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DEVELOPMENT OF A SET OF FORAGE STANDARD TO ESTIMATE IN VIVO DIGESTIBILITY OF FORAGES AND PREDICTION OF FORAGE QUALITY OF DIETS CONSUMED BY CATTLE GRAZING NEBRASKA SANDHILLS RANGE PASTURES

by

Bobbi Gene Geisert

A DISSERTATION

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Under Supervision of Professors Terry J. Klopfenstein and Don C. Adams

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DEVELOPMENT OF A SET OF FORAGE STANDARD TO ESTIMATE IN VIVO DIGESTIBILITY OF FORAGES AND PREDICTION OF FORAGE QUALITY OF DIETS CONSUMED BY CATTLE GRAZING NEBRASKA SANDHILLS RANGE PASTURES

Bobbi Gene Geisert, Ph.D
University of Nebraska, 2007

Advisors: Terry J. Klopfenstein and Don C. Adams

In beef cattle productions systems, feed costs accounts for the majority of production costs. In year-round grazing systems, knowledge of diet quality is important for supplement formulation and predicting animal response in order to meet production goals without increasing feeding costs. The objectives of these two trials were: to develop a set of feed standards to use in *in vitro* laboratory procedures to estimate *in vivo* digestibility of forages, determine the effect of moisture, day, and grazing level on diet quality, and develop prediction equations to estimate diet quality using the variables moisture, day and grazing level. Trial 1 used 8 crossbred yearling steers to determine in vivo digestibility of 5 chopped hays (Malf, Ialf, Mbrome, Ibrome, and prairie). Feces, feed, and feed refusals were analyzed for DM, OM, CP, NDF, IVDMD and protein fractions. Feed samples were included in 21 separate IVDMD runs and regressed against the *in vivo* digestibilities. As hay digestibility increased DMI increased ($P<0.01$). Slopes of the 21 regression equations did not differ; however, there were differences between the individual IVDMD runs. In vivo and *in vitro* digestibilities were correlated and the average for all 21 runs was $r = 0.831$. In trial 2, diet samples were collected using esophageally-fistulated cows from pastures varying in grazing pressure from May 2003 through November of 2005 in the Nebraska Sandhills. Diet samples were analyzed for CP, IVOMD, NDF, and protein fraction. In Vitro OMD was adjusted to in vivo digestibility using the regression equations generated from the hay standards within each run. Diet digestibility and CP were used in a series of multiple regression equations to

predicted diet quality using the variables moisture, day and grazing pressure. Diets were higher in CP and OMD during the growing season and remained constant during the dormant season. Predicted digestibility and protein were correlated to observed values.

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The Lord has blessed my life in so many ways that I can not list them all. I have been blessed with all of my family, friends, professors, who have supported and encouraged me, good health, and Gods unconditional love. I am extremely thank full for all of the gifts that I have received.

First on the list to thank for all of there love and support is my parents I could not have accomplished so much without you. Also their ability to move large quantities of furniture and house hold belongings (usually on short notice) very well (9 times to be exact). I know we are all hoping we do not have to load and unload the car hauler any time in the near future. It is amazing as I look back over my life so far and see all of the things that I have gained from both mom and dad I couldn't list them all. Some things I don't particularly care to admit to. The learning process starts at such a young age it still amazes me the lessons you learn when you are young that carry with you for what seems like forever. For example I remember when I was young I was "helping" dad with chores and got stuck in the mud out in the middle of the cow correl. I ended up walking out of my boots into the mud. Dad dropped what he was doing to dry my tears and clean me up. I learned from that lesson that no matter how messy life gets my parents would always be there to pick me up, clean me off, and set me back on my way. I know this because I have been stuck in the mud several times not to mention all of the times life dealt me a hand I wasn't prepared to play. Also maybe the most important lesson learned was that if the mud is deep and it looks like you might get stuck choose an alternative path. I would

