-season

grass species (various wheat grass, needle grass, brome grass, bluegrass) begin growth in early spring as soon as the soil is above freezing and daytime temperatures are favorable to growth. Cool-season grasses produce high-quality forage early in the growing season (Minson, 1990). However, they do not grow during the hot periods in midsummer, and often become semi-dormant. They may grow again in the fall as temperatures cool and late summer precipitation replenishes soil moisture. Thus, CSG can present two periods of growing: early spring and late fall. Buxton and Casler (1993) reported that optimal temperature for biomass production of cool season grasses is 12 to 30°C.

In contrast, warm season grasses (WSG) start their growth in spring, growing throughout the summer and into early fall. Biomass production is higher with WSG; however, quality forage is lower than CSG. Additionally, quality forage declined faster in WSG than in CSG, because temperature increases the lignin deposition. Warm-season (blue grama, buffalo grass, bluestems) grow during warmer periods when temperatures are 20 to 35°C. Warm-season grasses use soil moisture more efficiently than cool-season species and often can withstand drought conditions. Different growth habits and requirements of CSG and WSG

species can be used to extent availability of forages throughout year.

Biochemistry of C_3 and C_4 plants

The classification of grasses in WSG and CSG is based in metabolic pathway by which atmospheric CO_2 is fixed. Carbon dioxide is fixed in the compound ribulose 1,5 bisphosphate, a 5-carbon carbohydrate, which after CO_2 is fixed, splits in two molecules of three-carbon compound, 3-phosphoglycerate (C_3). The grasses that use this metabolic route are called C_3 (or cool-season grass). The CO_2 fixation is catalyzed by ribulose 1,5-bisphosphate carboxylase (Rubisco), which is the most abundant protein in biosphere. When temperature increases, Rubisco increases its oxygenase activity, which results in the loss of fixed CO_2, this phenomenon known as photorespiration. Photorespiration decreases net biomass formation (Lehninger et al., 1993). Grasses and legumes developed a mechanism that concentrates CO_2 to decrease photorespiration. In C_3 plants, CO_2 reaches carbon fixation site by simple diffusion through a specialized structure, called stomata. Although, pCO2 (330 ppm in atmosphere) favors photosynthetic CO_2 fixation over photorespiration, when temperature increases, more 0_2 enters into solution increasing the oxygenase

activity of Rubisco. As more CO_2 is dissolved, the affinity of Rubisco for CO_2 decreases. In contrast, carbon dioxide fixation begins with condensing CO_2 with phosphoenolpyruvate, producing oxaloacetate (OOA), which, in turn, is reduced by a NADPH to malate (C_4) in the mesophyll cells, which lack Rubsico. Then, malate is shuttled to bundle sheath cells. In the bundle-sheath cells malate is oxidatively decarboxylated by NADP+ to form pyruvate and release CO_2. Finally CO_2 enters to Calvin cycle. Plants that have this physiological mechanism are called C_4 plants.

Although, C_4 plants use two additional ATP per CO_2 fixed, they have an advantage: CO_2 concentrating mechanism lets C_4 plants keep their stomata close for longer, reducing the water losses. As consequence, C_4 species, such as blue grama, buffalo grass, bluestems, grow during hot and dried climate.

This characteristic explains geographical distribution of C_3 and C_4 plants. Plants with C_4 mechanism are general absent from grasslands in the north hemisphere at higher elevations, and in the Mediterranean climate regions. African grasslands are largely C_4 and Asian grasslands are largely C_3. While in North and South America, grasslands are a mix of both C_3 and C_4.

Plant tissues that impact nutritive value

Maturity is probably the main factor affecting nutritive value of forage, which is generally appreciated as the increase in fiber concentration (Buxton and Fales, 1994). Fiber is probably the most important factor affecting dry matter digestibility of growing animals (Buxton and Redfearn, 1997). The increase in fiber concentration in the plants is related to changes in the leaf/stem ratio and increases in proportion cell wall to cell content.

At the cell level, digestibility changes as proportion of cell content and cell wall fractions change. As the plant matures, cell walls become thicker and more lignified, decreasing cell content fractions. Stems mainly consist of phloem and xylem tissues, which are resistant to microbial attack and become lignified. For example, digestibility of alfalfa stem decreased by lignified of vascular tissue of xylem (Grabber, 2002). Usually, as plants mature, stems are less digestible compared to leaves and the leaf:stem ratio decreases, explaining the declination in nutritive quality of the plant.

Changes in chemical composition

During plant development, amino acids, carbohydrates are in constant movement from storage points in leaves and, roots to new growth points, or re-located in leaves to compensate the losses by defoliation (Manske, 1998). In consequence, the chemical composition of aerial parts of plants is changing throughout the year. At the beginning of growth season, photosynthetic resources, such as Rubisco enzyme, nitrogenous precursors, and glycosides are abundant in cell contents. In addition, proportion of cell walls, which are low in lignin at that moment, is small compared to cell content. On the other hand, cell walls are highly lignified and thick in dormant plants, with a minor proportion of cell contents. In dormant plants, the photosynthetic apparatus has been mobilized from leaf tissues to other tissues, decreasing crude protein concentration in vegetative aerial parts. Minson (1990) reported that mean rate of decrease in crude protein, as forages mature are 2.2 g/d/kg DM.

Season

As temperatures increase in spring, warm-season, perennial forages grasses begin to growth. Thus it was observed that diet quality improved during April

independent of supplements because environment conditions favored the beginning of growth for wheat-grass (Hess et al., 1994). After growing season has begun, crude protein and IVOMD of smooth brome and big bluestem pastures grazed by steers from March to July decreased, but escape protein content increased (Blasi et al., 1991). It is well known that the amount of Rubisco in a fully expanded leaf may account for at least 50% of the total soluble protein (Mangan, 1982). As the plant cells mature, photosynthetic capacity decreases because less Rubisco is present, so this can explained why escape protein increases with the advances of maturity. However, Karn (2000)found that protein supplementation (soybean meal) had little effect on weight gains or forage intake of yearling steers grazing native rangeland from June to September because pasture showed adequate N concentration during those months. Although, degradable protein content of native range can be adequate during summer, microbial protein synthesis may be insufficient to satisfy the metabolizable protein requirements (Karges et al., 1992). In spite of the excess of nitrogen, animals respond only to degradable and undegradable protein supplements, but not to energy supplements (Hafley et al., 1993).

Johnson et al., (1998) determined that crude protein and IVDMO of mixed-grass prairie in the Northern Great Plains declined from June to November. No additional change was observed between November and December. The IVOMD declined faster than CP during June to November, but small changes were observed between September and December. In contrast, escape protein and soluble protein increased as plant became dormant. Similarity, Park et al., (1994) reported that total N and available N in masticate samples of wheat grass decreased from May through November. In contrast, bound nitrogen content of masticate samples for September increased by almost 5 percentage units (4.9 to 9.3 % of total N) with respect to previous months. A higher increase of bound nitrogen was detected between September and November (Park et al., 1994). In winter native sandhills grazed by gestating beef cows, CP and degradable intake crude protein decreased in December compared to November. No more changes were observed in January and February, but IVOMD (56%) did change during study period (Hollingsworth-Jenkins et al., 1996). In contrast, McCracken et al., (1993) reported that total N and available N contents of masticate samples of un-irrigated, endophyte-free tall fescue from April to December were not affected by maturity forage, but they

detected that bound N to cell wall increased through grazing period. In vitro OM disapperance (IVOMD) decreased significantly in September, but IVMOD did not significantly change, al least, until November. Although, protein supplementation increased forage intake of intermediate wheat grass range during February to April, supplementation did not affect diet quality (Hess et al., 1994). Data suggest that animals selected their food, resulting in a better composition than biomass in range or pastures; however as season advances, changes in composition of grasses can be affected chemical composition of what animals are ingesting.

Sampling method

In grazing research one of main concerns is to obtain representative samples of what animals are consuming. The main sampling methods include hand-clipped method or animals fitted with esophageal or ruminal cannula.

Compared with hand-clipped method, esophageal extrusa of animals grazing have showed higher CP and *in situ* DM digestibility, and lower fiber content compared with samples collected by hand-clipped method (Coleman and Bart 1997; Dubbs et al., 2003). Masticate samples have been utilized to determine changes in diet quality by

supplementation, season, management, stocking rate, pastures, etc.

Physiological control of voluntary intake

Eating behavior

Voluntary intake more than being a reflex that is induced peripherally is an emergent property of the central nervous systems (CNS). It is frequently forgotten that stimuli or mechanisms that initiate feeding behavior have their origin in CNS. The CNS integrates many afferent signals coming from receptors in gastrointestinal organs, storage tissues, liver, kidney and, chemical compounds that have their origin in peripheral organs. From a mechanistic point of view, the sum of stimulating and inhibiting signals determines the type of response that CNS will trigger, stopping or beginning the eating behavior (Klemm, 1993).

Hypothalamus

Traditionally, hypothalamus has been considered to be the physical place for the integration of stimulating and inhibiting signals. When bilateral lesions were produced on lateral areas of hypothalamus of rats, rats developed aphagia and lost weight. In contrast, when the ventromedial nuclei of hypothalamus were damaged, rats

became hyperphagia and developed obesity. Early experiments interpreted these effects as presence of two hypothalamic centers controlling satiety and eating (Williams et al., 2000). It is now clear that the control of feeding behavior is more complex. There have been discovered a multiple of factors that initiate to begin or to stop eating activity. A numbered of functional aspects of hypothalamus concerning to the control of feeding behavior has been described: satiety information generated during meal intake is largely conveyed to the hindbrain by means of afferent fibers of the vagus nerve from the upper gastrointestinal tract. The signals from these receptors are neural while other products of digestion stimulate the release of humoral agents, such as cholecystokinin, which may act locally, in the liver, or on the central nervous system. For example, proopiomelanocortin neurons are actived by leptin to liberate alfa-MSN that inhibits neurons expressing melanin concentrating hormone (MCH) and orexins, peptides that are know that stimulate appetite (Cupples, 2003). Another example (Blevins et al., 2002) involves the peptide cholecystokinin (CCK), which is released from the gut following ingestion of a meal. Since it was discovered that glucose and free fatty acids had a role in controlling feed intake, the list of chemical

compounds that influences food intake has grown enormously. Some of the most important are: Insulin, Growth hormone, Estrogenic compound, Propionates, Lactate, CCK, Opiates, etc (NRC, 1987). In spite of the intensive research, feed intake control is still not well understood, however, physiological integration in hypothalamus is now better understood.

Ruminal distension

Ruminal distension is the more probable dominant factor in control of feed intake in animals fed low quality forages (Forbes, 1996). In the evolutionary process, grass-selector ruminants developed large and complex set of stomachs to maximize ruminal fermentation (Van Soest, 1994). However, because fibrous material is retained for more time in rumen, the increment on the amount of energy extracted per unit of mass conveys an "extra pay", a reduction in forage intake (Fisher, 2000). Mean retention times vary from 16 to 50 h (Owens and Goetsch, 1988). Mean retention times are longer in cattle than sheep or goats (Van Soest, 1994). It is clear that ruminant can manage great amounts of feed. Mature cattle fed high quality hay can accommodate 39 to 84 kg in its reticulum-rumen. . However, quantity of material that the rumen can load is

controlled by mechano-receptors located in rumen wall (Iggo, 1956). The functions of receptors are to prevent excessive distension of the viscera and to initiate the reflexes of saliva secretion, rumination, eructation and reticulum contractions. The receptors are found on the region of the reticular wall adjacent to reticular groove and the wall of the cranial ruminal sac (Grovum, 1987). These distension sensitive receptors stimulated generate satiety signals that are carried by vagus nerves to hypothalamus to the control of voluntary food intake (Forbes and Barrio, 1992). Even though, the ruminant can manage great amount of fiber in rumen, when fed low quality forage, physical capacity of rumen limits the forage intake (Campling and Baile, 1961; Grovum, 1987).

Therefore, if rumnal distension is alleviated, voluntary intake can be increased. For example, reducing particle size by grinding and palletizing forage, Minson (1990) reported that sheep and cattle consumed more forage, 41 and 23 %, respectively. The effect of particle size is smaller when high quality forages are pelleted. Possibly ruminal distension is lower with high quality forages. Mambrini and Peyraud (1997) compared fecal excretion of dairy cows fed perennial ryegrass hay, long or ground, and concentrate-based diets. The analysis of the excretion

curve showed that small concentrate particles passed the gastro intestinal tract faster than the particles of long or ground hays. In turn, particles of ground hay passed the rumen faster than the particles of long or ground hays. Reduction of particle size of long hay particles however was slower as shown by the declining phase of the fecal marker curve. This faster passage has been associated to higher roughage intake. Comparatively, in horse and pigs, the colon and ceccum are well developed to digest fibrous material, however transit time is still faster than that in ruminant species (Van Soest, 1994). These animals therefore can eat more forage than ruminant, but sacrifice digestibility.

Nitrogen deficiency in low quality roughages

Low quality forages

Corn stover, wheat hay, and oat hay are good examples of low quality roughages. They are characterized by a low energy digestibility and protein content. The low digestibility of forages is a result of low cell content and highly lignified cell walls. Van Soest (1994) reported that digestibility and lignin content are related, but intake and digestibility are less correlated. Highly

lignified cell walls are a determinant of forage intake because the more lignified cell wall, the more NDF content of forage and the more bulkiness. Osbourn et al., (1974) found that 6-h *in vitro* digestion residue was more correlated to intake than to longer incubation times. In contrast, after incubating for 1 h, very little wall matter has disappeared. They concluded that short incubation times were correlated to bulkiness and longer incubation to digestibility. Bulkiness of forage is related to cell walls, measured as NDF and lignin content (Van Soest, 1994). It is known that animals ruminate in proportion to cell wall content of the forage (Welch and Smith, 1969; Beauchemin, 1991). This is important because rumination is the most important factor in reduction of particle size (Kennedy, 1985). Thus, as volume of forage is reduced, the animal can accommodate more food in its rumen. Ruminal distension is surely one of the most important factor controlling voluntary intake in animals consuming low quality forages (Baile and Forbes, 1974; Allen, 1996).

In most low quality forages, low protein content is the main factor that limits forage intake. Large amounts of data have shown that forage intake is increased by protein supplementation (Neutze et al., 1986; Tillman and Sidhu, 1965; Garza et al., 1992; Bandik et al., 2001;

Köster et al., 1996). The increase in forage intake can be related to a higher microbial activity, contributing to increase cell wall degradation (Murphy and Nicoletti, 1984), and reducing rumination time (Campling and Freer, 1961). Most researchers pointed out that forage intake is limited when forages contained around 6 to 8 % CP (Minson, 1990; Van Soest, 1994). However, it is not clear how protein supplementation increases forage intake or why animals do not always respond to protein supplementation.

Several authors (Jordon et al., 2002, Freeman et al., 1992 and, McCollum and Horn, 1990) have reported that protein supplementation can improve animal performance through the following mechanisms: 1) Ruminal level: Increasing rate and extent of digestion and increasing energy ingestion; 2) Intestinal: Increasing metabolizable protein, and 3) Metabolic: Correcting amino acid deficiency or imbalances. Similarly, Van Soest (1994); describes three possible stages: 1) the nitrogen content is inadequate for microbes and animals: exogenous NNP can be beneficial for bacteria and animals, but adding starch or sugar to diet it can be detrimental reducing more forage intake 2) the nitrogen content is in excess for microbes and animals; exogenous NNP is not beneficial for bacteria and animals, but adding starch, sugar or protected protein

improves animal performance and 3) Intermediate level of nitrogen content, but inadequate level for higher requirement (for example for milk production): exogenous NPN can be beneficial for bacteria production; adding energy improves animal performance; exogenous NPN is detrimental for animal; positive animal responses to protected protein; adding energy, the recycling of urea is increased.

Ruminal ammonia

Crude protein content in native pastures is frequently below than 6 % of DM, resulting in low ruminal ammonia concentration (Sunvold et al., 1991; Freeman et al., 1992; Lintzenich et al., 1995; Köster et al., 1996; Bandyk et al., 2001). The ammonia concentrations were below of 5 mg NH_3-N/dl, which is considered as the adequate minimum for microbial growth (Satter and Slyter, 1974). Many studies have demonstrated that nitrogen supplementation to animals consuming low quality forage results in increases of forage intake (Campling and Freer, 1961; Garza et al., 1992; Bandyk et al., 2001) and digestibility (Scott and Hibberd, 1990). However, Freeman et al. (1992) and Jordon et al. (2002) did not observe any increase in forage intake. In both studies, forages were probably only marginally

It has been postulated that synchronization of degradation rate of protein and energy source increases N utilization by bacteria, resulting in lower ammonia accumulation (Firkins, 1992 Sinclair et al., 1993). Sinclair et al. (2000) found that plasma ammonia was lower when nitrogen release matched energy release rate, independent of release rate of the carbohydrate source. Nitrogen recycling back to gastro intestinal can be considered as a synchronization method because N is made available for ruminal fermentation hours or days later after having been ingested. When urea was infused every other day, steers had similar microbial production than those infused daily (Garza et al. 1992; Henning et al. 1993) reached a similar conclusion when microbial production was not improved by the synchronization of release rate of nitrogen and energy sources. Kim at al. (1999) found that sucrose infused in rumen increased microbial protein synthesis in cattle consuming grass silage; however, synchronization of sucrose infusion and feeding time for silage did not improve the microbial synthesis. In lactating cows, the substitution of dry rolled grain with extruded corn grain decreased ruminal ammonia and plasma urea concentration (Shabi et al., 1998).

Similarity, Köster et al. (1997) did not detect effect of substitution of soybean meal by urea on duodenal microbial protein, non-ammonia nitrogen or on microbial efficiency in the rumen of steers fed tall grass prairie hay.

Energy for microbial growth

Energy is probably the main factor that determines ammonia concentration in the rumen. As energy availability increases in the rumen, more bacteria grow, resulting in a greater microbial nitrogen uptake (Satter, 1980). Roffler and Satter (1975) reported that ration CP and total digestible nutrient (TDN) explained 92% of variation in mean ruminal ammonia concentration in cattle in 35 trials. In a batch culture study, Bach et al. (1999) determined that energy supplements (beet pulp, molasses, cracked corn and soybean hulls) increased nitrogen utilization by bacteria fed with a mixture of high quality grass and legume pasture (50:50 ratio). This was a result of decrease ammonia concentration in media and an increase in non-ammonia nitrogen flow as a percentage of nitrogen supply. Obara and Dellow (1993) found that addition of sucrose to rumen of sheep fed lucerne hay, decreased ruminal ammonia, plasma urea and urinary excretion of urea.

nitrogen deficient, which possibly decreased the responses to protein supplementation. Supporting this, Köster et al. (1996) reported that responses to protein supplementation decreases when crude protein of forage is near 6 % DM. However, there are reports that forage intake increased when CP was over 6% DM (McCollum and Galyean, 1985). It has been suggested that the protein/energy ratio can affect responses to N supplementation. The NRC (1976) reported that urea supplementation had positive effects on forage intake and/or animal performance, when dietary protein constituted about 12% of TDN. Also, it is likely that animal responses can be affected by factors than other CP level. The amount of ammonia that can be utilized by bacteria depends on the number of bacteria and how rapidly they are growing, which, in turn, is determined by the amount of fermentable energy available in the rumen (Satter, 1980). The urea fermentation potential system recognized that efficiency in urea N utilization depends on TDN and protein degradation (Burroughs et al., 1975). Although, Kropp et al. (1977) could not detect differences in microbial production when soybean was substituted by different levels of urea in diets with positive urea fermentation potential. Additionally, total digestion of organic matter was affected but not ruminal digestion.