like to think that dad was the one that taught me how to be tough and strong but I think that mom had a pretty strong hand in those lessons. Mom may not look really strong and tough but don't let her fool you she is pretty tricky. She can load and unload a 18 foot trailer full of heavy furniture and belongings or drive all night to get you to the rodeo on time just to sit in the hot and dusty sun all day to cheer you on. Mom you have taught me that the most important strength comes from the heart. You both have showed me how to work hard, stand up for what you believe in, and to go after your dreams no matter how big you dream. Growing up it may have seemed like I did really want a younger sister. But who knows where I would have ended up without you. Together we have learned how to laugh, love, and box. I love the idea that I get the opportunity to look up. I truly admire the honesty and strength. We have lived so far apart for so long I am excited to have the opportunity to spend more time together again. Georgina, I could not leave you out of this section. You are a amazing gift from God. I have learned so much about unconditional love from you. You have showed me how to believe and have faith in the Lord. You are my angel on earth. I would like to thank Mark for bringing me into his life and family. You have given me much more support and love than I could have dreamed of. I really enjoy the time we spend together and look forward to the future. I also look forward to all of the future fishing and hunting adventure as longs as I still get to bring home some big ones.

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Love,

Bobbi Gene

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Introduction

There are approximately 4.5 million ha of Sandhills in the state of Nebraska which is more than 25% the land surface area in Nebraska. This region is located above the largest aquifer in North America known as the Ogallala Aquifer. The 4.5 million ha are primarily dominated with native, warm and cool season grasses(USDA - Census Agriculture; Nebraska Agricultural Statistics, 1997). This vast and unique resource gives Nebraska the opportunity and capacity for turning low protein and high cell wall forages into high quality protein (beef) for human consumption. In Nebraska, the state's economy depends on the beef cattle industry as it is the single largest industry in the state with 1.97 million head of beef cows and 4.85 million head of fed cattle (USDA - Census Agriculture; Nebraska Agricultural Statistics, 1997). These numbers make Nebraska the number 2 beef producing state in the US. Four counties in Nebraska are ranked in the top 10 US counties, holding the top three positions in the US for the number of beef cows (Cherry County-number 1, Holt County-number 2, Custer County-number 3, and Lincoln County-number 10) (USDA - Census Agriculture; Nebraska Agricultural Statistics, 1997). All of these counties are located in the Sandhills and depend heavily on the native grasses as feed resource. Proper management of the natural resources is important in the sustainability of the cattle industry in the state and the sustainability of Nebraska.

When formulating rations and supplements for grazing livestock it is important to know the protein and energy of the forage they are consuming. It is difficult to determine the energy and diet that grazing animals consume because forage quality is in general

constantly changing throughout the year and from year to year. It is also difficult to quantify diets consumed and the total intake of the animal. With over 200 plant species growing in the Sandhills, all of which differ individually in nutrient content, availability, and palatability, knowing exactly which plants and how much are consumed through out the grazing periods is difficult. When using the NRC models for diet formulation knowing accurate CP and TDN values is critical to formulating diets that supply enough nutrients to the animal to maintain production without supplying too much (Lardy et al., 2004; Patterson et al., 2006). Over supplementation of grazing livestock can be financially costly to the producer especially when the cost of energy and protein feeds are high.

Literature Review

Diet Sampling

Obtaining accurate diet samples from grazing cattle can be challenging, especially in mixed grass prairies were the cattle can select not only different plant parts but different plants in different proportions. Different methods have been established to estimate diet chemical and botanical composition. These include hand clipping, hand plucking, and animals fitted with fistulas (esophageal and rumen). There has been some debate and concern over which method more accurately samples the grazing animals' actual diet.

Using live animals for diet collection allows for researchers to account for animal selection of specific plants or plant parts, whereas hand-clipping or plucking samples

may not account for what the animal actually consumes in a natural setting. Hand-clipping or plucking techniques have advantages of eliminating the use of animals which reduces cost, labor, and care of those animals. Little equipment is needed for clipping or plucking, and it is easy to obtain a sample. Clipping large numbers of quadrats require large amounts of labor and time.

The use of esophageally fistulated animals is not a new technique. This technique has been reported as early as the late 1800's by Claude Bernard (Bernard 1855 sited by Van Dyne and Torell, 1964) and Pavlov (Pavlov 1887 sited by Van Dyne & Torell, 1964). Surgical procedures have been reported in mature animals by several researchers (Van Dyne and Torell, 1964 and Bishop and Forseth, 1970). Adams et al. (1991) discussed successful surgical establishment in suckling calves. Surgical procedures have been altered over the past 100 years in order to reduce stress to the animal and improve the success rate. Success is not only safe and harmless establishment in the animal, it is also measured in the ability to utilize the animal to collect representative samples. Torell (1956) tested methods for successful esophageal fistula surgery and collection of consumed forages. Success rates have been estimated to be at least 90% (Van Dyne and Torell, 1964). Animal longevity has been reported to be more than 4 years (sheep) and greater than 6 years (cattle) (Langlands, 1969; Grings et al., 1995). Another method for collecting diet samples is using ruminally fistulated animals. This method for collecting a diet sample also allows for use of an actual animal, however, as opposed to hand clipping or plucking, it is very labor and time extensive as compared to esophageal fistulated collections. Rumen contents must be evacuated, safely stored while out of the

rumen, and replaced in a reasonable amount of time. Exposure to temperature outside the animals' body temperature, oxygen, and sun light can have detrimental effects on the rumen microbes. The use of ruminally fistulated animals also limits the number of collections which can be performed within a short amount of time, and they may not be suited for cold, open, winter range conditions. There is also the possibility of decreased selectivity due to an empty rumen (Olson et al., 1991).

Olson (1991) evaluated different collection techniques in steers which were fitted with both esophageal and rumen fistula. Grazing diets were collected using 3 different procedures (rumenally, esophageally with rumen evacuation, esophageally without rumen evacuation). Chemical variables of diets indicated no differences between any of the sampling procedures suggesting that empty rumens did not affect diet selectivity. No difference between ruminal or esophageal samples suggests that researchers can use either to obtain diet samples with confidence that diet chemical variables are not impacted. Olson concluded that choice of collection method using live animal models should not be based on rumen evacuation decreasing selectivity and altering dietary chemical variables. It should however be based on resources and labor availability. Diet collection through the use of rumen fistulation has several management disadvantages as compared to esophageal fistulas (Olson, 1991). These disadvantages include time and labor evacuating and cleaning the rumen, limited number of times an animal can be used within a week or day, unsuitable for cold, open winter ranges, potential decreased diet selectivity due to an empty rumen, difficulty in determining size of sample while grazing, and potential microbial contamination (if rumen is not properly cleaned).

Olson et al. (1991) showed that there were no differences in diet nutrient content in samples collected from rumen fistula and esophageal fistulas (with and without rumen contents). Arnold et al. (1964) studied the differences in behavior and production measures of non-fistulated ewes and esophageally fistulated ewes. Data indicated no difference in lamb birth weight, growth weight or mortality from ewes with or without esophageal fistula. No differences were observed for wool production, grazing time, grazed herbage or herbage intake. Animals with esophageal fistulas could remain productive throughout their life without major negative effects on the dam or their offspring.

One major concern with esophageally and ruminally collected samples is salivary contamination. Saliva can contaminate the sample with protein, minerals and moisture (Lesperance et al., 1960a; Hoehne et al., 1967; Barth et al., 1970; Scales et al., Little, 1972; 1974; Cohen, 1979). Bovine saliva is 1.02 % DM (Baily & Balch, 1961) and contains 0.85 (Lesperance et al., 1860a) to 0.89 % (Baily & Balch, 1961) ash. Nitrogen content of cattle saliva when fed alfalfa hay ranged from 0.007 to 0.27% and 0.003% to 0.018% when grazing desert range (Galt et al., 1976). These values are higher than results published by Bailey and Balch (1961) who reported salivary N levels of 0.003% to 0.007% in steers fed alfalfa. Salivary N content of cattle grazing mountain summer range have also been reported as 0.04% (Cook et al., 1964) which is higher that the N content in saliva from cattle grazing desert range (Galt et al., 1976). Salivary contamination does not appear to significantly alter N content of diets collected from fistulated animals (Bath et al., 1956; Galt et al., 1976; Lesperance et al., 1960). Wallace

et al. (1972) evaluated the effects of salivary contamination on esophageally collected diet samples. Salivary contamination increased the ash content of samples. However, it did not alter nutritional components of the samples when calculated on a OM basis. The same results were seen when hand clipped samples were compared with hand clipped samples soaked in saliva. Expressing data on an ash free basis minimizes the effects of salivary contamination of minerals and soil contamination (Van Dyne & Torell, 1964; Wallace et al., 1972). Using collection bags with screens in the bottom to allow for saliva to drain from the sample as the animal grazes and squeezing the extrusa sample can reduce the amount of contamination from saliva in the sample collected. Conclusions from Barth and Kazzal (1971) indicated that the leaching of N from screen bottom bags equaled salivary N contamination. Samples in their study collected in solid bottom bags had higher N. Researchers should be aware of the risks associated with salivary contamination in the sample when designing and conducting grazing experiments including diet collection from animals.