Protein nitrogen sources

When mixed ruminal bacteria were incubated with a pancreatic casein hydrolysate and free amino acids of a similar composition, rates of ammonia production were much greater for peptides than for amino acids. The pancreatic digest of casein was then fractionated with 90% isopropyl alcohol. Hydrophobic peptides, which dissolved in alcohol, contained an abundance of phenolic and aliphatic amino acids, while the hydrophilic peptides, which were precipitated by alcohol, contained a large proportion of the highly charged amino acids. The Km values of the mixed ruminal bacteria for each fraction were similar (0.88 versus 0.98 g/liter), but the Vmax of the hydrophilic peptides was more than twice that of the hydrophobic peptides (18 versus 39 mg of NH_3 per g of bacterial protein per h). Pure cultures of ruminal bacteria had a similar preference for hydrophilic peptides and likewise utilized peptides at a faster rate than free amino acids. Since peptide degradation rates differed greatly, hydrophobicity is likely to influence the composition of amino acids passing unfermented to the lower gut of ruminant animals.

Nitrogen intake

Ammonia in excess of nitrogen needs of rumen bacteria is absorbed by rumen wall, and then, it enters the portal vein. Portal circulation takes ammonia to liver where it transformed to urea. Ammonia also leaves the rumen in digesta flowing into duodenum. Later, this ammonia will be also absorbed into portal circulation and taken to liver. Huntington and Archibeque (1999) demonstrated that nitrogen intake and absorbed nitrogen as ammonia through portal-drained viscera had a high correlation (0.88). Varying nitrogen intake from 34 to 380 and 12 to 25 g N/d for cattle and sheep, respectively, in cattle, ammonia N absorbed ranged from 20 to 157 g of N/, while in sheep, from 4 to 25 g N/d. Minson (1990) summarized the effects of several forages on the ruminal absorption of ammonia N, confirming that nitrogen absorbed was related positively (r=0.74) to nitrogen content in forage. From this data, it can be concluded that when forage CP content is over 137 g CP / kg, net loss (ammonia N absorption) of nitrogen become positive. However, this is only a general guide, suggesting that ammonia N loss become important when diet contains more than 13.7% CP. Roffler and Satter (1975) regressed ruminal ammonia concentration over dietary CP

concentration and found that they are related quadratically: $y=10.57-2.50x+0.159x^2$, ($R^2=0.95$).

Because NPN rapidly increases ruminal ammonia concentration, it is expected more ammonia be absorbed, and in turn increased urinary N excretion. However, ammonia levels in rumen are also increased by protein sources feeding and by feeding levels. In cows ded native grass hay, supplements based soybean meal produced higher ammonia levels than supplements based blood meal and corn gluten meal (Scott and Hibberd, 1990). Similarity, increasing the amount of a protein supplement increased rumen ammonia (Scott and Hibberd, 1990). So it is expected that urinary nitrogen excretion also increase.

Relationship between plasma urea and N excretion

Increased loss of nitrogen through urine and feces as a result of feeding excess N resulting in low efficiencies in N utilization and could pose environmental effects. Jonker et al. (2002) reported that farmers fed 6.6% more N than recommended by the NRC (2001), resulting in a 16% increase in urinary N and a 2.7% increase in fecal N over the expected by using values recommended by NRC (1989). Huntington and Huntington and Archibeque (1986 and 1999)

reviewed several studies in beef steers and found that more N intake and urinary N excretion are related positively.

Normally ammonia, urea, allantoin, creatinine, and, amino acids are nitrogenous compounds excreted in urine. The proportion of these compounds is not constant, depending on N status of animals, dietary CP concentration, etc. Elliot and Troops (1964) found that distribution of nitrogenous compounds in urine changes as nitrogen intake increased. While urinary urea increased with the increases in nitrogen intake, ammonia concentration decreased to very low levels. At standard conditions of feeding, urinary nitrogen is constituted mainly by urea N. Bristow et al. (1992) reported that dairy cattle excreted 3.8 to 21 g N/d, of what urea accounted for 68%. In studies with NPN, urinary nitrogen excretion is higher, even when NPN was infused continuously (Thornton, 1970; Owens et al., 1973). It is clear that as nitrogen intake increases, ammonia absorption goes up too. Based on data from ^{15}N-tracer methodology in sheep, Parker and et al. (1995) showed clearly this relationship between N intake and ruminal N absorption. Several studies showed that ruminal ammonia concentrations are related positively to blood urea levels (Thornton , 1972 Huntington and Archibeque, 1999).

Unfortunately, there is a positive relationship between plasma urea and urinary excretion. Thornton and Wilson (1972) found that above 10 mg / ml plasma urea nitrogen, urinary urea nitrogen excretion was well correlated. When blood urea N (BUN) is lower than 10 mg/100ml, BUN is retained, decreasing urinary nitrogen excretion. Ford and Milligan (1970) observed that within the physiological range (up 21 mg urea N/100 ml blood), recycled urea and plasma urea concentration fit a linear function. That tempts one to think that kidney play can play a role in N recycling. Some researchers (Marini and Amburgh, 2003) support that kidney has a role in nitrogen conservation. However, it is frequently forgotten that urea has a role in water conservation in kidney, for which is retained in kidney medulla. Similarity, Kenny et al. (2001) found a similar relationship (R^2=0.95) plasma urea and pasture crude protein concentration (from 10% to 35% CP) in dairy cows grazing *Lolium perenne* fertilized or not.

<u>Animal response to protein supplementation</u>

The most common response to protein supplementation in ruminants given low quality forage or grazing dormant pastures is the increase in forage intake (Romero et al., 1976; Scott and Hibberd, 1990; Garza et al., 1992; Bandyk

et al., 2001; McCollum and Galyean, 1985). This results in a higher energy intake, even if digestion of forage does not change. Data suggest that energy available to animal is significantly increased by nitrogen supplementation. Egan (1965) suggested that intake of low quality forage could be limited because energy utilization is affected by a low concentration of gluconeogenic precursors. Nitrogen supplementation improves efficiency of energy utilization by increasing the flow of amino acid of microbial or dietary origin. Also, animals respond by increasing their forage intake when NPN sources are given. Romero et al. (1976) supplemented urea (50 g/d) in different ways to steers consuming spear grass (2.3% CP). Brahman x Shorthorn steers (220 kg BW) increased their forage intake by 27 %, but the greater response in forage intake was when urea was fed more uniformly, twice a day or sprayed on forage versus every other day.

It is generally recognized that proteins are degraded to different extents in the rumen. Soybean meal is considered to be extensively degraded in rumen, releasing amino acids and peptides into ruminal fluid; In contrast, fish meal or dried brewer grains are little affected by microbial activity in the rumen. Bandyk et al. (2001) found that Angus x Hereford steers fed tall grass prairie

hay (3.6% CP and 76 5 % NDF) increased their forage intake when sodium caseinate was infused in rumen as degradable intake protein (DIP) or in abomasum as undegradable intake protein (UIP). Forage intake increased to a lesser extent when casein was infused in the abomasum. The authors suggested that differences in forage intake were related positively to ruminal NH_3 concentrations. However, animals do not always responses to DIP supplementation by increasing forage intake. Steers fed brome grass (5.9 % CP) did not increase their forage intake by supplementing DIP, but total digestible organic matter intake was increased. No differences in forage intake were detected when forage intake when 600 g of cottonseed meal were offered to beef steers consuming western wheat grass (Freeman et al., 1992; Mathis et al., 2000). It is clear that usually protein supplementation increases forage intake. The lack of an intake responses to protein supplementation in some situations suggests that others factors are involved.

It has been discussed that rumen distension could be alleviated by a faster passage of rumen digesta. Ruminal digestion affects rate of passage but also depends on the absorption rate. A low activity of rumen bacteria by the deficiency of nitrogen could delay the digestion process.

Smith (1979) concluded that microbial protein synthesis in general was the factor most likely to limit maximum microbial growth rate. In low quality forage, nitrogen deficiency surely limits protein microbial synthesis. Scott and Hibberd (1990) reported that in non-lactating cows degradable intake protein linearly increased digestion of organic matter of native grass hay. Mathis et al. (1999) also reported that protein supplementation to steers consuming tall grass prairie hay (5.3% CP and 64.4% NDF) increased ruminal and total digestibilities of organic matter. Substitution of soybean meal with urea reduced OM and NDF digestion, mainly when urea was the only DIP source. Minson, 1990 and Köster et al., 1997 summarized the effect of protein or urea-based supplements in protein deficient forages. Increases in voluntary intake in response to protein supplements varied from 14 to 77% (average 40%) over forage intake of controls. On the other hand, the responses to urea supplements varied from 8 to 104% over forage intake of control, on the average 34% over the control.

Unless it can be re-utilized, all ammonia absorbed from rumen can be considered a waste for animal economy. Nolan and Leng, (1974) considered that ammonia converted to

urea represented the major nutritional inefficiency in ruminant animals.

Efficiency in dietary protein utilization

Although amino acids serve in the biosynthesis of many important substances for diverse physiological functions, the main role of amino acids is to serve as building blocks in protein synthesis. Thus most of the total nitrogen requirement of animals are amino acids (McDonald et al., 1996). MacRae (1997) reported that 62 % of total net accretion occurs in the saleable tissues in ruminants. In dairy cattle, just milk protein synthesis represented, on average, 28% of protein intake (NRC, 2001) or 40% of the total of nitrogen absorbed (Smith, 1980). It has been accepted that the maximum efficiency of incorporation of absorbed protein could be reached when absorbed amino acid match the AA profile of protein synthesized, reducing AA catabolism at a minimum. The use of ideal protein is based on this concept. The efficiency in protein deposition has been increased greatly; for example, until 87% of absorbed amino acids were retained when diets were formulated in relation to the AA profile of muscle (Chung and Baker, 1992). In contrast, the efficiency in the corporal incorporation of dietary protein is low in the ruminant

varying from 0-35% (Lobley, 1996). However, efficiency of absorbed amino acids is as good as that observed on pigs, varying from 40 to 80% (Lobley, 1996; MacRae, 1997). Several factors can explain the lower protein efficiency of ruminants: 1) Absorbed ammonia in rumen can constitute until 50% of ingested N (Lobley, 1992), 2) the constancy of the amino acid composition of rumen microbial protein (Chalupa, 1972), and 3) high basal N requirements of the ruminants (Inkster et al., 1989). That means that ruminants could reach efficiencies as good as the efficiencies of non-ruminants if dietary protein utilization is improved. As in non-ruminants, protein synthesis and protein degradation in ruminants increase in response to protein supplementation (Wessels et al., 1997), resulting in net deposition because synthesis is greater than degradation rate. In addition, amino acid catabolism is also increased, resulting in a greater urea production. Because nitrogen atoms of urea comes from AA catabolism and/or ammonia produced in gastro intestinal tract, in general a strategy would be to decrease absorption of urea by reducing dietary degradation or increasing ammonia uptake and reducing AA catabolism in organism (Lapierre and Lobley, 2001). In addition to ammonia produced by the microbial flora in gastrointestinal tract, amino acid

catabolism is also an important nitrogen source for urea synthesis in the ruminants. Rumen ammonia concentration depends on microbial degradation rate of endogenous and dietary protein, ammonia absorption rate, and the rate of uptake of ammonia by rumen microbes. Any ammonia accumulation in the rumen suggests that ammonia is being released in excess of what microbial ammonia can utilize. It is very important to understand factors that determine ammonia concentration in the rumen.

Control of urea production

Regulation of urea production

Functions of the urea cycle are: 1) prevent the accumulation of the ammonia, a toxic nitrogenous compound and 2) *de novo* synthesis of arginine (Meijer et al., 1990) and, possibly 3) the disposal of bicarbonate for pH homeostasis. However there are opposite points of view over the last function (Walser, 1983; Atkinson, 1992).

In 1932, Hans Krebs and Kurt Henseleit found that three compounds, citrulline, ornithine and arginine stimulated urea production from ammonia, more than any other nitrogenous compound and that these compounds shared a similarity in their chemical structure. This suggested

that they could be related in a sequence, which was later found to be urea cycle (Lehninger et al., 1993).

Amino acids undergo oxidative degradation in three different metabolic circumstances: 1) Protein turnover: normal process during synthesis and degradation of body protein 2) When the ingestion of amino acids is in excess of body's needs for protein synthesis and 3) Ingestion of diet that is rich in proteins.

A starting point for the analysis of nutritional effects on urea synthesis is that equal amounts (stoichiometric) of aspartate and carbamoylphosphate are required for urea synthesis (Krebs at al, 1973). During normal conditions for human and non-ruminant species, surplus nitrogen that is going to be converted into urea becomes available primarily either as NH4+ or as glutamate. Primary sources of NH4+ are the amide groups of glutamine and asparagine (Krebs et al., 1973;) and guanine and the reactions of degradation of serine, threonine, histidine, glycine, and methionine. Some ammonia comes from bacterial degradation in the colon and can account for up to 20% of urea formed by liver. On the other hand, glutamate comes mainly from intestinal digestion, and transamination reactions between alfa-ketoglutarate and the majority of the amino acids (alanine, aspartate, ornithine, lysine,

leucine, isoleucine, valine, tyrosine, phenylalanine, proline, histidine, glutamine, and arginine). Krebs (1973) calculated that more glutamate N is formed than ammonia N on the degradation of bovine alfa-casein. (182 moles of glutamate by 69 moles of ammonia N per mol of casein degraded). In this situation the ammonia must be formed from the glutamate dehydrogenase. In contrast, when ammonia is the sole source of nitrogen, the proportions of glutamate and ammonia changes drastically; however, urea nitrogen comes completely from ammonia. When alanine was added, eventually all alanine N appeared in urea, but alanine N passed through the stage of glutamate. Glutamate dehydrogenase equilibrium favors the formation of glutamate, helping to keep a low ammonia concentration. Glutamate dehydrogenase is inhibited by GTP and activate by ADP in vitro. However, in vivo studies indicate that enzyme works close to equilibrium, so the flow is controlled more concentration of substrates and products.

Nitrogen recycling determination

Considering that plasma urea and urea excretion are well correlated, production of urea could be calculated measuring total urine production times urea concentration in urine. However, this method underestimates urea

production because it does not account for urea that is recycled into gastro-intestinal tract.

Empirical estimates of N recycling can be obtained from duodenal flow. Minson, 1990 and Egan (1974) summarized duodenal nitrogen flow of ruminants receiving forage differing on protein content and found that when diet crude protein content is lower than 12%, it is possible detect a net nitrogen movement into rumen. Roffler and Satter (1975) mentioned that 10 to 15 % of dietary nitrogen intake was recycled to tract gastro-intestinal tract and concluded that the influence of nitrogen recycled could be small on contribution of ammonia concentration. Duodenal nitrogen flow changes depending on degradability of protein supplements, being higher for those supplements with low degradability protein sources (Coomer et al., 1993).

Empirical equations to re-entry of N in rumen

Because the determination of urea recycling is not an easy task, some empirical formulas have been developed from tracer studies or from portal-drained viscera (PDV) studies.

Several equations can be found to estimate N recycling form nitrogen intake or BUN (Ford and Milligan, 1970):

$$Y = 0.1117 + 0.37X \quad (s.e. = 2.27 \; r=0.85)$$

Where:

Y= g urea recycled per day

X= plasma urea concentration (up to 45mg urea / 100 ml).

From portal-drained viscera (PDV) studies, it was demonstrated that gastro-intestinal tract removes blood urea and more urea is removed as BUN increases (Lobley, 1988).

Methods to measure urea kinetics

There has been a lot studies where the changes in the plasma concentrations, urinary urea production, as well as changes in ruminal concentrations are described, however it is not possible to draw conclusions regarding of nitrogen release or uptake rates. Changes in concentrations of urea can give some information about ability of microbes or cells to uptake the substrate, but it does not give any information about the absolute rate of either release or uptake, which can differ at different concentrations of substrate (Wolfe et al., 1974). To measure in vivo the release and uptake rates, two general techniques are available: 1) Veno-arterial differences (AV) and 2) labeled urea approaches (Wolfe, 1974; Lapierre and Lobley, 2001).

Artery-venous differences studies (AV) have been utilized to determine net uptake or release by measuring arterio-venous difference of a substrate across an organ and by determining blood flow. The main disadvantages of AV studies are: 1) Major surgical intervention required, 2) Measurements of blood flow are variable 3) The differences in the arterial and venous concentrations of substrate can be smaller than variations by detection levels and, 4) It does not give information about metabolic source and fate of urea N (Lapierre and Lobley, 2001).

Methods to estimate protein degradation

In vivo method

Evaluation of forage protein in ruminant nutrition includes the partition of dietary protein in a fraction that is utilized by rumen bacteria, degradable intake protein, and a fraction that resists microbial degradation, undegradable intake protein (Broderick, 1994), which most of protein feeding systems are based (NRC, 1996; Waldo and Goering, 1979). Ideally, determination of protein degradability should carry out using *in vivo* trials where plant factors and animals interact. However, *in vivo* trials are expensive and time-consuming, because they require of animals fitted with cannulae in rumen and

duodenum, trained personal, and considerable amount of laboratory work (Broderick, 1994).

In vitro and situ methods

There has been an extensively research to find a single method that provides rapid and reliable estimates of protein degradation (Stern and Satter, 1984). Although, in early studies (Wohlt et al., 1976; Krisnamoorthy et al., 1982) reported that protein solubility in solvents could be a good estimate of ruminal degradation, however, Stern and Satter (1984) reported that solubility and ruminal degradation are poorly correlated (7% of variation was explained by solubility). Solubility depends on solvents (Waldo and Goering, 1979; Krishnamoorthy et al., 1982), protein composition, pH and ionic strong of solution (Owens and Goetsch, 1993). It is little probable that a single solvent simulates conditions of ruminal fluid. Thus, it has been developed methods that try to simulate the rumen environment: the methods that try to simulate the conditions of rumen fluid: two stage *in vitro* method (Tilley and Terry, 1993) and *in situ* procedure (McDonald, 1981). *In vitro* method has been widely utilized in the analysis of forages; its values and *in vivo* values have showed a strong statistical correlation (Weiss, 1994). In

contrast, the results form *in situ* method have been variable, mainly because the lack of standardization in those factors affecting, pore size, samples characteristics, location where insert bags, raising technique (Weiss, 1994). However, Broderick (1994) considered that *in situ* values are biologically meaningful. However, *in situ* and *in vitro* methods require of fistulated animal and trial are time-consuming. Proteins are degraded in rumen by proteolytic enzymes. Luchini et al. (1996) isolated and preserved proteolytic enzymes to determination of protein degradation. However, proteolytic activity of rumen fluid varies with type of diets (Falconer and Wallace, 2000). Enzymatic methods, such as protein degradability by *Streptomyces griseus* protease (Roe et al. 1991), have become more widespread because of good correlation with ruminal degradation (Krishnamoorthy et al. 1982), low cost, easy to standardization, and no requirement of fistulated animals. Although, there are many approaches to make degradation protein estimation easier and faster, many studies lack of *in vivo* data to support or disapprove the new approaches.

Summary review of literature

Nitrogen metabolism in ruminants is linked to changes in CP content of forages. Plants with a defined growth season have great variation in its N content. In the growing phase they accumulates nutrients in leaves, stem or roots or when they defoliated, mobilizes their resource to increase their photosynthetic capacity to compensate the loses of resources. As plant matures, digestibility of plant decreases as consequences of increment of cell wall lignified and less cell content. After plants complete their reproductive cycle, they enter in a senescence period, which let them survive to low temperature. Carbohydrates and amino acids have been mobilized to roots leaving fibrous material very small nitrogen that possible is mainly indigestible (protein incrusted in lignified cell wall. Several studies have showed the low CP content of masticate samples for winter months. So it is very important to nutritionist to know when animals could not get sufficient nutrients to rumen digestion or for them. Although, *in vivo* trials should the best way to do it, it is expensive and variations due to actual techniques limited its use as standard values, researchers have looked alternative methods to estimate *in vivo* values. Although many methods works well ranked on basis of protein

degradation, they have not compared to *in vivo* values. In forage-based productive systems is essential to route that N follow after animals ingest their ration of pasture.