Another concern with using fistulated animals to collect diet samples are the sources of variation in chemical and botanical composition of the diet collected. These sources include day to day, within day, and animal variation (Arnold, et al., 1964; Obioha et al., 1970; Torell et al., 1967). Data from 10 different trials (Obioha et al., 1970) indicated that morning samples contained slightly more N than those collected in the evening (during summer experiments). This relationship was reversed in trials during the fall (only 1 trial). Researchers attributed the fall results on N content of the diets to the cattle attempting to satisfy hunger in the morning and consequently consuming fewer

forbs and more grasses at this time. No differences were detected in this trial on lignin content between samples collected in the morning verses those collected in the evening. Torell et al. (1970) collected the same pasture for 10 consecutive days and noted a decrease in CP from day 2 through 10. They attributed this decline to the advancing maturity in the forages available for grazing or the animals were becoming less selective. They however, noted that the pasture had been grazed a total of 40-d by collection d 10 which could change total forage availability. Obioha et al., (1970) indicated significant differences between day in dietary N content with increasing numbers of grazing days. They indicated the difference in daily N content was due to changes in animal preference. As preferred plant species are removed cattle shift their consumption to other plant species

Obioha et al. (1970) suggested that 3 animals per treatment for 4 different days would allow one to detect 10% difference in N with an 85 % confidence interval and 10% probability. In a study by Torell et al. (1967) researchers concluded that the number of animal days (animal x day) needed to predict (95 % confidence interval) CP was 5.6 %, ether extract was 28.7 %, and crude fiber was 4.1 %. The variation between days can also alter not only diet chemical composition but also dietary botanical composition. The number of diets needed to accurately determine botanical composition is greater than the number needed to measure chemical composition (Galt et al., 1969; Harniss et al., 1975; Holecheck & Vavra et al., 1983). Galt et al., (1969) reported that 6 animals would adequately predict botanical composition in grazing situtations. Holecheck et al. (1983) stated 5 animals and 6 collections are needed to accurately determine forage classes (in

forest and Grasslands in Northeastern Oregon) but more animals and collections are needed to determine forage species (90% confidence interval).

Other sources of possible variation are sex, age and breed differences in diet selection (Langlands, 1969; Ferrell et al., 1979; Hodgson and Janieson, 1981; Miller and Gaud, 1990; Grings et al., 1995; Hollingsworth-Jenkins et al., 1995; Mohammad et al., 1996). Hollingsworth-Jenkins et al. (1995) collected diet samples from nursing calves and lactating cows in the Nebraska Sandhills range pastures during the summer. Calves consumed diets higher in CP and escape protein as compared to the mature cows. *In vitro* OMD was similar between the diets collected by calves and cows. Similar results (Hodgson and Jamieson, 1981) also were seen between calves and mature cows (lactating and non-lactating), where calf diets were higher in digestibility than cow diets. No differences were seen between lactating and non-lactating cows. When calves that were not experienced grazers were used no differences were detected in digestibility between calf and cow diets.

Langlands (1969) summarized data from 8 trials including 120 different esophageally fistulated sheep. In three of the trials, N content of diets were compared between 6-month-old and 66-month-old Merino sheep. Numerical differences were observed in N content between the two age groups in 2 of the 3 trials. Statistical differences were observed in the other trials. When evaluating of diets collected from 6 and 18 month old Border Leicester sheep no differences were seen in N content of diets. Researchers concluded that immature sheep tend to select diets higher in protein than mature sheep. Grings et al. (1995) found similar results between 9 month old nursing

calves and mature steers. Researchers concluded that calves selected diets which were significantly higher in protein, lower in ADF, and lower ADL as compared to the diets selected by the steers. Botanical composition of diets collected in eastern Colorado from calves and cows, differed (Walker et al. 1981). Calves consumed 4 percentage units more sand bluestem as compared to mature cows.