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CHAPTER III

NUTRITIVE VALUE OF COMMONLY GRAZED FORAGES BY BEEF CATTLE IN OKLAHOMA ACROSS THE CALENDAR YEAR

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ABSTRACT: Monthly forage samples were collected from Old World bluestem (OWB), midgrass (MGP), and tall grass (TGP) prairie, by fistulated steers over 5 yr to determine the effects of year, month, and forage on changes in crude protein content (CP), degradable and DIP (as a percentage of total CP), and *in vitro* organic matter digestibility (IVOMD). Additionally, the ratio (DIPrt) of DIP supplied by forage to theoretical DIP requirement for rumen bacteria was calculated. The DIPrt was calculated as the DIP supplied divided by IVOMD*microbial growth efficiency (MOEFF), assuming that DIP requirement was 11.5 % of IVOMD. Data were analyzed with a model that included month, forages, and year, using GLM procedures of SAS and prediction equations were generated with proc REG. No interaction between year and month was noted in regards to

forage type. Old world bluestem tended to be higher in CP from May to July and in DIP during fall and winter compared to both native grasses. Overall, OWB was higher ($P < 0.05$) in CP, DIP and IVOMD than TGP; MGP was similar ($P > 0.05$) to OWB and TG in CP and IVOMD. Content of DIP was similar among pastures ($P > 0.05$). Compared to OWB and TGP, MGP was lower ($P < 0.05$) for DIPrt, but OWB and TGP did not differ ($P > 0.05$). With regards to the month effect, CP content was highest ($P < 0.05$) in April and May, then, slowly declined through December. From June to July, no differences were detected in CP, then it declined from August to December. Crude protein did not change during December to February. Crude protein concentration began ($P < 0.05$) to increase in March for all pastures. Degradable intake protein was highest in masticate samples in April and May, after that few changes were detected. The IVOMD for the period April to October was higher ($P < 0.05$) than for November to March. In regards to the DIPrt, pasture masticate samples DIPrt were greater than one, meaning that DIP supplied by forage was in excess relating to energy during April and May. In contrast, DIPrt for native grasses and OWB pastures was less than one after May and July, respectively. Year, month and forage type were the most important effects to predict changes in CP, DIP, IVOMD

and DIPrt of masticate samples. Prediction equations that included pastures and month effects predicted more of the variation observed for CP and DIPrt (R^2=66 and 59%), but for DIP and IVOMD, the variation predicted was lower (R^2=38 and 46%, respectively). Based on the DIPrt, data support observed responses during summer and winter to protein supplementation. These values provide needed information about forage nutritive values used to evaluate possible supplementation strategies utilizing the metabolizable protein systems.

Key Words: Forage, Degradable Protein, *in vitro* digestion, Native range, bluestem grasses.

Introduction

Proteins, nucleic acids and chlorophyll are the main constituents of total N in plant tissues. Total N content can vary between 0.5 and 5% on a dry weight basis. Mangan (1982) classified proteins of fresh forages in: 1) A fraction that is constituted mainly by ribulose 1,5 bisphosphate carboxylase (Rubisco), 2) A fraction that is a mixture of proteins and enzymes of chloroplasts and cytoplasm, 3) A fraction that are chloroplast membrane proteins. From a nutritional point of view, the first two fractions constitute 75 % of total leaf protein and are

soluble in water; chloroplast membrane proteins that are insoluble in water constitute the remainder of CP (around 25 % of the total). As a plant develops, the proportions of these fractions change, affecting possibly protein degradation by rumen bacteria.

It is recognized that better prediction of forage intake, and therefore, animal performance, can be done if the fraction of forage protein that is degraded in the rumen and the fraction that will resist bacterial attack are known, dividing forage protein in degradable intake protein (DIP) and undegradable intake protein (NRC, 1996). In addition, a minimum amount of energy is required to elucidate an animal response to protein supplementation (NRC, 1976, Egan, 1972). Due to the fact that forage provides the major portion of grazing cattle diets, it is imperative to understand the protein and energy relationships of the forage throughout the year. Accurate estimates of the DIP content of forages, and an understanding of how DIP may vary over the course of a grazing season are essential for evaluating supplement strategies. Therefore to gain some knowledge how protein and energy changes in forage samples collected by animals, monthly changes in DIP, UIP, and forage digestibility for Old World bluestem and tall grass prairie and mid grass

prairies were evaluated from samples collected in different years and generate a calendar of forage quality over time.

Materials and Methods

Pastures and samples

Esophageal and ruminal forage samples (n=237) from tall grass, midgrass prairies, and old bluestem pastures collected in 1993, 1994, 1995, 1998 and 1999 were analyzed for crude protein (CP), degradable intake protein content (DIP), and *in vitro* organic matter digestibility (IVOMD) to determine the adequacy of degradable intake protein (DIP) respect to available energy throughout the year. Samples were collected from three experimental stations of Oklahoma Agricultural Experiment Station: 1) Southwest Agronomy Research Station (WW) located in Woodward, OK, 2) Marvin Klemme Research Station (MKRS) located in Bessie, OK and, 3) Oklahoma Agricultural Experiment Station Research Range approximately to 11 km southwest of Stillwater, OK (STL). In WW station, pastures were of Old World Bluestem grass (OWB) and midgrass native pastures; in MKRS, pastures are mainly midgrass prairie range. The pastures at STL station has plains Old World Bluestem and tall grass prairie. Samples were collected during the course of several independent trials using steers fitted with either ruminal

or esophageal fistulae. Ruminal samples were collected by removing the entire reticulo-ruminal contents, allowing animals to graze for 1.0 to 1.5 h, then removing the masticate from the rumen and replacing the previously removed reticulo-ruminal contents (Ackerman et al, 2001). Approximately 500 g (wet) of masticate from each animal was placed over a 2-mm screen to remove salivary contaminants and frozen.

Laboratory analysis

The frozen samples were thawed at room temperature in trays and dried in at 55°C in a forced-air oven for 96 h. In accordance to AOAC (1996), dry matter of 55°C-dried samples was determined by oven drying at 105°C overnight and ash content of dried samples was determined by ashing at 550°C for 6 h. Crude protein (CP) in samples dried at 55°C was determined by a combustion method (Leco NS200, St Josephed, MI).

Degradable intake protein (DIP) of samples was determined by an *in vitro* method using *Streptomyces griseus* proteases as described by Roe et al. (1991) and Mathis et al. (2001). The amount of nitrogen incubated was adjusted to be 15 mg in 40 ml of borate-phosphate buffer (pH 7.8 - 8.0) for 1 hour and then, 10 ml of protease solution (330 x

10^{-3} units/ml) was added. The samples were incubated in water bath at 39°C for 48 h and continuously shook. Then, samples were removed from water bath and filtered through filter paper (Whatman # 54), using a funnel with vacuum suction. Residues were re-suspended with 400 ml of distilled water. Nitrogen residue was determined by the combustion method (Leco NS200, St Josephed, MI). The percentage of degradability of total crude protein (%) was calculated as: Initial crude protein (mg) - crude protein (mg) in the protease incubation residue divided by initial crude protein amount (mg).

The TDN content of masticate samples were estimated way the *in vitro* organic matter disappearance (IVOMD), following a 48-h in vitro procedure similar to the method of Goering and Van Soest (1970). Ruminal fluid for the *in vitro* digestibility procedure was obtained from steers fitted with ruminal cannula penned in pen 4x4 m pen fed a maintenance diet based on prairie hay and two pounds of soybean meal. Rumen fluid preparation consisted in taking whole rumen content, squeezing and filtering through for four layers of cheesecloth. After that, fluid was transported to laboratory in a thermal container with a CO_2 cap. In the laboratory, rumen fluid was mixed with buffered solution (1:4), bubbled with CO2 gas, continuously

shaken at 37°C (Bodine et al., 1999). Masticate samples (0.5 g) were incubated in buffered ruminal fluid for 48 h. Microbial activity was stopped by putting fermented sampled in freezer. Samples were thawed and an NDF extraction was performed on the residue, which was dried and ashed mentioned formerly.

Calculations of protein/energy ratio

In vitro OMD was calculated by subtracting the post-NDF residual OM (g) from the initial OM (g), divided by the initial OM (g), and multiplied by 100 to express it as percentage OM.

To estimate if DIP content of masticate samples was adequate or deficient, the ratio of DIP supplied in masticate sample (g/kg) to DIP requirement for bacteria growing with an assumed efficiency (MOEFF) of 11.5% of TDN. Degradable intake protein requirement was estimated by multiplying MOEFF by actual IVOMD. Degradable intake protein requirements for microbial protein production ranges from 9% to 13% of TDN (NRC, 1996). If the calculated ratio is smaller than 1, this means that the masticate sample is deficient in DIP. In contrast, if ratio is bigger than one means DIP is in excess of available energy.

Statistical analysis

The observed CP, DIP, IVOMD and ratio data were analyzed with a model that included pastures, year, month, interaction month x pastures using the GLM procedures of SAS (1991). A multiple comparison test (Bonferroni t-test) was utilized to separated sample means with $\alpha=0.05$ (Kuehl, 2001).

In the development of prediction equations, indicator variables for pasture were created as described by Neter et al. (1996). Month was recoded because the highest values studied variables occurred in April and May. Therefore, April was codified as month=1, then May as month=2 and so on. Initial equations included the linear, quadratic and cubic terms for month, pastures, year, monthly rainfall and temperature. Then variables that less contributed to R^2 were removed of model; then the model was re-run to observe changes in R^2. If there was a change in explained variation (5 %), the variable was returned to model.

Results and Discussion

Pasture and month interaction

Although, it was expected that there would be interactions between grasses and month, no interactions ($P > 0.05$) were detected for any responses studied in the present study

(data not showed). Patterns for crude protein concentration was similar for masticate samples of TGP, MGP and OWB. The highest crude protein contents of masticate samples were for April and May, and then CP declined as growing season advanced.

Effect of year

Unfortunately, not all months were represented within all years; therefore values reported for year is biased by the season in which samples were taken. However, it is possible to describe some tendencies. The CP mean for 1993 was lower ($P < 0.05$) than that for other years (Table 1). This difference is explained because masticate samples for 1993 included only November and December, which is dormancy period these grasses. More masticate samples of native grasses were taken at the end of year for years 98 and 99, explaining the lower DIP content. Grings et al (1995) reported that year affected CP content in native grass grazed by esophageal fistulated mature steers and suckling calves during June to November in two successive years. Although, plants are well adapted to their environment, the effect of year probably is through extreme changes in climate patterns. For example, lack of rain affects mainly biomass production, with little effect in chemical

composition. Low temperature delays maturity of grass value, increasing nutritive value (Hakkila et al., 1987) or at temperatures below the optimum for growth, soluble carbohydrates are accumulates (Buxton and Fales, 1994).

These changes in chemical composition could affect digestibility of whole plant. Coleman and Barth (1973) reported that variation in dry matter digestibility of fescue-lespedeza and Orchard pastures during May to November for three consecutive years, year and month accounted for 84 % of the total variation. Although, most studies include data from at least two years, effect of year is described, but not discussed. Changes in chemical composition by effect of year could be related more to years with unusual rainfall patterns.

Pasture type

On average, crude protein in Old World bluestem pastures (OWB) was higher ($P < 0.05$) than tall grass prairie (TGP) (Table 2), but CP content of midgrass prairie (MGP) was similar ($P > 0.05$) to CP content for both OWB and TGP. It is well established that warm-season grasses (WSG) dominate 65 % of area in Great Plains and the cool-season grass (CSG) the remainder, 35% (Epstein et al., 1997). Also, Tieszen et al. (1997) suggested that grassland in

Oklahoma consists of as much of 80% of C_4 plants species, dominated by Bluestems, switch grass, and Indiangrass. However, during the spring and fall CSG can be an important source of forage. On other side, it is known that CSG, such as brome grass, blue grama, buffalo grass, tobasa grass, have a higher CP content than WSG. For example, Blasi et al. (1991) reported that extrusa of steers grazing smooth brome had more CP (17 - 25 %) than extrusa from animals grazing Big bluestem (10.9 to 16.6 %) for the period May to July. In the present data, CP began to increased in April that corresponds more to CSG than WSG, because WSG begin to growth later than April.

Several studies confirm that native grasses have a low nutritive value compared to improved grasses. At a similar growth stage, Orchard grass and Timothy (C_3 grasses) had more protein, 12.4 % CP, than native grasses Switchgrass and Flaccidgrass, 7.6 % CP (C_4 grasses) (Reid et al., 1990). Mullahey et al. (1992) also found that Smooth brome grass had a higher CP (11.2 % CP DM) in whole plant than Switchgrass (7.7% CP DM) at comparable stages of growth. Dubbs et al (2003) also reported that masticate samples of fescue had almost 25% CP during April, after declined CP content, but no lower than 15% CP during May to October. Kloppenburg et al. (1995) reported that wheat grass, Fescue

and Range were similar in CP during the spring, but in the fall, wheatgrass and Fescue had almost twice what that of range pastures. In contrast, during summer, native grass and bermdudagrass and bluestem were similar in CP. The range of temperature in Oklahoma can suggest that pastures production can be given for plant communities that include warm- and cool-season grasses.

With respect to DIP, MGP had a lower DIP than OWB and TGP, and DIP was higher in OWB than in TGP ($P < 0.05$). Mullahey et al. (1992) reported that 59% of total CP was DIP in switchgrass, warm-season grass. In contrast, in smooth brome grass and tall fescue grasses, extent of protein degradation is estimated as 65% of CP (Elizalde et al., 1999). Redfearn and Jenkins (2000) showed that WSG have more escape protein per kg than CSG. Although MPG had more CP concentration than TGP, MGP resulted to be more deficient in nitrogen when expressed in terms of DIPrt. This is a result of the high IVOMD content, compared to others pastures. In contrast, tall grass was less ($P > 0.05$) deficient than MPG, but similar to OWB. On average, OWB had DIP supplied was enough for the energy content of forage for microbial growth (11.5 % efficiency). In contrast, tall grass prairie was slightly deficient and midgrass was considerably deficient in DIP supplied.

Although, data support the idea that Old World Bluestem is an improved pasture with higher nutritive value than native pastures, these mean values do not show the fluctuations that take place through of calendar year.

Month effect

Monthly means for crude protein (CP), degradable intake protein (DIP), *in vitro* OM digestibility (IVOMD), and DIP supplied: DIP required ratio (DIPrt) content in masticate samples are showed in Table 3.

Nutrient concentrations in plants usually change through life cycle of plants. Concentration is usually high at the beginning of growth phase and then decline as plant matures. The changes in nutrients affect nutritive value of plants through the year. Thus, crude protein concentration of masticate samples began to increase during March compared to January and February ($P < 0.05$), and it reaches its maximum ($P < 0.05$) in April and May. In the Table 4, it can appreciate that where April and May intercept there is a 'B', that means that they do not differ ($P > 0.05$), but in the others intercepts of April and May with the remainder months an 'A' was located there, which means that mean for April or May is different to month which is intercepting. For example, CP mean for May

and June is an 'A' in the intercept, that means that they are different, but it does not say nothing about other comparison. In regards to crude protein means for April and May, they were concentrations were higher ($P < 0.05$) than CP means for all other months. Then, CP declined rapidly (0.8 g CP/d) until August ($P < 0.05$). Although in September CP content increased slightly, CP content went on declining from September to November. During the period from December to February, which corresponds to dormancy period for grasses, CP content of masticate samples was at the lowest level (around 6 % CP) for the remaining of the year

Crude protein changes are more notable in all forages as maturity advances (Minson, 1990; Nelson and Moser, 1994). On average for all forages, monthly CP mean declined around 11% percentage units (17.7 to 6.0%) from May to December, this equates to lose of around 0.56 g CP/kg OM/d. Minson (1990) reported that CP content decreased by 2.2 g/kg DM/d in several studies. Probably, the difference is due to the database in Minson (1990) comes mainly from clipping samples, while in the present trial, the forage samples were selected by grazing animals. It is known that quality diet of grazing ruminants is higher than available forage in range (Jefferies and Rice,

1969; Coleman and Barth, 1973; Hafley et al., 1993; Dubbs et al., 2003). Dabo et al. (1988) reported that CP content in the whole plants of several varieties of Old world bluestem declined constantly for the first eight weekly sampling periods from May-Jun, but in the last two sampling periods, CP content did not decline more. In the actual study, CP continued to decline until December, when it reached the lowest point (Table 3). In contrast, Mullahey et al. (1992) reported that CP content in clipping samples of switchgrass and smooth brome declined during June to September from 12% CP to less than 4 % CP in DM. Teague et al. (1996) reported that CP of pastures clipping samples of Old world bluestem grew in spring, CP was highest (10.7% CP) in May then decreased until a 4.9 % at the end of July. In contrast, masticate samples of cattle grazing a mixed stand of primarily big bluestem and switchgrass during June to August, did not have significant changes in CP concentration (Hafley et al., 1993). The smaller changes during this time in nutritive value of masticate samples are related probably to forage selection by animals that selected better forage. However, in dormancy period, animals have less opportunity of selected better forages. Unless, animals can find some cool season winter grasses, diet quality for that period is poor. In the present

study, CP content of the samples was around 6 % CP from December to February. Crude protein content decreased rapidly as forage matures, however in masticate samples CP decrease more slowly. When, grasses are dormant, animal do not have many opportunities of selected better forages than the standing forage. As forage matures, the proportion of cell wall increases, so that fractions bound to it, such as bound protein.

Monthly means for the degradable intake protein (DIP) in masticate samples are shown in Table 3. In general, DIP mean for April was higher ($P < 0.05$) than the other months (Table 5). Although DIP mean for March was lower ($P < 0.05$) than April mean, it was higher ($P < 0.05$) than DIP means for period June to October. The DIP mean for May was higher ($P < 0.05$) than period of June to July. Few changes in the DIP content can be appreciated during the period of December to February (Table 5). Dubbs et al. (2003) reported that DIP for masticate samples of based-fescue pastures were between 72.4 and 74.6% of CP during April and October, when vegetative growth was occurring, and declined to 65.0% in June when the forage was at maturity. The higher DIP at the beginning of growing season can be related to higher content of nitrate, amino acids and rubilose 1,5, bisphosphate (Rubisco; Feller and Fischer,

1994; Masclaux et al., 2001). Rubisco is an abundant protein in the plant that is rapidly degradable by rumen microbes (Nugent and Mangan, 1981; Elizalde, 1999). In addition, most of CP in leaves is true protein (75%) and 25% is NPN (Mangan, 1982). Therefore, increase in DIP in March, April and May can explain for these nitrogenous fractions. It can be appreciated that the changes in DIP are less consistent that the changes in CP. Such inconsistence can be explained because soluble and insoluble nitrogenous components are mobilized constantly in plants. For example, when a ruminant animal defoliates a plant, the plant responds mobilizing protein from storage sites to compensate the loss of photosynthetic material. In contrast, chloroplast membrane protein, extensins, proteins covalently linked to wall cells probably constitute s pool less ruminally degradable and less mobilized (Van Soest, 1993). Thus, DIP and UIP fractions depend on several protein pools and the physiological stage of plants. In general, changes in DIP (or UIP) are smaller than observed for protein or digestibility.

It is well established that as plants mature, digestibility of forages decreases due an increase in fiber content or reduction of cell contents (Buxton and Redfearn, 1997). In the present study, it could appreciate that *in*

vitro organic matter digestibility (IVOMD) during the period April to August were higher than means for period November to March. The means for September and October were intermediate (Table 3). Table 6 confirms this observation; where it is possible determine that IVOMD for the period from November to March are different ($P < 0.05$) to means for the period April to August. In the same way, means for September and October were ($P > 0.05$) similar to all other means. Although, it has been mentioned that digestibility of forage decreases as plants matures (Buxton and Redfearn, 1997), IVOMD exhibited no large changes for period from April to October, compared to changes in CP content decays rapidly as growing season advances as shown in several studies. It seems that animals were able to select plant material of high quality before that IVOMD decreased. The reduction in digestibility due to maturity is explained by the fact that the leaves:stem ratio decreases and stems usually have lower digestibility than leaves. Additionally, cell content decreases as wall cell increases, and more tissues in stems become lignified. Therefore, it is expected that digestibility decreases as plants matures. Tremblay (2002) reported that plant IVDMD of alfalfa was in function of stem digestibility and the leaf weight ratio. Titgemeyer et al. (1997) reported that

in a legume, clover, the reduction of leaf:stem ratio was substantial, from 0.53 to 0.13. Hendrickson et al., (1997) reported that IVDMD of the leaf in two Sandhill grasses (sand reed and sand bluestem) was caused by reduction in digestibility of cell walls throughout the summer. Hollingsworth-Jenkins et al. (1997) reported that IVOMD did not changed in masticate samples of Sand hills range for period November to February. In the present study, IVOMD was higher ($P < 0.05$) in the period from April to October. From November to March, IVOMD tended to be lower. In addition, it seemed that animals had enough grass to select the best material before a change in the digestibility could be detected.

The utilization of forage protein depends on the energy available for rumen bacteria. As more energy bacteria can get more nitrogen bacteria will capture to grow. Changes in DIP supplied to DIP required ratio (DIPrt) through calendar year are showed in Table 3. As expected, DIP supplied exceeded DIP required in regarding to available energy of forage at April and May when the CP is high and CP is highly degradable. Although, both CP and IVOMD increased in March, the increase in CP was greater, so an improvement in DIPrt was observed ($P < 0.05$), but DIPrt was still slightly limiting (DIPrt=0.89). In the

period of June to November, DIPrt means did not differ (P >0.05). Finally, DIPrt dropped in the winter months to their lowest values. Nitrogen is the factor limiting of low quality forage (Egan, 1965; NRC, 1985) and grazing situations (Lardy et al., 1999). Nitrogen deficiency decreases microbial activity, which could affect breakup of cell walls and digestion, in turn, limiting forage intake because less forage can be accommodate in the rumen (Baile and Forbes, 1974; Grovum, 1987). Utilizing a ratio that puts DIP and energy into one common term, it allows us to evaluate these interrelated nutrients that must be closely balanced for optimal animal performance. For example, DIPrt for period of November to Febraury indicated that there was a nitrogen deficiency, which can be alleviated by nitrogen supplementation. As resulted of supplemented protein to animals grazing Sandhills range (6.2 % CP and 52.3%) during winter, resulting in an increase in digestibility (Lardy et al., 1999). Hess et al. (1994) did not find effect by protein supplementation on dormant intermediate wheatgrass, but harvesting efficiency was increased by protein supplementation. In contrast, energy supplementation results in a negative effect, decreasing fiber digestion and feed intake (Sanson et al, 1990), because deficiency of nitrogen is increased (Bodine et al,

2000). In contrast, energy supplementation could be beneficial on April and May when DIPrt was bigger than one, indicating that energy contained in the forage could support a higher microbial growth. In accordance with these results, it is interesting that supplementation programs usually begin in September when DIPrt begins to decline.

Prediction of nutritive value

Prediction equations generated in the present study are shown in the Table 8. All partial coefficients in the regression equations were significant ($P < 0.01$) in the reduction of variation. For the content of crude protein content, pastures had different intercepts was different ($P < 0.01$) and the best equations included factors quadratic and cubic for month, explaining about 63% of variation. The intercept for degradable intake protein of Old World Bluestem and midgrass prairie was similar, but the intercept of tall grass prairie was different ($P < 0.01$). Prediction equation for DIP required quadratic terms for year and cubic, quadratic and lineal terms for month; however, terms considered were less effective to explain variations in DIP ($R^2 = 0.38$). The intercepts for IVOMD did not differ. Therefore, only one prediction equation was

generated which explained 46% of the variation. Year and month had negative coefficients, indicating that IVOMD decreased through year and month. Respect to DIPrt, forages had different intercepts and linear and quadratic terms for month were included, explaining about 57% of variation.

Maturity is the factor that more affects quality forage probably because it integrates the effects of many effects (Fick et al., 1994). In the present studied, re-coding month, in some way age and maturity were considered in the model because months with forage less matures were included at the beginning of calendar year, so that prediction equation developed explained considerable variation with few variables. Predictions equations for forage in agronomic studies have been developed with highly precise. To develop equations more precise for masticate samples could be taken more variables, such as temperature, height of stand, day of the year when esophageal samples are taken. Figure 1 shows the values predicted for DIPrt. It can appreciate that DIPrt for Old World bluestem (OWB) was adequate for April to July, then, it became deficient in August. Compared to OWB, tall grass prairie became nitrogen deficient in July. Finally, midgrass only one month is adequate in DIP based on its IVOMD. Thus, data

suggested that producer grazing cattle in OWB can delayed the protein supplementation one or two months compared to producer grazing cattle in native pastures. That means less money inverted.

Implications

It would appear that both protein and energy supplementation could be used at different times during the year to improve nutrient balance of the forages. Overall these values should assist in development of supplementation strategies that attempt to match forage nutritive value with the addition of energy or DIP. The development of prediction equations should help producers better understand supplementations needs. Energy and protein in one term (DIPrt) may allow us to choose the right combination of energy and protein to optimize microbial efficiency and animal performance.

Table 1. Effect of year on the mean crude protein, degradable intake protein, *in vitro* digestibility and ratio[1] of Old World bluestem, tall grass, and midgrass masticate samples

	Year						
Variables	1993	1994	1995	1998	1999	MSE[2]	P < X[3]
N	12	159	53	17	33		
Crude protein	7.1[b]	11.7[a]	12.1[a]	12.6[a]	12.4[a]	8.31	0.05
DIP	61.0[a,b]	61.4[b]	64.6[a]	54.6[c]	53.9[c]	34.39	0.05
In vitro, % OM	66.1[a]	70.9[a]	74.2[b]	69.4[a,c]	67.9[a,c]	34.19	0.05
Ratio	0.59[a]	0.91[a]	0.96[a]	0.90[a]	0.88[a]	0.62	0.05

[1] DIP: (in vitro * 11.5% of microbial efficiency) ratio that measures adequacy of DIP content for bacteria with an estimated microbial efficiency of 11.5%

[2] MSE: Mean square error. Standard error of mean= $\sqrt{}$ (MSE *(1 / n_1 + 1 / n_2)

[3] Bonferroni t test

[a,b,c] Year means without common superscripts differ ($P < 0.05$)

Table 2. Effect of pasture type on mean crude protein, degradable intake protein, *in vitro* digestibility and DIP supply to DIP required ratio of Old World bluestem, tall grass, and midgrass masticate samples

Variables	Pastures			MSE [2]	P <X [3]
	Tall grass prairie	Midgrass prairie	Old World bluestem		
N	119	30	125		
Crude protein, % OM	10.5^{b}	$11.8^{a,b}$	12.8^{a}	8.31	0.05
DIP, % of total N	59.9^{b}	56.3^{c}	62.3^{a}	31.34	0.05
In vitro, % OM	69.7^{b}	$71.5^{a,b}$	71.9^{a}	34.19	0.05
Ratio[3]	$0.9^{a,b}$	0.8^{b}	1.0^{a}	0.62	0.05

[1] DIP: (in vitro * 11.5% of microbial efficiency) ratio that measures adequacy of DIP content for bacteria growing at 0.115%

[2] MSE=Mean square error. Standard error of means: $\sqrt{(MSE^* (1 / n_1 + 1 / n_2))}$

[3] Bonferroni t test

[a,b,c] Means without common superscripts differ ($P < 0.05$)

Table 3. Monthly means for CP[1], DIP, IVOMD and ratio of Old World bluestem, tall grass, and midgrass masticate samples

Month	N	CP	DIP	IVOMD	Ratio
January	24	6.7	62.4	64.0	0.59
February	15	6.3	60.5	63.3	0.55
March	26	9.6	65.1	64.8	0.89
April	22	17.6	71.0	75.7	1.51
May	33	17.7	61.9	76.8	1.29
June	41	14.1	57.1	74.0	0.99
July	25	11.9	54.5	73.4	0.81
August	38	10.7	57.8	74.0	0.76
September	7	12.8	56.2	71.3	0.96
October	16	10.4	63.7	70.4	0.86
November	15	8.6	57.3	65.1	0.69
December	12	6.0	60.6	63.9	0.52

[1] CP: Crude protein (x6.25) on organic matter basis, [2] DIP: Degradable intake protein, *Streptomyces griseus* protease method (Roe et al, 1991; Mathis et al., 2001), [3] IVOMD: in vitro digestibility of organic matter, and [4]ratio: Degradable intake protein supplied / Requirement of degradable intake protein for bacteria growing with an efficiency of 11.5% of IVOMD ratio.

Table 4. Multiple comparisons for CP[1] of masticate samples from steers grazing native grasses and Old World Bluestem (Bonferroni t-test [2])

Month											
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jan	B[4]	A[3]	A	A	A	A	A	A	A	B	B
	Feb	A	A	A	A	A	A	A	A	B	B
		Mar	A	A	A	B	B	B	B	B	A
			Apr	B	A	A	A	A	A	A	A
				May	A	A	A	A	A	A	A
					Jun	B	A	B	A	A	A
						Jul	B	B	B	A	A
							Aug	B	B	B	A
								Sep	B	B	A
									Oct	B	A
										Nov	B
											Dec

[1] CP: Crude protein (x6.25) on organic matter basis.
[2] Bonferroni test (Kuehl, 2000)
[3] The literal A in the interception between two months means that they are different ($P < 0.05$)
[4] The literal B in the interception between two months means that they are not different ($P > 0.05$)

Table 5. Multiple comparisons for DIP[1] (as % total crude protein) of masticate samples from steers grazing native grasses and Old World Bluestem (Bonforroni t-test [2])

	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jan		B[4]	B	A[3]	A	A	A	B	B	B	B	B
Feb			B	A	B	B	B	B	B	B	B	B
Mar				A	B	A	A	A	A	B	A	B
Apr					A	A	A	A	A	A	A	B
May						A	A	B	B	B	B	B
Jun							B	B	B	A	B	B
Jul								B	B	A	B	B
Aug									B	A	B	B
Sep										B	B	B
Oct											B	B
Nov												B
Dec												

[1] DIP: Degradable intake protein, Streptomyces griseus protease method (Roe et al, 1991; Mathis et al., 2001)
[2] Bonferroni test (Kuehl, 2000)
[3] The literal A in the interception between two months means that they are different ($P < 0.05$)
[4] The literal B in the interception between two months means that they are not different ($P > 0.05$)

Table 6. Multiple comparisons for IVOMD[1] of masticate samples from steers grazing native grasses and Old World Bluestem (Bonforroni t-test [2])

	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jan		B[4]	B	A[3]	A	A	A	A	B	B	B	B
Feb			B	A	A	A	A	A	B	B	B	B
Mar				A	A	A	A	A	B	B	B	B
Apr					B	B	B	B	B	B	A	A
May						B	B	B	B	A	A	A
Jun							B	B	B	B	A	A
Jul								B	B	B	A	A
Aug									B	B	A	A
Sep										B	B	B
Oct											B	B
Nov												B
Dec												

[1] IVOMD: in vitro digestibility of organic matter
[2] Bonforroni test (Kuehl, 2000)
[3] The literal A in the interception between two months means that they are different ($P < 0.05$)
[4] The literal B in the interception between two months means that they are not different ($P > 0.05$)

Table 7. Multiple comparisons for ratio[1] of masticate samples from steers grazing native grasses and Old World Bluestem (Bonforroni t-test [2])

	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jan		B[4]	A[3]	A	A	A	B	B	B	A	B	B
Feb			A	A	A	A	B	B	B	A	B	B
Mar				A	A	A	B	B	B	B	B	A
Apr					A	A	A	A	A	A	A	A
May						A	A	A	A	A	A	A
Jun							B	A	B	B	A	A
Jul								B	B	B	B	A
Aug									B	B	B	B
Sep										B	B	A
Oct											B	B
Nov												A
Dec												

[1]ratio: Degradable intake protein supplied / Requirement of degradable intake protein for bacteria growing with a microbial efficiency of 11.5% of IVOMD
[2] Bonforroni test (Kuehl, 2000)
[3] The literal A in the interception between two months means that they are different ($P < 0.05$)
[4] The literal B in the interception between two months means that they are not different ($P > 0.05$)

Table 8. Regression equations for CP[a], DIP and IVOMD for grazed Old World bluestem (OWB) and tall grass prairie (TGP)

Pastures	Prediction equation[b]	SEM[c]	R^2
TGP	$CP = 17.24 - 0.36X^2 + 0.02X^3$	2.95	0.63
MGP	$CP = 13.96 - 0.36X^2 + 0.02X^3$	2.95	0.63
OWB	$CP = 18.84 - 0.36X^2 + 0.02X^3$	2.95	0.63
TGP	$DIP = 80.79 - 0.25y^2 - 10.00X - 1.40X^2 - 0.06X^3$	6.42	0.38
OWB/MGP	$DIP = 83.92 - 0.25y^2 - 10.00X - 1.40X^2 - 0.06X^3$	6.42	0.38
TGP/MGP/OWB	$IVOMD = 74.37 - 0.20y^2 - 4.20X + 1.03X^2 + 0.52X^3$	6.11	0.46
TGP	$DIPrt = 1.63 - 0.26X + 0.15X^2$	0.24	0.57
MGP	$DIPrt = 1.39 - 0.26X + 0.15X^2$	0.24	0.57
OWB	$DIPrt = 1.77 - 0.26X + 0.15X^2$	0.24	0.57

[a] CP=Crude protein, DIP=degradable intake protein, IVOMD=Degradable intake protein; DIPrt=DIP supplied:DIP required ratio.

[b] X=month, where X=1 is April, X=2 is May, etc

[c] Standard error of mean

[d] Both pastures, no pasture effect on IVOMD.

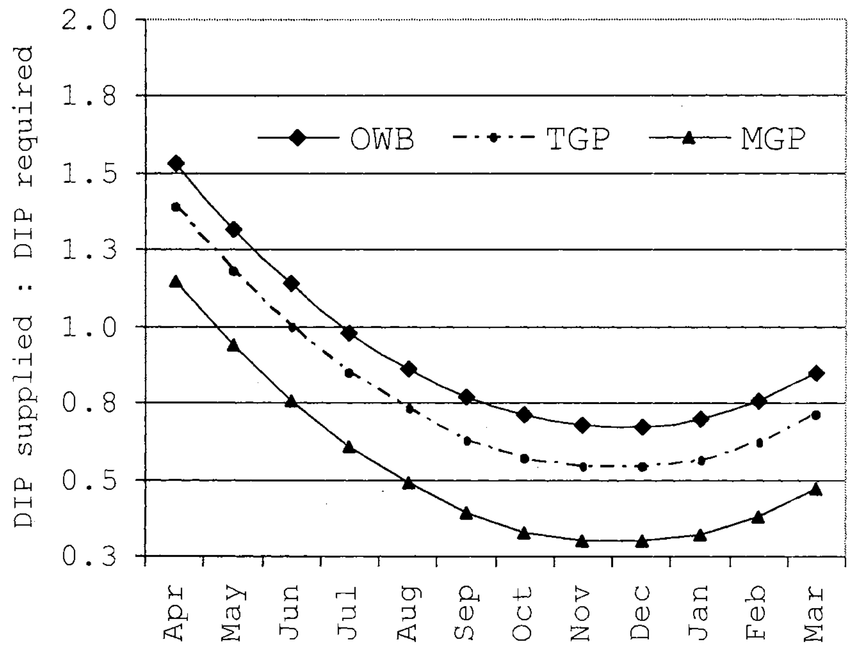

Figure 1. DIP supplied to DIP required ratio in grazed Old World bluestem, tall grass and midgrass prairies when microbial efficiency is 11.5% of TDN.

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Chapter IV

COMPARISON IN VIVO DETERMINATION OF THE DEGRADABLE INTAKE PROTEIN OF FORAGES vs. IN SITU AND IN VITRO METHODS

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ABSTRACT: To estimate *in vivo* degradable intake protein (DIP) of high, medium and low quality forages, six crossed Angus steers were fed *ad libitum* alfalfa hay (ALFA), bermudagrass hay (BER) or prairie hay (PH) in a replicated 3 x 3 Latin square design with 21-d experimental periods. Ruminal digestion and microbial protein production were determined by chromic oxide as indigestible marker of duodenal flow and purines as bacterial marker; bypass protein fraction was estimated by subtracting bacterial N and ammonia N from total duodenal N flow. In addition *in vivo* DIP estimation, an *in vitro* method and *in situ* procedures were compared. *Streptomyces griseus* protease method (SG) was the *in vitro* method; *in situ* methods were: by incubating forage samples for 16 (IS_{16}) and 20 h (IS_{20}), adjusted by ADIN residue and by estimating effective degradability of neutral detergent insoluble nitrogen(NDIN)

pool, which was estimated by using slope-intercept form of straight line passing through 2 and 12 h of incubation times, adjusted by NDIN residue after 96 h incubation (NDIN-1) or taking the actual laboratory value of NDIN in forage (NDIN-2), adjusted also by NDIN residue after 96 h incubation, were compared. In vivo DIP values for ALFA, BER and PH were: 84, 79 and 49.7 % of the total crude protein. It was calculated that g DIP/100 g TDN were for ALFA, BER and PH: 28.4, 14.9 and 5.2, respectively. Prairie hay is nitrogen deficient and ALFA has an excess of nitrogen in relation to TDN. The estimations of *in vivo* values using *in vitro* and *in situ* methods were affected by forage type. For ALFA, all methods studied predicted DIP similar to *in vivo* values. However, IS_{16} and IS_{20} underpredicted DIP of BER or PH in -16 and -9 for BER and -25 and -17 percentage units for PH. The procedures based on NDIN (NDIN-1 and NDIN-2) predicted better DIP values. The prediction of NDIN-1 and NDIN-2 differed of *in vivo* DIP values in +2.4, 9.0, +6.7 and -7.2, -0.3 and +5.0 percentage units for ALFA, BER and PH, respectively. The deviation of estimates of DIP values using protease method were consistently lower: -7.2, -12.9 and -10.9 percentage units for ALFA, BER and PH, respectively. Variation among runs was the lowest for protease methods. Because of

extensive protein degradation of CP, different methods can be used to estimate actual DIP content of alfalfa. In contrast, methods that included passage and digestion rates gave good estimates of DIP for bermudagrass and prairie hays. SG method tended to give low estimations, however, it is a simple method.

Key Words: Forage, Degradable Intake Protein, Digestion Rate, Passage Rate, Microbial protein

Introduction

The evaluation of forage protein in ruminant nutrition is the partitioning of dietary protein into degradable intake protein (DIP), the fraction that is utilized by rumen bacteria, and undegradable intake protein (UIP), the fraction that resists microbial degradation (Broderick, 1994). Most of protein feeding systems are based (Waldo and Goering, 1979; NRC, 1996) on metabolizable protein. Ideally, protein evaluation should be determined using *in vivo* trials; however, *in vivo* trials are expensive and time-consuming. Additionally, due to inaccuracies in determination of duodenal flow, the partition of dietary protein, animal variability, *in vivo* values serve only as reference values more than as actual values (Tamminga and Chen, 2001). There has been an extensive research effort

to find a single method that provides rapid and reliable estimates of protein degradation (Stern and Satter, 1984). Although, early studies (Wohlt et al., 1976) reported that protein solubility in solvents could be a good estimate of ruminal degradation, Stern and Satter (1984) reported that solubility and ruminal degradation are poorly correlated. It is not probable that a single solvent simulates conditions of ruminal fluid. On the other hand, other methods try to simulate the conditions of rumen fluid, for example, two stage *in vitro* method (Tilley and Terry, 1993) and *in situ* procedures (McDonald, 1981). *In vitro* methods have been widely utilized in the analysis of forages; their values and *in vivo* values have showed a strong statistical correlation (Weiss, 1994). In contrast, the results from *in situ* methods have been variable, mainly because the lack of standardization of factors affecting, pore size, sample characteristics, location where insert bags, and rinsing techniques (Weiss, 1994), however, *in situ* values had biological meaning (Broderick,1994). Enzymatic methods, such as protein degradability by *Streptomyces griseus* protease (Roe et al, 1991; Mathis et al., 2001), have become more widespread because of good correlation with ruminal degradation (Krishnamoorthy et al, 1982), low cost, easy standardization, and no requirement of fistulated

animals. The objectives of the present study were to estimate *in vivo* DIP of forages, to determine microbial growth efficiency, and to compare *in vitro* and *in situ* methods to estimate DIP of forages.