Young calves are able to be more selective and are better equipped to pick specific plants and leaves as mature animals due to the smaller size of the muzzle. The smaller muzzle size of younger animals allows them to maneuver and choose higher quality material easier. The sex of the animal may also effect diet selectivity. Diets collected from rams and ewes differed (Ferrell et al., 1979; Miller and Gaud, 1990), likely because of differences in maintenance energy requirements and DMI between the different sexes effecting diet selectivity. Langlands et al. (1969) documented no differences between N content in diets collected from rams and ewes. Mohammad et al. (1996) tested the difference between mature cow and steer diets. No differences were found across season in total number of grasses, forbs, and shrubs consumed between fsteers and cows. Within seasons cows selected more grasses and less Forbs and shrubs as compared to the steers. In most cases these differences were small. Differences between botanical composition of diets were greatest between the sexes during periods of unfavorable forage conditions (late winter and early spring).

Walker et al. (1981) compared botanical composition of diets collected from 3 different breeds of cows and calves (Hereford, Angus X Hereford, and Charolais X Hereford). No differences were detected between breed within age group. Herbel et al.

(1966) also reported no difference in botanical composition between Hereford and Santa Gertrudis.

Methods for Assessing Dietary Botanical Composition

Botanical composition of grazing animals' diets can be determined through several different techniques all of which have their own benefits and limitations (Holechek et al., 1982). These techniques include diet observation, utilization techniques, fistula sampling and fecal analysis. Direct diet observation requires minimal equipment and time thus decreasing experimental monetary costs. However, accuracy and precision of botanical composition estimates are compromised, especially in wildlife species and non-tame domestic animals. Direct observation makes it difficult to quantify how much of a plant was consumed (Holechek et al., 1982). The oldest procedures used to estimate diet botanical composition are utilization techniques and include evaluating differences between grazed and un-grazed plots, evaluation differences before and after grazing, general observation, and cage plots. The advantages of utilization techniques include time and information is provided on location of grazing and the extent that the range and range species are being used. One major limitation with utilization techniques during the growing season is that plants are continually growing and utilization techniques do not account for grazing of plant regrowth (Holecheck et al., 1982). Also other losses from weathering and animal trampling could confound results (Cook and Stoddart, 1953). The use of cages could also create some challenges in altering microclimates that could change forage growth (Grelen, 1967 and Owensby, 1968 as reported by Holechek et al., 1982).

Stomach analysis has been used in wildlife research. The major disadvantage of this approach is animal sacrifice is required. This approach is impossible for endangered species and areas of low populations not to mention the loss is costly for owners. Fecal analysis allows researchers to sample large numbers and areas. This method works well in areas of low animal populations, and does not interfere with normal animal habits, can compare several animal species, and requires little equipment. Major disadvantages include accuracy because forage species passed in feces are often not proportional to consumption, no knowledge where the forage was consumed, feces identification, requires extensive reference plant collection, aging of feces effects on identification, and plant identification is tedious and time consuming (Holechek et al., 1982).

Diet Selectivity

Reports of diet selectivity of grazing livestock under a variety of management and environmental conditions are common (Weir and Torell, 1959; Reppert, 1960; Cable et al., 1966; Langlands, 1966; Bredon et al., 1967; Bedell, 1968; Langlands, 1969; Barth & Kazzal, 1971; Rao et al., 1973; Vavra et al., 1977; Taylor et al., 1980; Judkins et al., 1985). There are several variables that affect diet selection such as grazing pressure and weather conditions. Diet collection using hand-plucking or clipping techniques do not represent the diets that the animal actually consumes (Edlefsen, et al., 1960; Cook, 1964; Kiesling et al., 1969; Jefferies et al, 1969; Rao et al., 1973; Blümmel and Grings, 2000). Blümmel and Grings (2000) evaluated diets collected by esophageally fistulated heifers and hand-plucking from May through September. Samples from esophageally fistulated

animals were higher in both CP and IVDMD as compared to hand-clipped samples. Chemical variables and IVOMD of diets from esophageally-fistulated heifers were more closely related to animal weight gain than variables from hand-plucked samples. Kiesling et al. (1969) showed that diets collected via esophageally-fistulated steers were higher in protein and ash, but lower in fiber compared to the hand-plucked samples. The higher fiber in the hand-plucked samples suggests that the steers were selecting diets higher in digestibility. Jefferies et al. (1969) collected diet samples from esophageally - fistulated steers and compared nutrient content of those samples to clipped samples. Data from this trial indicated that in years of abundant moisture, steers consumed diets higher in crude protein compared to the hand-clipped samples. Researchers attributed this difference to selectivity for forbs (*Snapis arvensis,annual* mustards; *Kochia scoparia*, fireweed summer cypress; and *Salsola kali*, Russian thistle) in high rainfall years when forbs were abundant. Sheep selected different plants *Lothium perenne-Trifolium subterraneum* and *Festuca arundainacea-Trifolium subterraneum* (ryegrass-subclover and tall fescue-subclover, respectively) pastures with advancing seasons (Bedell, 1968) . They selected higher amounts of subclover during spring in both pasture types. In the summer they preferred tall-fescue to subclover, they still preferred the subclover in the ryegrass-subclover pastures. Wallace et al., (1972) reported similar chemical analyses variables and digestibility between diets collected from hand-clipping and esophageally-fistulated steers.

Plant species preference can also vary between different seasons (Cook et al., 1958; Heady and Torell, 1959; Galt et al., 1969; Rosiere, et al., 1975). Data from the

Semidesert Grassland indicated that cattle grazed 28 of the 52 different plant species present (Rosiere et al., 1975). Twenty of the 28 species comprised 84-95% of the steers' diet in all seasons. Steers consumed the highest amount of grass species in the summer and lowest in the spring. Shrub portions were highest in spring (*Yucca elata,* soaptree yucca). Forb fractions did not vary greatly between seasons but were highest in the winter. These botanical composition changes were observed in both none-grazed and grazed pastures. Grazing specific plants during earlier seasons reduces their availability later in the grazing period. This forces animals to graze other available species later in the grazing period (Galt et al., 1969).

Differences in dietary quality (chemical composition) among animal species have been documented (Cook et al., 1963; Van Dyne and Heady, 1965; Ngugi et al., 1992). Diets collected from sheep were higher in quality as compared to cattle grazing in common on the same dry annual range Cook et al. (1963). Botanical composition also differed between the two species(Cook et al., 1963). Sheep diets contained 35% grass, 40% forbs, and 25% browse whereas, cattle diets contained 55% grass, 25% forbs, and 20% browse. Ngugi et al. (1992) studied the differences in dietary composition of 5 major ungulates (*Antilocapra americana*, pronghorn; *Oclocoileus hemionus*, mule deer; *Cervus elaphus*, elk; *Bos taurus*, domestic cattle; and *Ovis aries*, domestic sheep) living in southcentral Wyoming. During the spring, sagebrush (*Artemisia tridentata*) was more abundant in pronghorn than elk diets, whereas elk were consuming a larger amount of graminoids. In the summer pronghorn diets were higher in sagebrush as compared to deer and cattle. The deer and pronghorn consumed larger amounts of bitterbrush

(*Purshia tridentata*) than did cattle and the cattle consumed more graminoids. In the fall pronghorn, deer, and elk diets contained similar amounts of sagebrush and forbs. Deer and pronghorns consumed more bitterbrush than elk. The differences in animal species preference for plant species consumed makes it important to use the appropriate animals to collect diets for research comparisons. Assumptions in dietary botanical and chemical composition between species should not be used.

Variables Effecting Diet Quality and Plant Nutritive Content

Plant species, stage of plant growth, and weather conditions during different stages of growth impact forage quality. Mixtures of cool and warm season plants can extend the grazing season and impact forage quality due to differences in the growing season between these plants. Plant species vary between regions because of adaptation of plants to specific environmental varieties (e.g., moisture, temperature, growing degree days). Management of forage allocation and utilization also impact quality.