Material and Methods

Animals

The Oklahoma State University Animal Care and Use Committee approved the experimental protocol. A 63-day metabolism-digestion trial was conducted in the Nutrition Physiology Research Center in Stillwater, OK with six steers (491 kg) fitted with ruminal and duodenal cannulae in a replicated 3 x 3 Latin square experimental design. During each experimental period, animals were kept in individual metabolism crates (2 x 4 m) in a room with a controlled environment (20°C and continuous light). While in the metabolic crates animals had access to fresh water all the time.

Experimental period

Each experimental period lasted 21 d and consisted of an 11-day adaptation phase of animals to experimental forages. After the adaptation phase, sampling for determining the digestibility was carried out during 5 days (from d 12 to 16). Then, an *in situ* digestibility was

performed in the last four days of each experimental period. On last day (d 21) of each period, rumen contents were removed, weighed and sampled.

Changes in body weight

At the beginning and the end of each experimental period, body weight was individually recorded, without previous food withdrawal. Body weight on d 21 was recorded before rumen evacuations were carried out.

Experimental forages

Because nutritional quality of forages varies greatly, three forages were chosen to cover that range. Forages were: 1) alfalfa hay represented the high quality forage, 2) bermudagrass hay represented the medium quality forage, and 3) prairie hay represented the low quality forage (Table 1).

Forage feeding

Hays were ground through a hammer mill with a 3-cm screen to avoid animal selection of more leaves than stems, especially with alfalfa hay (ALFA). Forages were offered daily at 0800 in a plastic feeder. To assure an *ad libitum* voluntary intake, 2.5 kg more forage was offered over previous day's forage intake. Except for adaptation phase,

refusals were weighed for all hays daily. All hays were offered with no mineral, protein or energy supplement.

Forage intake

Forage intake was measured as the difference between average weights of offered forage minus average weights of refusal. A representative sample of whole material of hays was taken before the beginning of digestion trial. Therefore, only the refusals were sampled. Approximately, 500 - 600 g of refusal of all hays were dried at 55°C in forced-air oven during 96 h and stored. At the end of each period, the samples were ground and composed for animal-period, then, stored until analyzed in the laboratory.

Duodenal flow determination

From d 7 through 15 of each experimental period, each animal received 10 g of chromic oxide (Cr_2O_3) daily, as an indigestible marker to measure duodenal flow. Chromic oxide was dosed intra-ruminally in gelatin capsules containing 5 g Cr_2O_3 at 0800 and 2000. To estimate daily duodenal flow, duodenal samples were taken every 6 h, advancing two hours every day, until completing a 24-h period. Duodenal pH was recorded immediately using a portable, combination electrode pH meter (Corning 870,

Corning, NY). Duodenal samples were kept frozen (- 20 C) until analyzed in the laboratory.

Fecal collection

Daily total fecal output per animal was collected, weighed, mixed by hand, and sampled on d 13 to 17. The sample per animal (about 500-700 g) was weighed in aluminum pan and dried at 55 C for 96 h. After drying, samples were ground and stored at room temperature until analyzed in the laboratory.

In situ digestibility

To determine the rate and extent of digestion of nitrogen in forages, 2-mm screen-ground hay samples (5 g) were placed inside of dacron bags (10 cm x 20 cm, 53 ± 15 μm inc pore size, Ankom, Firport, NY) with heat sealed edges. Bags with samples were pre-soaked in warm water (37°C). All dacron bags were located in rumen at 0800 on d 17 for 0, 2, 12, 16, 20 and 96 h. The bags were placed together in nylon mesh bags under the ruminal mat. After removal, bags were rinsed in tap water to remove particles adhering to the outside of bags and stored frozen (-10 C). When trial was finished, all bags were washed using a washing machine following the procedure described by Bodine (1999), which decreases the variation associated with washing.

Briefly, the washing machine was set in delicate cycle with one-minute rinsing and two-minute spinning and repeated ten times. Washed bags were oven-dried (55°C for 96 h) and weighed to determined dry matter at 55°C.

Fluid passage rate

After an intra-ruminal infusion of Co-EDTA (0.5 g Co in 200 ml (Udén et al., 1980) on d 16, ruminal samples at 0, 2, 4, 8, 12, 16 and 24 h pos-infusion were taken to determine fluid passage rate. Ruminal samples were filtered through four layers of cheesecloth. Exactly one ml of 7.2 N of H_2SO_4 was added to 9 ml of filtrate to stop microbial activity. The acidified samples were stored frozen until analyzed in laboratory.

Ruminal evacuation

On the last day of each experimental period, all rumen contents were completely removed, weighed, mixed by hand in a container before forage feeding (0 h) and 4 h pos-feeding. In each evacuation time, two samples of whole ruminal contents were taken: 1) one-kg sample was weighed and dried at 55 C for 96 h. Dried samples were ground through a 2 mm screen with Wiley mill. The second one-kg sample was mixed with one L of cold 40% formaldehyde solution, then, stored frozen until analyzed in laboratory.

Laboratory analysis

After grinding, refusal, fecal, and duodenal samples were composited by animal within each period. For fecal and refusal composite samples was considered the weight of total fecal collection and refusal weights in each periods. Forage and fecal samples dried at 55°C, ruminal samples and, *in situ* residues were analyzed for dry matter and organic matters by oven drying at 100°C over night and by ashing at 550°C for 6 h in a muffle furnace, respectively. Crude protein (N x 6.25) of forage samples, dried fecal samples, lyophilized bacteria pellets, duodenal samples, *in situ* residues, DIP residues were determined by Kjeldahl method using Kjeltec 2400(Foss Tecator, Höganäs, Sweeden). Forage DIP was determined by an enzymatic procedure that uses the *Streptomyces griseus* protease (Roe et al., 1991; Mathis et al., 2001). Neutral detergent indigestible nitrogen (NDIN) of forage and *in situ* residues of 2, 12 and, 96 h of ruminal incubation were determined using NDF procedure, then N residue was determined by Kjeldahl method (Mass et al., 1999). Concentration of acid detergent insoluble ash (ADIA) was determined in ruminal samples and forage samples as suggested by Van Soest el al. (1991). Ruminal fluid samples were thawed at room temperature and centrifuged at 11,000xg for 10 min. The supernant was

analyzed for cobalt concentration by atomic absorption spectroscopy (Perkin Elmer Model 4000, Norwalk, CT), and with air plus acetylene flame (Hart and Polan, 1984). To yield a bacteria pellet, ruminal samples with formaldehyde were thawed at temperature room. After that, to detach particle-associated bacteria, mixture was blended with a high-speed blender for two minutes. Then, fluid was passed through two layers of cheesecloth; next, strained fluid was centrifuged at 150xg for 10 minutes to remove large particles and protozoa. Supernatant was decanted into a centrifuge bottle; using rubber policeman and 0.9% saline solution, re-suspended was again centrifuged at 15,000xg under refrigeration for 20 min. The last procedure was repeated twice. The bacteria pellet was freeze-dried and crushed with a pestle in mortar (Vanzant et al., 1996). Duodenal samples were thawed, composited and freeze-dried and crushed with a pestle and mortar (Gunter et al, 1997). Chromic oxide concentrations in dried duodenal samples were determined by bromate-phosphoric acid technique method of Williams et al. (1962) and digested in open flasks on a hot plate. The freeze-dried duodenal samples were re-constituted in distilled water and analyzed for NH_3-N as suggested by Murphy et al. (1994). Bacteria pellet and duodenal samples were analyzed for purine concentration by

the method of Zinn and Owens (1988;) with the modifications suggested by Aharoni and Tagari (1991).

Calculations

Forage voluntary intake was calculated as the differences between offered forage minus refusal weights. By multiplying dry matter, organic matter and nitrogen intake contents of forage times forage intake was estimated the consumption of those nutrients. Apparent digestibility was calculated by subtracting fecal output from forage intake, then, divided by forage intake. Fluid passage rate was considered to be the slope of the linear regression of logarithm of cobalt concentration in rumen regressed over sampling time. Assuming a steady state in forage intake, passage rate was equal to amount of ADIA fed per hour divided by average amount of ADIA g in rumen digesta at 0 and 4 h post-feeding (Waldo and Smith, 1972). Duodenal flow was calculated as: chromium oxide dose (10 g d^{-1}) divided by chromic oxide concentration in duodenal samples. To estimate microbial flow, it was assumed that bacteria isolated from ruminal samples and bacteria flowing into duodenal have the similar purine:nitrogen ratio. Therefore, it is possible to estimate how much of duodenal N is bacterial nitrogen. Dietary N (or bypass protein) can

be calculated subtracting bacterial N and NH_3-N from total duodenal N. Then, in vivo estimation of degradable intake protein was calculated by subtracting bypass N flow from N intake. Estimates of in vivo DIP were obtained as the difference between initial forage N minus ADIN fraction and N in residue after incubating forage for 16 or 20 h, assuming that acid detergent insoluble N is indigestible. A second approach to estimate in vivo DIP assumed that residual NDIN at 96 h of incubation is an estimate of indigestible N of forage and NDIN is the pool of undegradable intake protein as suggested by Broderick (1994) and Van Soest (1994). Therefore, residual NDIN at 96 h was subtracted from residual N at 2 and 12 h. Nitrogen residues were transformed to a log value. Then, digestion rate was assumed to be the slope, which was calculated from equation of slope: $m=(Y_{12}-Y_2) \div (X_{12} - X_2)$ and intercept (initial NDIN pool; NDIN-1) was calculated from point-slope form, $Y_0 - Y_{12} = m\ (X_0 - X_{12})$. In addition, the direct NDIN value of forage in laboratory was considered to be an estimate of initial NDIN pool (NDIN-2). To estimate forage UIP, it was considered that effective degradability of NDIN (UIP) was: kd/(kd+kp) times NDIN-1 or NDIN-2, where kd is the particle passage rate (ADIA) and kd

is digestion passage (slope m). DIP estimates were: Initial nitrogen - (1- effective degradability of NDIN).

In Addition, in the present study, two incubation times were selected 16 and 20 h post-feeding (IS_{16} and IS_{20}) to be used as single point estimate of protein degradation. It was assumed that microbial N contamination was minimum and was not considered in calculation. The acid detergent insoluble nitrogen of forage (ADIN) was subtracted from N residue after incubation.

Statistical analysis

Response variables were analyzed as a replicated 3 x 3 Latin square experimental design using the GLM procedure of SAS (SAS, 1991). The model included square, period and animal nested in square and hays. The pairwise comparison of the means were performed with LSMEANS and ADJUST=Bonferroni options (Kuehl et al, 2000;).

Results and Discussion

Chemical composition

The chemical analysis of experimental forages is shown in Table 1. As expected, alfalfa hay (ALFA) was high in crude protein (CP) content, low in ADF and acid detergent insoluble nitrogen (ADIN). In contrast, prairie hay (PH) was low in CP and high in NDF, compared to ALFA. Bermuda-

grass hay (BER) was intermediate in crude protein and ADIN, compared to other forages.

Forage intake

Steers fed ALFA consumed more forage intake ($P < 0.05$) on DM basis than those fed BER or PH; and BER steers had higher forage intake than those PH steers (Table 2). In addition, BER and ALFA differed ($P < 0.05$) in DM intake, but not on OM intake ($P > 0.05$). Bermudagrass was higher in OM than ALFA (91.1 vs 95.4 OM %). It is widely accepted that voluntary intake and digestibility are directly correlated, where forage intake depends on structural volume and digestibility on cell wall and lignification (Minson, 1990; Van Soest, 1994). Legumes are highly digestible because their greater leaf:stem ratio and higher cell content (Minson, 1990; Meissner and Paulsmeier, 1995). Tremblay et al. (2002) reported that *in vitro* digestibility/NDF ratio explained 67% of alfalfa intake. Van Soest (1994) indicated that there is direct relationship between NDF and forage intake, where as NDF of forage decreases, typically forage intake increases. Soeane et al. (1982) reported that voluntary intake is reduced by 0.15 g as NDF increased (g/kg); the increase in NDF explained 81% of variation in voluntary intake. In the

present study, even though BER and PH had a similar NDF content, forage intake was greater ($P < 0.05$) for BER than that for PH. Possibly a faster digestion rate of NDF (West et al., 1997) and the faster reduction of particles of BER (Fisher, 1991) would favor a higher intake of BER.

In addition, the lower intake of animals consuming PH can be explained by a higher content of lignified fiber and the N deficiency of forage (Campling and Free, 1966; Van Soest, 1994). With grass species there is a negative relationship between voluntary intake and lignin content. However, voluntary intake of legume is less affected by lignin content (Van Soest, 1994). This could explain that in spite of similar ADF content in ALFA and PH, voluntary intake was so different. Also, the lower intake with low quality forage (or high ADF intake) could be related to more indigestible matter content in rumen, increasing the retention time. In addition, more material that is indigestible reduces fermentable digesta and, hence the total amount of microbial activity (Grovum, 1988).

Digestibility

Apparent digestibility coefficients for DM (DMD), OM (OMD) and CP (ACPD) are in Table 2, and the coefficients ranked as expected. For all variables, ALFA had the higher

digestibilities ($P < 0.05$), compared to BER and PH. In turn, the DMD, OMD and ACPD were greater ($P < 0.05$) for BER than for PH. As previously mentioned, digestibility is more related to lignification and cell content (Moore and Jung, 2001). For a wide range of grasses and legumes total digestibility and ADF content were negatively related ($r=-0.79$; Minson , 1990). As cell wall content increases, cell content decreases, resulting in a lower digestibility (Buxton and Redfearn, 1997).

Apparent crude protein digestibility of ALFA and BER were almost 3.5 and 1.3 times higher ($P < 0.05$) than that of PH, respectively. It is well known that CP content in forage and CPD are highly correlated (Milford and Minson, 1965; Minson, 1990). More protein is bound to wall cell in mature grass and cell content is lower in protein, thus less potential degradable protein exists in mature forages (Elizalde et al., 1999). The differences observed in digestibility can be explained by protein intake, CP content and, protein degradability.

Ruminal digestion

Parameters for ruminal digestion are shown in the Table 3. Apparently ruminal fermented OM (ARFOM), as a percentage of total OM intake (OMI), was similar ($P > 0.05$)

among three forages. Truly ruminal fermented OM (TRFOM) was higher (P > 0.05) in steers consuming ALFA than those steers consuming BER or PH. In turn, TRFOM was higher (P > 0.05) in steers consuming BER than in those consuming PH. No differences were detected (P > 0.05) in efficiency of microbial growth (MOEFF; Table 3). Although it is possible that BER had numerically better MOEFF than ALFA and PH had the lowest MOEFF, the coefficient of variation (SEM) for this variable was the highest observed in the present study. This shows the variability in this measurement. Cell content includes all dry matter constituents other than fiber and is considered to be completely digestible and not time-dependent (Buxton and Fales, 1994). In contrast, fiber digestion is a time-dependent process. Therefore, it is expected that digestibility decreases with increases in the amount of fiber in forage (Buxton and Redfearn, 1997). Ruminal fermentation can be limited by other limiting factors such as available N. Steers fed dormant bluestem forage (2.3% CP and 79.1% NDF) had a low ARFOM, 31.8% and TRFOM, 38.2%, but N supplements with 27% CP or alfalfa dehydrate increased ruminal digestion and microbial production them (Hannah et al., 1991).

In the present study, PH showed numerically a lower ARFOM than ALFA and BER, but no difference was detected as

observed in TRFOM. Compared to those reported in the literature, ARFOM and TRFOM reported here seem to be overestimated. Archimede et al. (1997) summarized 553 observations from 157 references and reported that mean for ARFOM was 45.16 % with SD=11.02. From Archimede et al. (1997) one can conclude that ARFOM for forage based diets range from 30% to 50% of OM intake. The main limitation of *in vivo* method is variability in estimates of duodenal and microbial flows as the observed in the present study.

Energy and protein relationship

Taking digestible organic matter as an estimate of TDN and CP content, the calculation of DIP g/ 100 g TDN were for ALFA, BER and PH: 28.4, 14.9 and 5.2, respectively. Poppi and McLennan (1995) suggested that when forages have more than 210 g CP/kg of DOM, animals will suffer significant losses of ingested protein in rumen. In the same sense, NRC (1996) suggested that DIP requirement for rumen bacteria could be around 13 g bacteria CP/100 g of TND. If N supply were higher than DIP required, losses of N in rumen would occur. Several DIP/TDN values has been reported for low quality forages varying from 40.4 to 8.8 g DIP/ 100 g TDN (NRC, 1996; Bodine et al., 1999; Olson et al., 1999). It is clear that PH is nitrogen deficient

forage, while BER has a slight excess of nitrogen, and ALFA has an excess of nitrogen.

Passage rate

The data for intake and average in ruminal content at 0 and 4 pos-feeding of acid detergent insoluble ash (ADIA) are shown in the table 3. There were great differences in ADIA intake, which surely reflected fiber composition of ALFA, BER and PH. Animals fed PH had highest ($P < 0.05$) ADIA intake and ruminal ADIA content compared to ALFA and BER. Animals fed BER were intermediate on ADIA intake and ruminal ADIA content. In contrast, animals fed ALFA had the lowest ADIA concentrations in rumen. It has been suggested other internal markers, such as lignin (Fahey and Jung, 1983) or acid insoluble ash (Van Keulen and Young (1977), however, their low concentration and variation in analytic procedure limit their use in digestion studies, mainly when forages are high quality (Van Soest, 1994). It has been proposed indigestible NDF and ADF as markers (Lippke, 2002; Sunvold et al. 1991) or insoluble ash in acid detergent solution as internal markers because of higher percentage of dry matter (Van Soest, 1994).

In the present study, ALFA and BER had similar ($P < 0.05$) passage rates (kp), which were higher ($P < 0.05$) than

that for PH. The kp values observed in the present study are similar to others previously reported for PH for cattle consuming PH without supplements (Freeman et al., 1992; Bandyk et al., 2001). As expected, increase in alfalfa intake increased kp, by using indigestible ADF, in cattle fed *ad libitum* dormant prairie hay (Vanzant and Cochran, 1994). Passage rate ranged from 2.7 to 3.4 %/h for alfalfa hay when radio-labeled herbage was used as passage marker (Holden at al., 1994). Prigge et al. (1990) reported that steers consuming alfalfa hay *ad libitum* had 4.3%/h, which was higher than switchgrass and their mixes. The values reported for alfalfa tend to be more variable amount trials. For bermudagrass (8.2 % CP and 71% NDF), Mathis et al. (2000) reported that ADIA passage rate varied from 3.5 to 4.1%/h. This reported value is higher than determined in this study. Bermudagrass of actual study apparently had a better composition (8.2 % CP and 37.2 % ADF). Burns et al. (1991) reported that kp of bermudagrass was 3.1 and 2.0 %/h for cattle (285 kg BW) grazing in June and July, respectively. Passage rate for PH and bermudagrass are within range of studies in the literature. Passage rate for alfalfa has been variable in the literature and the current study tended to be higher than those reported.

Comparison of methods

In vivo degradable intake protein (DIP) values are showed in the Table 5. Compared to BER and PH, ALFA had higher ($P < 0.05$) DIP content. The lowest DIP was ($P < 0.05$) for PH. The DIP value for BER was intermediate ($P < 0.05$). The protein degradability increased as protein content increased. It is well known that protein content and protein digestibility are well positively related (Minson, 1990). Protein digestibility for ALFA and BER were 84 and 79%, respectively, which are similar to NRC values (NRC, 1996). In contrast, DIP value given by NRC (1996) for PH is higher than values estimated (49.7%) in the present study.

The estimates of *in vivo* DIP values from laboratory and *in situ* methods are presented on the Table 5. The methods IS_{16} and IS_{20} estimated DIP of ALFA accurately, but they sub-estimated ($P < 0.05$) DIP of BER and PH. Blasi et al. (1991) reported that ruminal escape protein was higher in big bluestem (15.1 to 38% of total CP) compared to smooth brome (7.0 to 14.5% of total CP) and increased as growing season advanced. The sub-estimation could be suggested that longer incubations are required, however, hays tend to be more contaminated by rumen bacteria as incubation time increases (Nocek, 1988). However, the sub-estimation with IS_{20} decreased as incubation time increased.

It is possible that CP content and residue size (as determined by difference) can determine the degree of effect of microbial contamination. For example, forages with little CP content and large bulkiness, the proportion microbial contamination could have larger effect on magnitude of nitrogen content of residue after incubation, compared to forage with high CP content, but small residues after incubation. It seems that IS_{16} and IS_{20} determination are affected by microbial contamination and other factors. Craig et al. (1987) reported that 50-65% of particle nitrogen and 17-27% of particle dry matter was of microbial origin, and microbial contamination differs among forages. Dixon and Chanchai (2000) reported that rumen degradability of alfalfa hay N was underestimated by 4 to 12 %, while protein degradability for oat hay and barley straw N were underestimated by 26 and 75% units. Similar results were found by Wanderley et al., (1993), using 15N-labled forage samples. Nocek (1988) suggested that the use the neutral detergent solution when protein degradability of low quality forage is going to be estimated with *in situ* methods. The methodology is attractive because the estimation of undegradable intake protein requires less time and resources than *in vivo* determinations.

Predicted values for ALFA and PH from method NDIN-1 and those for *in vivo* method did not differ (P > 0.05). In contrast, method NDIN-1 over-estimated (P < 0.05) *in vivo* values for BER. The method NDIN-2, which is taking NDIN pool equal to actual laboratory value, predicted *in vivo* DIP correctly (P< 0.05) for all forages. Overestimation of bypass protein can results from microbial contamination, especially of forages. Purines determination (Zinn and Owens, 1986) has been utilized estimate microbial N contamination, however, it is timing consuming and imprecise. Mass et al. (1999) determined that neutral detergent solution was effective to detach microbes on in situ residue

In this study, *in vitro* method with protease from *S. griseus* (SG; Roe et al., 1991) sub-estimated consistently (P < 0.05) *in vivo* DIP values for all three forages. Variations for SG among runs (SEM=0.77) and among forages (SEM=0.54) were low. The use of protease methods has becoming widespread because it has good correlation to *in vivo* DIP values. Coblentz et al. (1999) reported that in vivo DIP values for alfalfa and prairie hay and those from using protease from *S. griseus* were highly correlated (r=0.95). Abdelgadir et al. (1996) reported that *S. griseus* protease method gave similar results to in situ

values after a carbohydrase pretreatment. This method has been adopted for several protein systems to estimate protein degradation (Sniffen et al, 1992). It has been mentioned that protease from *S. griseus* does not have the same specificity of bacterial protease of rumen and that proteolytic activity is variable in the rumen. However, the method is simple and does not need fistulated animals.

From Table 6, it is possible appreciated that all methods studied estimated very well protein degradation of ALFA. In contrast, NDIN-2, where NDIN is actual laboratory number for BER and it uses *in vivo* values for passage rate and it was the only method that estimated well degradable intake, the others underestimated protein degradation. Protein degradation of prairie is variable because its percentage in DM is low, however, NDIN-1, similar to NDIN-2 and passage rate for *in vivo* values. The single point methods, IS_{16} and IS_{20}, underestimated protein degradation.

It can be concluded that *in vivo* trails involved a lot of work and variation in duodenal flow and protein microbial production. With less work, some alternative methods can predicted with certain confidence level the *in vivo* DIP values. Although protease method sub-estimated consistently *in vivo* DIP values, it was very precise

between assays and it is easy to standardize. The type of forages affected predicted values from in situ methods.

Implications

Values for protein degradation coming from *in vivo* trials are the most adequate because they result from protein and animal interactions. However, variability in the determination and labor needed to conduct *in vivo* trials, justify the search for more simple methods to estimate DIP values. Although, *in situ* methods give good estimation, fistulated animals and standardization of the method are required. Protease method is the easiest to standardize, most repeatable, cheapest and, fast, compared with other methods.

Table 1. Chemical composition of experimental forages offered to beef steers

Variable[1]	Hays		
	Alfalfa	Bermuda	Prairie
DM	90.9	91.9	92.8
		%, DM basis	
OM	91.1	94.5	91.8
CP	20.7	11.48	4.78
NDF	54.0	70.32	70.43
ADF	39.2	31.57	42.59
NDIN	1.03	0.91	0.48
ADIN	0.59	0.41	0.29

[1] DM: Dry matter, OM: Organic matter, CP: Crude protein (x 6.25), NDF: Neutral detergent fiber, ADF; Acid detergent fiber, NDIN: Neutral detergent insoluble nitrogen, ADIN: Acid detergent insoluble nitrogen

Table 2. Intake and digestibility coefficients of alfalfa, bermuda-grass, and prairie hay consumed by beef steers

Variable	Hays			SEM
	Alfalfa	Bermuda	Prairie	
DMI, kg/d	13.2[a]	11.1[b]	7.6[c]	0.4
OMI, kg/d	12.1[a]	10.5[a]	7.0[b]	0.4
DMD, %	62.0[a]	53.7[b]	45.1[c]	1.5
OMD, %	61.5[a]	54.2[b]	46.0[c]	1.4
ACPD, %	71.4[a]	53.4[b]	20.2[c]	3.5

[1] DMI: Dry matter intake, OMI: Organic matter intake; DMD: Dry matter digestibility, OMD: Organic matter digestibility and ACPD: Apparent crude protein digestibility

[2] SEM: Standard error of mean

[a, b, c] means within the same row without common subscripts are different $P < 0.05$

Table 3. Ruminal fermentation and microbial efficiency in beef steers consuming alfalfa, bermudagrass, and prairie hay

Item[1]	Hays			SEM[2]
	Alfalfa	Bermuda	Prairie	
ARFOM, %	69.0	57.9	62.5	3.1
TRFOM, kg d^{-1}	9.4[a]	7.1[b]	5.0[c]	0.5
MOEFF, g N / TRFOM kg	11.7	14.4	8.9	2.0

[1]ARFOM= Apparent ruminal fermented organic matter, TRFOM= Truly fermented organic matter in rumen, MOEFF=Microbial efficiency

[2]SEM: Standard error of mean

[a, b, c] means within the same row without common subscripts are different $P < 0.05$

Table 4. Particle passage and digestion rates of NDIN in animals fed alfalfa, bermudagrass or prairie hay

Variable[1]	Hays			SEM[2]
	Alfalfa	Bermuda	Prairie	
ADIA intake, g/h^{-1}	4.24[a]	10.36[b]	16.95[c]	0.75
ADIA in rumen, g	125.08	330.67	719.76	24.32
Particle passage, $\% \ h^{-1}$	3.61[a]	3.17[ab]	2.39[b]	0.23
Digestion rate $\% \ h^{-1}$	0.012[a]	0.015[a]	0.008[b]	0.001

[1] ADIA=Acid detergent insoluble ash of rumen digesta at 0 and 4 h pos-feeding. Particle passage rate calculated as: ADAI intake (g/h^{-1})/ (average ADIA g in rumen at 0 and 4 h posfeeding), Waldo and Smith, 1972;47; Digestion rate calculated from *in situ* NDIN residue at 2, 12 and 96 h of incubation in rumen, then slope equation was applied

[2] Standard error mean

[a, b, c] means within the same row with uncommon superscripts differ $P < 0.05$

Table 5. Comparison of methods to estimate *in vivo* DIP of alfalfa, Bermuda-grass and prairie hay.

Forage	Methods						
	In vivo, %	IS_{16}	IS_{20}	NDIN-1[1]	NDIN-2[1]	SG[2]	SEM[3]
	Differences in percentage units (estimate - in vivo)[1,2]						
Alfalfa	84.3	+0.7	+1.9	+2.4	-7.2	-7.2	2.3
Bermuda	70.3	-15.6[a]	-8.5	+9.0	-0.3	-12.9	2.3
Prairie	49.7	-24.9[a]	-17.2[a]	+6.7	+5.0	-10.4	2.3

[1] Undegradable intake protein (UIP)=NDIN * kp/(kp+Kd); where NDIN was considered to be equal to the intercept of regression equation of NDIN digestion (NDIN-1) or direct determination of NDIN in the forages (NDIN-2), kp=passage rate and kd=digestion rate

[2] *Streptomyces griseus* protease method (Roe et al., 1991)

[3] Standard error mean

[a] Mean difference does not included value zero (P < 0.05)

Table 6. Simultaneous confidence intervals at 95% for the comparison of methods to estimate in vivo DIP of forages of different nutritional value.

Method[1]	Alfalfa	Bermuda	Prairie hay
In vivo	79.4 - 89.2	65.4 - 75.1	44.8 - 54.5
IS_{16}	80.2 - 89.9	47.0 - 56.7*	19.2 - 29.7*
IS_{20}	81.4 - 91.1	54.1 - 63.6*	27.6 - 37.4*
NDIN-1	81.3 - 91.1	54.1 - 63.9*	51.5 - 61.2
NDIN-2	81.8 - 91.6	62.2 - 72.0	49.8 - 59.3

[1] IS_{16} and IS_{20}= In situ procedure for 16 and 20 h rumen incubation. NDIN-1= Effective degradability for NDIN pool estimated from the intercept of straight line. NDIN-2 is similar to NDIN-2, but NDIN pool is equal to forage NDIN determined in the laboratory. Effective degradability= NDIN*(kd /(kd + kp). Kp=passage rate determined by ruminal evacuation and ADIA as indigestible marker

* Within each forage interval confidence differs from interval confidence for *in vivo* DIP ($P < 0.05$)

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CHAPTER V

EFFECT OF RUMEN OR ABOMASUM INFUSION OF NITROGRN ON FORAGE INTAKE AND DIGESTIBILITY IN STEERS CONSUMING LOW QUALITY FORAGE

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ABSTRACT: To determine the effect of degradable intake protein (DIP) or undegradable intake protein (UIP) infusions on forage intake, digestion and N balance of cattle fed ad libitum coarsely chopped prairie hay (PH), eight ruminal and duodenal cannulated Angus steers were used in a replicated 4x4 Latin square experiment. Experimental periods lasted 16 d. All N sources were continuously infused isonitrogenous amounts (55 g N /d^{-1}) in the form either casein (DIP) or urea (UDIP) into rumen, or casein into abomasum (UIP). Control animals (CON) received water continuously infused into abomasum. Casein and urea infusions were dissolved in water, and a peristaltic pump was used to infuse each suspension at a rate of 2.5 ml/min. Refusal and total urine and fecal outputs were collected, weighed and samples. Data were analyzed using a model that

included square, pen, period, and treatment effects with the GLM procedure of SAS. When the F-test was significant ($P < 0.05$), non-orthogonal contrasts were performed: 1) Control vs. supplemental N, 2) Infusion site casein in the rumen (DIP) vs. abomasum (UIP), and 3) Type of protein in the rumen casein (DIP) vs. urea (UDIP). Compared to CON, N supplementation increased ($P < 0.05$) forage intake (FI), OM intake (OMI) and digestible organic matter intake (DOMI), but no differences were detected among supplemental N treatments. Compared to CON, digestibility coefficient for OM (OMD) was increased by supplemental N. Except urinary urea N, N infusions increased apparent N digestibility (AND), fecal N (FN), total urinary N (UN), total N excretion (TNE) and retained N (NR) compared to CON, which resulted in an improvement in N status. Nitrogen digestibility, TNE and NR were similar among N infusions; however, differences in excretion N routes among N infusions were detected. Urinary urea excretion was greater for UIP and UDIP compared to CON, but CON and DIP were equal. It is concluded that N content of forage limited voluntary intake by beef steers, however forage intake can be improved by the continuous infusion of N to the rumen or abomasum. The increase in forage intake was not accompanied by increased digestibility of forage

suggesting increased passage through GI tract. Additionally supplemental N improved N status and N retained.

Key words: Forage, Degradable Protein, Undegradable Protein, Low Quality Forage, Urea, Casein.

Introduction

Ruminants tend to maximize fiber digestion of low quality forages by increasing its digestive capacity and by decreasing passage rate, sacrificing total intake (Clauss et al., 2003). In grazing ruminants, voluntary intake is one the most important factors that limits animal productivity (Grovum, 1987; Lippke, 2000). However, morpho-physiology characteristics of ruminant stomach impose limits to forage intake and passage rates. Therefore, it is important for nutritionist to understand the factors that control voluntary intake in ruminants, especially when animals are consuming low quality forages. The most important factor that limits consumption of low quality forages by ruminants is N availability in the rumen (Balch and Campling, 1965; Egan, 1965a; NRC, 1985). Depending on magnitude of N requirements of rumen microorganisms and the ruminant animal, N supplementation can either increase forage intake and/or forage digestibility, thus impacting animal performance (McCollum

and Horn, 1990; Van Soest, 1994). Because of N deficiency of low quality forages, the increase in forage intake is the most common response to N supplementation. Non-protein N (Campling and Freer, 1961; Garza et al., 1992; Loest et al. 2001) and protein-N sources (DelCurto et al., 1990; Bodine et al., 2000; Bandyk et al., 2001) increase the voluntary intake of ruminants consuming forage.

A second mechanism by which N supplementation could increase food intake is by increasing the supply of amino acids flow into intestine, stimulating microbial production (Fleck et al., 1988) or dietary amino acids (Chermiti et al., 1994; Bandyk et al., 2001) or alleviating an AA deficiency (Keery et al. 1993; Volden, 1999). Responses to UIP sources are more probable in cattle consuming forage high in protein (Donaldson et al, 1991; McCann et al, 1993) where degradable intake protein requirement have been met (Titgemeyer and Löest, 2001). However, it is not well understand how absorbed amino acids increase forage intake. It is has suggested that absorbed amino acids increase N recycling (NRC, 1996) or improvement in energy efficiency in the animals (Egan, 1965b).

The objectives of this study were to determine the effect of infusion site, ruminal or duodenal, of casein or

urea on forage intake and digestibility of prairie hay and on N balance.

Materials and Methods

Animals

The Oklahoma State University Animal Care and Use Committee approved the experimental protocol. Eight steers fitted with ruminal and duodenal cannulae, averaging about 544 kg were used in a replicated 4x4 Latin Square design (Steel et al., 1997). At the beginning of experiment, animals were assigned randomly to squares and to individual metabolism stalls with ad libitum access to fresh water (2 x 3 m) in an environmentally controlled room. When not in metabolism stalls, animals were move to individual pens (3 x 4 m).

Experimental periods

Each experimental period (Figure 1) consisted of a 10-d phase for adaptation to forage, management and infusion procedures. After the adaptation phase, refusals and total outputs of feces and urine were collected, weighed and sampled. At the end of each experimental period, animals were relocated in individual pens for 5 d for a period of rest between experimental periods; then, animals were relocated the same metabolism crates.

Experimental treatments

Sodium caseinate (New Zealand Dairy Board, Wellington, New Zealand) is a highly degradable protein source with a low percentage of escape protein (Hristov and Broderick, 1994; Köster et al., 1996;) and a highly absorbable protein source in intestine (Yu et al., 1996; Sarwar and Peace. 1994; Sarwar, 1997). Therefore, when infused in rumen it was assumed that casein supplied only degradable intake protein (DIP) and when infused in abomasum, casein supplied only undegradable intake protein (UIP). In order to evaluate the effect of type of protein (casein vs. urea), a fourth group was infused N in form of urea (55 g urea N/d^{-1} in 3.6 L of water) infused continuously into rumen. Thus, four experimental treatments were designed: 1) Animals were fed coarsely chopped prairie (5.0 % CP) and infused tap water (3.6 L) into abomasum (CON), 2) Control + casein (400 g/d dissolved in 3.6 L of water) infused continuously to abomasum via ruminal cannula (UIP), 3) Control + casein (400 g/d dissolved in 3.6 L of water) infused continuously to the rumen (DIP), 4) Control + urea (55 g urea N g/d dissolved in 3.6 L of water) infused continuously to the rumen (UDIP). The amount of N from urea was isonitrogenous to that of casein nitrogen. Because it had been mentioned that abomasum distension decreased voluntary intake in

sheep (Grovum, 1979), it was decided to keep abomasal cannula in all animals through the duration the entire trail.

Casein suspension

Daily, the casein was prepared as suggested Macleod et al., (1982): 400 g casein and 5.3 g of bicarbonate were mixed in warm water (80-90°C). Casein solutions were kept under continuous stirring in the lab until use. Urea solutions was prepared daily with tap water. A peristaltic pump (Model 205CA, Watson Marlow, Falmouth, England) was used to infusion dissolution at approximately a rate of 2.7 ml/min. Hays were offered with no mineral, protein or energy supplements. Due to the fact that urea-based diets are deficient in sulfur-amino acids (Hill et al., 1985; Ferreira and Nolte, 2002), steers in UDIP treatment received 20g/d of magnesium sulfate anhydrous (EM Science, Darastad, Germany) in gelatin capsules inserted into rumen twice a day.

Forage feeding

Basal diet consisted prairie hay, which was coarsely chopped in a hammer mill using a 3 cm screen. Hay was offered daily at 08:00 in plastic feeder. To assure an *ad libitum* voluntary intake, offered forage was increased 2.5

kg over the previous day's forage intake. Offered forage varied between 125 to 130% of previous day intake. Refusals were withdrawn from feeder daily, weighed and sampled, except for adaptation phase. Previous day's refusal was dried at 55 C for 72 h in a forced-air oven. Dried samples were ground in Wiley mill through a 2-mm screen and composed per animal.

Fecal collection

Daily total fecal output per animal was collected, weighed, mixed by hand, and sampled on d 12 to 16. The sample per animal (about 500-700 g) was weighed in aluminum pan and dried at 55 C for 96 h. After drying, samples were ground, composed per animal in each period, and stored at room temperature until analysis in the laboratory.

Duodenal flow determination

From d 7 through 15 of each experimental period, each animal received 10 g of chromic oxide (Cr_2O_3) daily, as an indigestible marker to measure duodenal flow. Chromic oxide was dosed intra-ruminally in gelatin capsules containing 5 g Cr_2O_3 at 0800 and 2000. To estimate daily duodenal flow, duodenal samples were taken every 6 h hours, advancing two hours every day, until completing a 24-h period. Duodenal pH was recorded immediately using a

portable, combination electrode pH meter (Corning 870, Corning, NY). Duodenal samples were kept in refrigeration (- 20 C) until their analysis in the laboratory.

Total urine collection

Total urine collection was carried out during d 16 to d 21. Urine outputs were collected in 20-L jar containing 6 N HCl to keep urine pH lower than 3.5. Acid inclusion into urine jars was adjusted on a steer basis to ensure adequate acidification of samples. The volume of added volume varied from 200 to 600 ml/steers. All jars with urine per steers were weighed and, if it was needed, total urine output was mixed in a single container; then a sample of urine (1% of volume) per animal was kept and stored at -10°C. The weight of one-L sample was recorded to calculate urine density. Urinary N excretion (g/d) could be estimated followed next formula: urine output in (L)= urine wt (kg) divided by density, then total urinary N excretion (g/g)= L*1000*(mg N/ml of urine).

Laboratory analysis

Sodium caseinate, forage, refusals and fecal samples dried at 55°C were analyzed for dry matter and organic contents by oven drying at 100°C over night and by ashing at 550°C for 6 h in a muffle furnace, respectively. Crude protein

(N x 6.25) of forage samples, refusals, fecal samples were determined for total nitrogen by Kjeldahl method using Kjeltec 2400(Foss Tecator, Höganäs, Sweeden). To determine urea N concentration, composed sample were thawed at room temperature and, then, centrifuged at 11,000g for 10 min. Urea N in the urine samples was determined by using urease type III (E.C.3515; 45,000 UI/g) based in a spectrophotometric method described by Fawcett and Scott, 1960.

Statistical analysis

The effects of square, animal and period nested in square, and treatment on forage intake, digestibility, fecal excretion, urine N excretion and N retained were analyzed using GLM procedure of SAS (1991)in a experimental design replicated 4x4 Latin square design (Steel et al., 1997). When the F-test was significant ($P < 0.05$) for treatment term (Kuehl, 2000), non-orthogonal contrasts were performed: 1) Control vs. supplemented N treatment, 2) Effect of site: casein in the abomasum (UIP) vs. casein in the rumen (DIP) and 3) Effect of type of sources Casein in the rumen (DIP) vs. urea in the rumen (UDIP). An animal receiving DIP treatment was retired during the first

experimental period due to low forage intake, so that data were missing for first period.

Results and Discussion

Chemical composition

Table 1 shows the chemical composition for prairie hay and sodium caseinate utilized in the present experiment. Prairie hay in the present study was higher in CP and lower in NDF and ADF fractions than prairies utilized by Köster et al. (1996) and Bandyk et al. (2001). In previous studies (Garza et al., 1992 and Basurto at al., 2003) reported similar CP concentration for prairie hay.

Forage intake

Compared with CON, supplemental nitrogen, independent of source or infusion site, increased ($P < 0.05$) forage intake (Figure 2). Forage intake in the UIP treatment was increased by 28 % over CON. In contrast, rumen infusion of N as casein or urea increased forage intake by 46% over CON.

Köster et al. (1996) reported that steers consuming tall grass prairie (1.9 % CP) increased forage intake by 64% with the lowest level of DIP supplementation (180 g casein/d), and with a highest level (540 g casein/d) forage intake was doubled over the control. Non-protein sources,

such as urea or ammonia sulfate, have also been effective in increasing forage intake of low quality forage. Romero et al (1976) supplemented urea N (50 g/head/d) over various delivering times to steers consuming spear grass (2.25% CP), which increased their forage intake in 27 % respect to control. Campling and Freer (1965) reported that cows increased forage intake of oat straw (2.94% CP) by 38% over control when urea (150 g/d) or urea (150g/d)+sucrose (500g/d) solutions were continuously infused in rumen.

Although, protein and non-protein N sources can increase the forage intake, the increase voluntary intake is by true protein sources are more consistent or higher than non-protein sources (Minson, 1990). Prior (1974) reported that lambs consuming diets based on corn, wheat straw, cornstarch, and cellulose with either soybean or urea twice a day or 12 times a day, DM intake and N retention were higher for those fed ration with soybean. During protein degradation in the rumen, protein sources release amino acids, peptides, and branched-chain amino acids into ruminal fluid, promoting microbial protein production. Several studies have showed that microbial growth is enhanced by amino acids and peptides (Wallace, 1996; Atasoglu et al., 1999). However, it has been mentioned peptide uptake by bacteria requires carbohydrates

(Argyle and Baldwin, 1989; Van Kessel and Russell, 1996) to maximize the utilization of amino acids. This suggests that potential of DIP to increase microbial production could be restrained by energy in animals consuming low quality forage. Hamali et al (2001) could not detect that that the supply of peptides from casein increased efficiency of microbial growth on diets based on brome and prairie hays, contrary to based-corn diets. It is probable that rumen bacteria could be catabolizing casein extensively to amino acids to get energy, reducing availability of amino acids to microbial growth.

With regards to pos-ruminal infusion of casein (UIP), UIP increased forage intake over CON. In a similar study, Bandyk et al. (2001) reported that casein infusion in abomasum increased forage intake of a low quality forage over CON animals. Similarly, Egan (1965c) that reported rumen ammonia increased when 10 g/d of casein N were infused pos-ruminally to sheep eating oat hay. It can be hypothesized that the increase in forage intake could be related to recycling of α-amino-N because as availability of amino acids increases, amino acid catabolism is too increased, elevating blood urea (Lobley and Milano, 1997). Kennedy (1985) reported that cattle given tropical pastures or alfalfa, transfer of endogenous urea to the rumen in

about 8.6 to 13.5 g N/d and 20.7 g N/g, respectively. Archibeque et al. (2001) estimated that approximately 28 and 32 g N/d are recycled into gastro intestinal tract of steers fed gamagrass or switchgrass hay. Forage intake was increased by 33% when 17 g urea-N/d was infused postruminally in single dose to steers consuming 5.8 kg of prairie hay (Garza et al., 1992). This suggested that N recycling contributes an important quantity of N the rumen. It is accepted that the increase in forage intake by N supplementation are related to a major activity microbial that accelerate the comminution of particle size (Campling and Freer, 1961), or increasing ruminal motility, forcing to pass particles size larger through reticulo-omasum orifice (Luginbuhl et al., 1990) or improving N status of animals (Egan, 1965b). However, this last factor is not known very well how it is involved.

Total tract digestion

Compared to CON, the total apparent digestibility of OM, N, NDF, and ADF were increased ($P < 0.05$) by N supplementation (Table 2), but there were no detected ($P > 0.05$) differences in the digestibility among supplemental N.

Smith (1979) proposed that if N supply to the rumen is inadequate, rumen function can be affected in three ways: 1) Digestion of starch and fiber is depressed, 2) ATP is utilized in the synthesis in the storage of carbohydrates, 3) Turnover of bacterial matter is increased, and 4) A major proportion of energy is utilized for bacterial maintenance. Certainly, the depression of digestion of fiber is an important issue with diets based on low quality forages. The low activity of rumen bacteria results in a decrease of the rate breakdown of particle size of plant material, reducing forage intake (Grovum, 1988). In current trial, digestion of prairie hay was depressed by a N deficiency, which was alleviated when protein supplementation was supplemented. When infused in the rumen, urea or casein increased digestion of prairie at a similar rate. Sites of infusion (rumen vs. abomasum) were not different in their capacity to increase the digestion of prairie hay. However, the differences in the forage intake, but similar digestibility, resulted in an increase in digestible organic matter intake in supplemented-N animals over CON (Figure 1).

Bandyk at al. (2001) reported that infusion of casein in rumen or in abomasum increased OM digestibility and tended to increase NDF digestibility of tall grass prairie.

The increases in digestibility reported by Bandyk et al. (2001) are similar to those reported here. The digestibility coefficients increased by 4 to 5 percentage units over control. Contrasting, Köster et al (1996) reported that as DIP level increased, microbial production increased, however, OM digestibility was not affected by DIP level. The increase in forage intake and OM digestibility resulted in a higher digestible organic matter intake (DOMI) compared to CON (Figure 2). In addition, DIP increased DOMI respect to UIP (Figure 2). As forage intake increased, fecal OM excretion was increased, as a reflex of forage intake.

Nitrogen digestibility was lower in the animals in CON treatment, compared to supplemented-N animals (Table 2). Nitrogen digestibility was higher ($P < 0.05$) for UDIP than for DIP treatment. The N digestibility did not was not affected ($P > 0.05$) by site where casein was infused. The low N digestibility in CON animals is related to low protein content, bound protein in fiber and high metabolic N excreted. Köster et al (1996) reported protein digestibility for tall grass prairie was -39.8; that is substantial lower to observed in the present study (14.7%). Tall grass prairie from Köster's study had a low CP content of 1.9% CP vs 5.0% in the present study. In a previous

study, Basurto et al. (2001) reported that N digestibility for prairie hay was 20.1%, under similar experimental conditions as this trial.

Fiber is the largest component of DM in low quality forage; therefore increasing the NDF digestibility can impact the animal productivity. In the present study, supplemental nitrogen increased NDF digestibility, independently of site or type of protein. Likewise, Köster et al. (1996) infused several levels of casein as DIP in the rumen in steers consuming tall grass with very low N content and could detect that NDF digestibility was increased by the first DIP levels (180g/d), but higher DIP levels (540 and 720 g/d) were ineffective to increase more NDF digestibility. Sunvold et al. (1991) reported that protein supplements increased DM digestibility of dormant bluestem range, but not NDF digestion. They suggested that N sources differ little in their effects on DM or ADF digestibilities of prairie hay (Younis and Warner, 1990). However, Köster et al. (1997) reported that the substitution of soybean meal by urea, as DIP source decreased DM and NDF digestibilities linearly. It has been mentioned that true protein supply branched chained amino acids (BCAA), which are required for cellulolitic bacteria; this can explained the reduction of NDF digestibility when

urea level is increased. However, in the present study, no difference was detected between DIP and UDIP, what does not support the previous statement. Köster et al. (1997) reported a linear reduction in BCAA as soybean was substituted by urea. Brondani et al (1991) suggested that after that N deficiency in the rumen by DIP was corrected, BCAA become limiting. However, it is possible that low passage rate or long retention time reduces the need of BCAA because of turnover of bacterial matter (Owens and Zinn, 1988). However, in the current study, no differences were detected in total microbial production (Table 2) or in the efficiency of microbial growth.

Although it was not measured in current trial, there are data that support that N infusion to animals fed low quality forages reduces retention time and rumination time and increases the reduction rate of particle size of forage. It can expect that N supplementation increases microbial activity making particle forage became weaken, so that particles are broken easier during rumination and mixing cycles of rumen (Grovum, 1988). Campling and Freer (1965) reported that cows eating wheaten hay decreased their rumination time when 150 g of urea was infused in the rumen. When proportion chopped/ground orchard hay was 90/10 was changed to 10/90, rumination retention time from

11 h to 7.3 h and rumen retention time from 47.5 to 31.2 h (Bernard et al., 2000). Sunvold et al. (1991) reported that a supplement with 20% CP increased passage rate of dormant bluestem forage, measured by using insoluble acid detergent fiber, from 1.5 to 2.05 %/h. Bandyk et al. (2001) reported that passage rate of tall grass prairie without supplements was 2.06 %/h, which was increased to 3.36 %/h when 400 g of casein were infused in the rumen and to 2.26 %/h when infused in abomasum. It can not be conclude that N supplementation increases forage intake by increasing efficiency of rumination to reduce particle size and increasing passage rate by increasing when microbial activity. The duodenal OM flow was lower in CON than N-supplemented steers what can suggested a faster passage rate of OM.

Nitrogen intake

In the table 3, N intake for and DIP and UDIP included N forage plus infused N in the rumen either casein or urea. Likewise, N intake for UIP included N forage plus infused N in abomasum. The differences in N intake were due to differences in forage intake. Therefore DIP increased N from forage by 12 g/d over UIP treatment. The differences in N intake were due to the differences in forage intake

(Table 3). As expected, N supplementation increased forage intake and casein was more effective to increase forage intake than casein post-ruminal. This observation supported what Bandyk at al. (2001) reported. They found that UIP increased forage intake in a smaller extent compared to DIP. It was expected that UDIP would be lower than DIP effect and higher than UDIP. It is accepted that urea N utilization is improved when feeding frequency is increased (Romero et al, 1976; Owens et al., 1980). However, in the present study, UDIP increased forage intake as the same level as DIP did. For, UIP is expected that N recycling increases with N supplementation, but it is not expected that it fill all N requirements of rumen bacteria. In the present study, UIP increased forage intake by almost 2 kg respect to CON; that is an important amount. Archibeque et al, reported that 28 to 32 g urea N were recycled in steers, that is an important amount, related to nitrogen intake.

Fecal nitrogen

The low protein content of prairie hay is accompanied by a high content of acid detergent insoluble N (ADIN) that includes heat-damaged protein and nitrogen associated to the lignin. It is considered to be indigestible in the

gastro-intestinal tract (Van Soest, 1994). The ADIN concentrations vary: in a forage of high quality, such as alfalfa and bromegrass, ADIN constitutes about 7% and 3% of total CP (Blasi et al., 1991; Elizalde et al., 1999), while in a low quality forage such as bahiagrass constitutes of 11.0%. Prairie hay used in a previous experiment (Basurto et al., 2001) had an acid detergent insoluble acid about 30.0% of total crude protein. Besides of low protein content and low availability of N in prairie hay, low quality forage could increase endogenous or metabolic N in feces. Fecal N could represent about 6 - 12 % of N intake (Van Soest et al., 1980; Ouellet et al., 1999). The low digestibility of the low quality forages is due to mentioned factors, the low protein content, low degradability and endogenous N, resulting sometimes in negative digestibility. Nitrogenous fraction in feces included indigestible feed N, endogenous or metabolic N and microbial remains from ruminal bacteria or bacteria that grew in cecum and rectum (Van Soest, 1994). Van Soest (1994) pointed that bacterial matter explained 80% of fecal N, which depends on intake, quality diet and animal species. It is probably that more feed N origin could be present in feces because as forage intake increased more undegradable N was ingested as ADIN. In the present study,

the excretion of ADIN (0.29 % in DM; Basurto et al., 2001) could be 21 g N/d (7.3 kg DM intake * 0.29 % ADIN DM basis), representing almost the half of N excreted. Changes in total fecal N excretion were related to forage intake.

Urinary N excretion

Urinary excretion was increased ($P < 0.01$) by supplemental N, but UDIP excreted ($P < 0.01$) more N than DIP. However, infusion site of casein did not affect urinary N excretion. Total N excretion was lower ($P < 0.01$) in CON animals, compared to supplemented animals. Total N excretion (urinary plus fecal excretions) in supplemented animals; animals that received UDIP excreted more ($P < 0.05$) total urinary N than animals receiving DIP or UIP. Finally, urea N excretion was lower in CON animals, compared to supplemented animals. Urea infused in the rumen resulted in a higher urea N production than casein infused in the same site. Urea N excretion was similar ($P > 0.05$) when casein was infused in the rumen or the abomasum.

Nitrogen balance

Table 3 shows N intake and N balance in present study. Nitrogen intake is the sum of N contained in ingested

forage plus infused N in rumen or abomasum. As expected, supplemental N increased N intake, respect to CON. However, N intake for DIP treatment was higher ($P < 0.05$) than N intake for UIP. Type of N source was not different ($P > 0.75$) Fecal N followed the same tendency for N intake, where CON excreted less ($P < 0.01$) through feces fecal N and animals in DIP excreted more N through feces than UIP. No difference in fecal N was observed between DIP and UDIP ($P > 0.87$). Independently of infusion site or type of source, absorbed N was higher ($P < 0.01$) in supplemented animals than in CON animals. Nitrogen retention was higher ($P< 0.01$) in supplemented animals, but similar among supplemented animals.

The increment in N digested in UIP is explained by highly digestibility of amino acids of casein and by increasing in forage intake in 2 kg. Several studies have showed that casein protein is highly digestible. Sarwar and Peace (1994) and Sarwar (1997) reported that in mature rats, true digestibility of casein was 95-95 %. The low urinary N excretion in CON animals is due to the low N intake. Although, DOMI was similar between DIP and UDIP, the greater urinary excretion in UDIP can be related to higher plasma urea N. Ruminal ammonia and plasma urea were not measured in the present study, but Köster et al. (1997)

reported that ammonia concentration in the rumen when casein was substituted by urea in N basis. They reported that concentration of ammonia was 65% higher in diets with 100% of substitution. On the other hand, there is a good correlation between ruminal ammonia and plasma urea (Thornton and Wilson, 1972; Huntington, 1986) and between plasma urea and urine N excretion (Thornton and Wilson, 1972; Van Soest, 1994).

That animals responded increasing their forage intake to different protein sources and infusion sites can suggest that more than one mechanism is involved in the control of low quality forage. Urea was as good as casein as degradable intake protein when degradation rate of urea is controlled, in this trial by supplying at low rate of infusion.

Implications

By altering the site of infusion and type of nitrogen infused, it is possible to manipulate routes of N excretion without affecting N retention of mature beef cattle. Urea can be an inexpensive way to supplement nitrogen to grazing cattle consuming low quality forages if ruminal degradation of urea can be controlled.

Table 1. Chemical composition of prairie hay and sodium caseinate

Item	Prairie hay	Sodium caseinate[1]
	%	
Dry matter	91.4	98.9
	Dry matter basis, %	
Organic matter	92.6	96.4
Crude protein	5.0	87.9
NDF	68.8	-
ADF	40.4	-

[1]New Zealand Dairy Board, Wellington, New Zealand)

ITEM \ DAY	Thr 1	Fri 2	Sat 3	Sun 4	Mon 5	Tue 6	Wed 7	Thr 8	Fri 9	Sat 10	Sun 11	Mon 12	Tue 13	Wed 14	Thr 15	Fri 16
Forage	Forage Adaptation										Forage intake					
Weight	X														X	
Protein infusion	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cr_2O_3 Dose										X	X	X	X	X	X	X
Duodenal sampling															X	X
Refusal											Refusal sampling					
Urine											Urine collection					
Feces											Feces collection					

Figure 1 Schedule for experimental periods

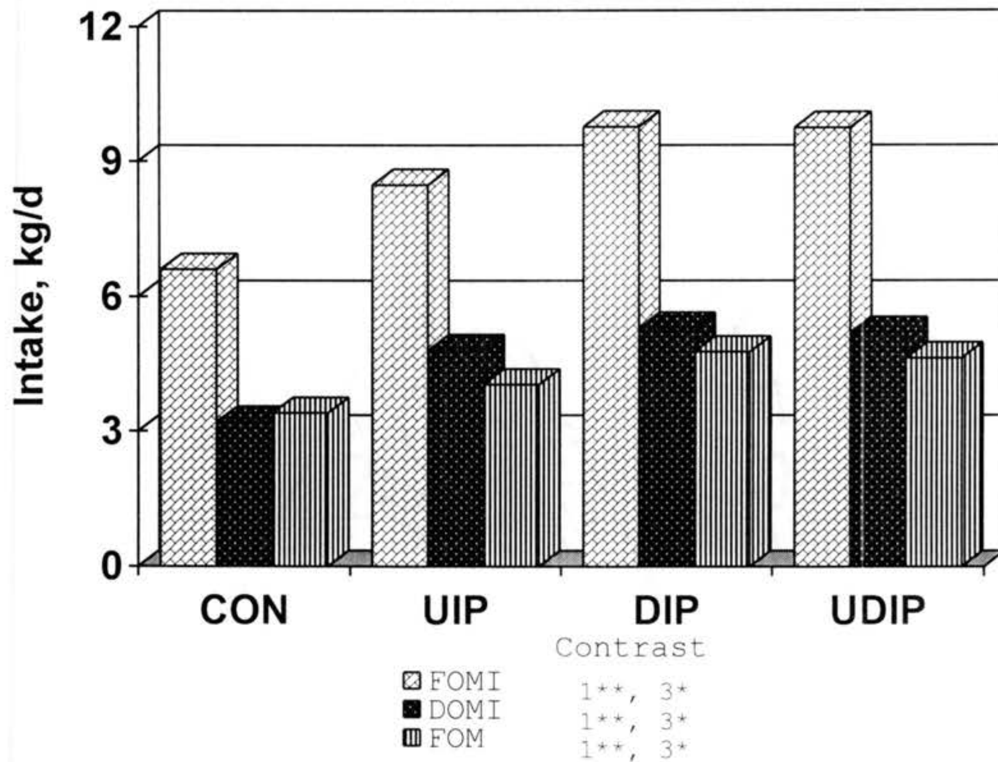

Figure 2. Effect of degradable or undegradable intake protein on forage OM intake (FOMI) and digestible organic matter intake (DOMI) and fecal OM output (FOM) of steers fed prairie hay

[1,2,3] Non orthogonal contrasts: 1) CON vs. supplemental N 2) Casein vs. urea in the rumen, and 3) Casein in abomasum vs. casein in the rumen *($P < 0.05$), ** ($P < 0.01$)

Table 2. Effect of continuous infusion of DIP or UIP on digestibility and ruminal digestion in steers fed *ad libitum* prairie hay

	TREATMENTS [1]					Contrast [3]		
Variable	CON	UIP	DIP	UDIP	$\sqrt{MSE}$[2]	CON vs ALL	DIP vs UDIP	DIP vs UIP
Dry matter, %	45.5	51.4	50.0	49.6	3.3	0.01	0.82	0.43
Organic matter, %	48.3	54.4	52.9	52.3	3.2	0.01	0.87	0.42
Protein, %	23.9	55.4	51.5	54.2	5.3	0.01	0.37	0.20
NDF, %	47.0	51.9	51.0	51.7	3.8	0.01	0.73	0.67
ADF, %	39.3	45.1	44.3	44.6	3.7	0.01	0.91	0.72
MICN[4] g/d	54.0	64.4	75.6	70.9	16.9	NS	NS	NS
MOEFF[4],g/100g ARDOM	9.6	10.8	12.6	9.5	3.9	NS	NS	NS

[1] CON: prairie hay; UIP: CON + abomasal infusion of casein (55 N g/d); DIP: CON + ruminal infusion of casein (55 N g/d) and UDIP: CON + ruminal infusion of urea (55 N g/d)

[2] $\sqrt{\text{Mean Square Error}}$

[3] Non orthogonal contrasts: 1) CON vs. supplemental N 2) Casein vs. urea in the rumen, and 3) Casein in abomasum vs. casein in the rumen (P < X)

[4] MICN=Microbial N in duodenal flow (g/d); MOEFF= g microbial N/100 g apparent OM digested in rumen (ARDOM)

Table 3. The effect of continuous infusion of DIP or UIP on intake, digestion, excretion, and retention of nitrogen in steers fed prairie hay (g/day)

Variable	TREATMENTS[1]				√MSE[2]	Contrast[4]		
	CON	UIP	DIP	UDIP		CON vs ALL	DIP vs UDIP	DIP vs UIP
N intake[3]	56.9	126.0	138.0	140.0	7.3	0.01	0.36	0.02
Fecal N	42.2	56.3	67.4	64.2	5.9	0.01	0.46	0.01
Urinary N	19.3	44.3	34.3	51.8	7.7	0.01	0.01	0.07
Total N excretion	61.6	100.5	101.6	116.1	10.4	0.01	0.05	0.69
Retained N	-4.7	25.4	36.3	24.0	8.8	0.01	0.07	0.11
Urinary urea N	1.6	10.9	7.9	13.5	4.3	0.01	0.05	0.49

[1] CON: prairie hay; UIP: CON + abomasal infusion of casein (55 N g/d); DIP: CON + ruminal infusion of casein (55 N g/d) and UDIP: CON + ruminal infusion of urea (55 N g/d)

[2] √Mean Square Error

[3] Nitrogen intake included infused N (55 g N d^{-1})

[4] Non orthogonal contrasts: 1) CON vs. supplemental N 2) Casein vs. urea in the rumen, and 3) Casein in abomasum vs. casein in the rumen ($P < X$)

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APPENDIXES

APPENDIX A. Climate data for Stillwater area from 1993 to 2000 and Normals for the period 1971 to 2000

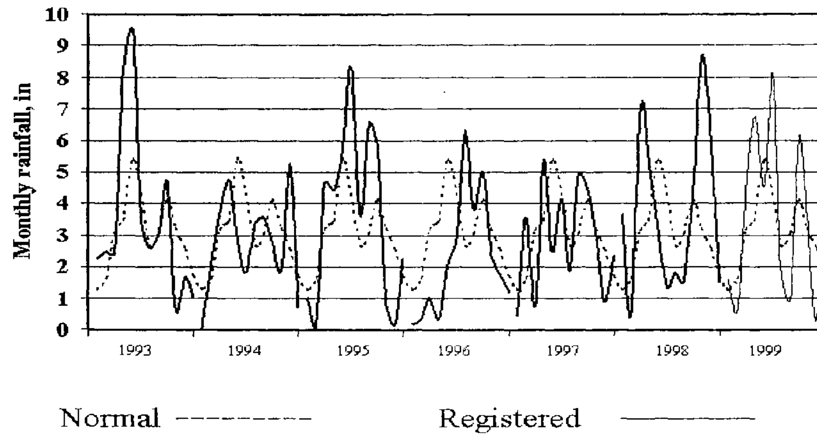

Figure 1 Monthly mean rainfall (inches) in the area of Stillwater from 1993 to 1999 and Normal rainfall for the period 1971 to 2000

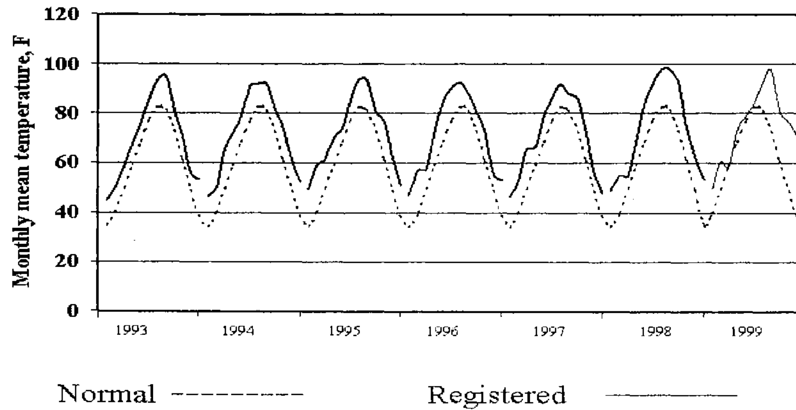

Figure 2 Monthly mean temperatures in the area of Stillwater from 1993 to 1999 and Normal temperature for the period 1971 to 2000

APPENDIX B. Relationship *in vivo* DIP values and DIP estimates from different methods to estimate protein degradation

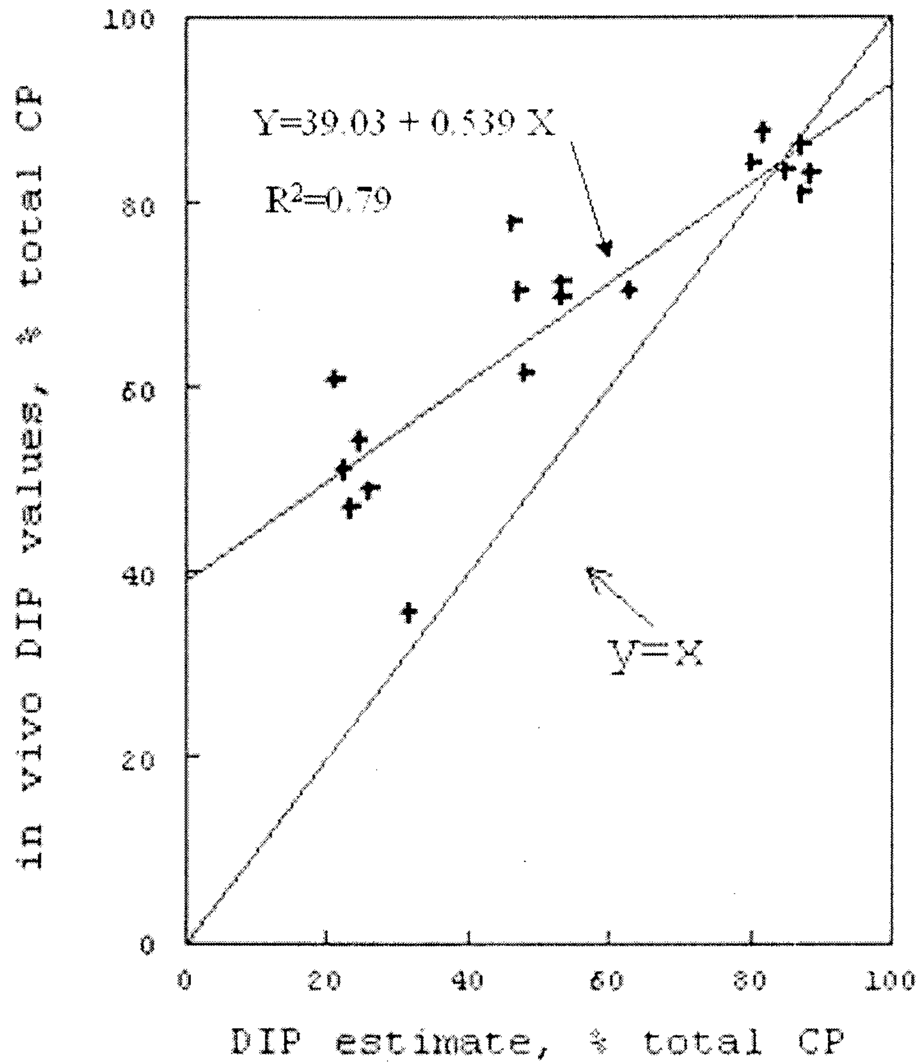

Figure 1. Relationship in vivo DIP values and DIP estimates` from *in situ* method by incubating samples for 16 h in the rumen (IS16)

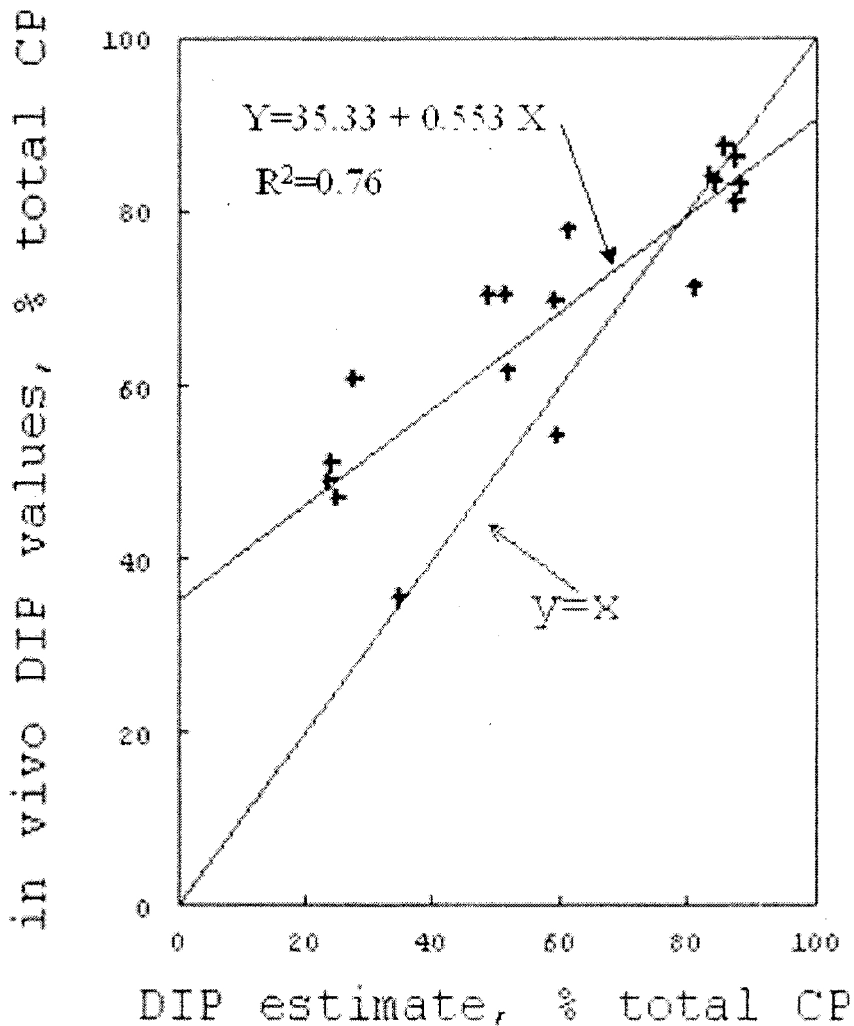

Figure 2. Relationship in vivo DIP values and DIP estimates` from *in situ* method by incubating forage samples for 20 h in the rumen (IS20)

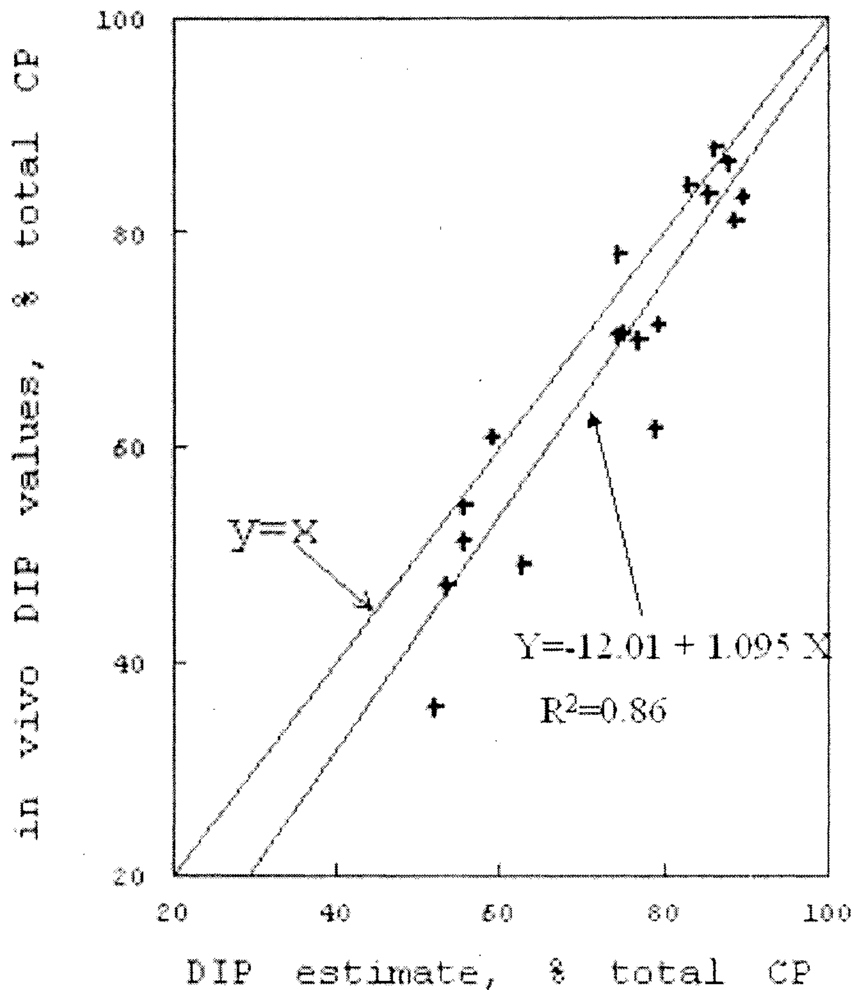

Figure 3. Relationship in vivo DIP values and DIP estimates` from *in situ* method by estimating initial NDIN pool and estimating digestion extent of NDIN (NDIN-1)

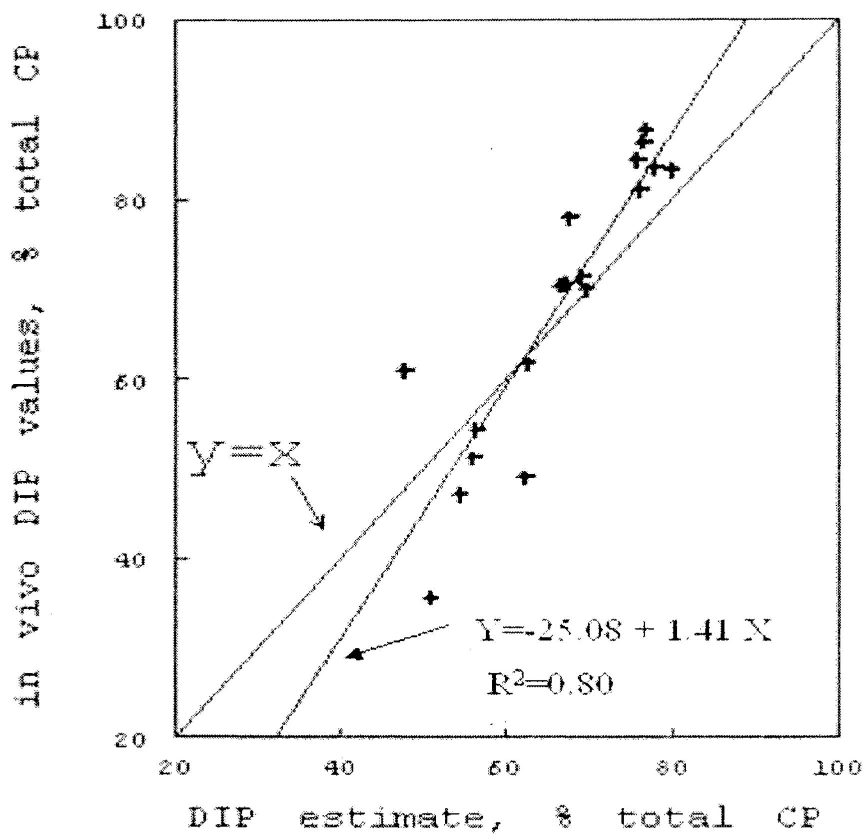

Figure 4. Relationship in vivo DIP values and DIP estimates from *in situ* method by using actual NDIN measured in the laboratory and estimating digestion extent of NDIN (NDIN-2)

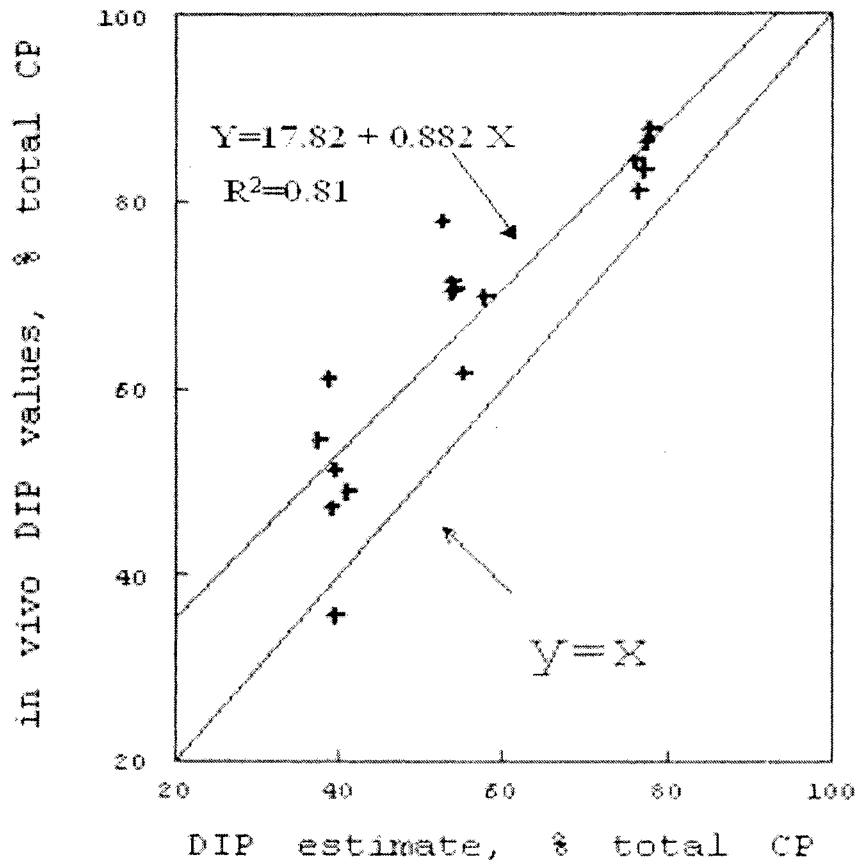

Figure 5. Relationship in vivo DIP values and DIP estimates from *in situ* method by Streptomyces griseus protease (SG; Roe et al., 1991; Mathis et al., 2001)

APPENDIX C Preliminary data for nitrogen recycling and endogenous production of urea

Table 1. Partial data for nitrogen recycling and endogenous production of urea (mmol/d) in steers fed *ad libitum* prairie hay

Obs	TRT[1]	PEN	UER[2]	GER[3]	%[1]
1	UIP	5	181.5	167.8	0.92
2	UIP	11	189.3	161.6	0.85
3	UIP	7	27.15	27.8	
4	DIP	6	87	70.0	0.80
5	DIP	13	53.5	49.1	0.92
6	CONTROL	5	74.9	71.7	0.96
7	CONTROL	6	30.6	28.6	0.94
8	CONTROL	11	39.7	38.0	0.96
9	CONTROL	13	30.1	28.8	0.96
10	UDIP	5	75.7	41.4	0.55
11	UDIP	6	152.4	101.5	0.67
12	UDIP	11	43.4	35.1	0.81
13	UDIP	13	149.9	143.5	0.96
14	UDIP	7	118.1	87.4	0.74

[1] UIP=Undegradable intake protein (400 g/d casein infused in abomasum); DIP= Degradable intake protein (400 g/d casein infused in rumen); UDIP= Degradable intake protein (123 g/d urea infused in rumen); CONTROL=3.6 Lt/d water infused in abomasum

[2] UER=Urea entry of urea (endogenous urea production), mmol/d

[3] GER=Gastrointestinal entry of urea (urea that enters to total gastrointestinal tract

[4] Percentage of endogenous urea production entering to gastrointestinal tract (GER/UER)

VITA (2)

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Doctor of Philosophy

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