Season and Plant Maturity

No single factor affects forage quality more than plant maturity. Plant nutritive attributes change throughout its life cycle (Kamstra et al., 1968; Wallace et al., 1972; Kamstra, 1973; Cogswell and Kamstra, 1976; Powell, et al., 1983; White, 1983; McCollum et al., 1985; McCollum and Galyean, 1985; Hakkila et al., 1987; Lardy et al., 1997; Johnson, et al., 1998). During vegetative stages, leaf:stem ratios are at their highest and decrease as the plants mature and reproduce. This decrease in leaf:stem ratio directly decreases forage quality. As plants mature, digestibility and CP decrease

whereas lignin, ADF, and NDF increase (Kamstra et al., 1968; Wallace et al., 1972; Kamstra, 1973; Cogswell and Kamstra, 1976; Powell, et al., 1983; McCollum et al., 1985; McCollum and Galyean, 1985; Lardy et al., 1997; Johnson, et al., 1998). In monoculture plant communities, there is a single peak in the spring or summer months (depending if C_3 or C_4) when plant material is at the highest nutritive quality (Cogswell and Kamstra, 1976; Kamstra, 1973). In mixed communities (mix of C3 and C4 grasses) there are generally two peaks of maximum nutritive quality, one for the cool season grasses during the later spring early summer and one for the warm season grasses during early to mid summer. Warm season grasses peak later in the growing season than do the cool season species (Cogswell and Kamstra, 1976). Cogswell and Kamstra (1976) showed a decrease in CP and digestibility and an increase in ADF from June to September in 2 warm season and 2 cool season prairie forage plant species. Similar results were seen in masticate samples collected from native range in western North Dakota (Johnson et al., 1998). Crude protein level decreased linearly and UIP increased linearly with advancing season (Mid-June through December). Dietary IVOMD decreased from June to October.

White (1983) studied seasonal changes of tillers from 2 grass species which indicated that reproductive tillers were 7-9 percentage units lower in DMD compared to vegetative tillers. Crude protein of the vegetative tillers was near 25% then decreased to 5.9% in the reproductive tillers. Lardy et al. (1997) evaluated chemical composition of diets collected from esophageally-fistulated cows from both Sandhills upland range pastures and subirrigated meadow during growing and dormant seasonal months. Crude

protein and IVDMD remained relatively constant throughout the dormant season (November through April in range diets and November to March in subirrigated meadow diets). Protein and IVOMD were highest in April for the subirrigated meadow and in June for range diets. This could be attributed the differences in cool season and warm season plants in each of the different plant communities. Subirrigated meadows used in this trial were predominately cool season grasses and legumes, whereas the upland range pastures consisted largely of warm season grasses with lesser amounts of cool season plants. Crude protein and IVDMD decreased from June to September, remained relatively constant through March, and then began to increase through June in upland range pastures. Samples from the subirrigated meadows followed the same pattern with the spike in CP and IVDMD occurring earlier in the year. Comparing the upland range samples to subirrigated meadow samples, upland samples were generally lower in CP and digestibility during the growing season.

In mixed grass prairies, diet protein and digestibility appear to be the greatest in late spring through early summer. In prairies where both cool and warm season grasses exist there are two peaks for IVOMD and CP, one in May (cool season species) and one in June-July (warm season species). Diet quality declines through the remaining growing season as the plant matures and reproduces. Cool season grasses will have a slight increase in protein and digestibility in late summer due to some regrowth, if there is soil moisture for growth. After the plants become dormant diet quality remains relatively constant. It will decrease with increasing grazing pressure due to the removal of higher quality plants and changes in dietary botanical composition.

Kamstra (1973) evaluated seasonal changes of four grasses, two warm season (little bluestem and blue grama) and two cool season (western wheatgrass and green needlegrass) species. Protein decreased linearly from early June through late August, early September for three of the species. Blue grama grass decreased from early June to early August, than increased slightly (~ 2 percentage units) in September. Lignin content of the four grasses increased with advancing stages of maturity. As lignin increased, digestibility decreased. McCollum and Galyean (1985) evaluated seasonal changes of diet in digestibility of blue grama grass in south-central New Mexico. Digestibility decreased from early August through late October sampling dates (66.5, 63.1, 51.6, and 47.9 % for , early August, late August, late September, and late October, respectively).

Seasonal changes in the nutritive value of bluestem pastures were evaluated (Roa et al., 1973) in the Flint Hills, near Manhatten, Kansas. Organic matter digestibility and DMD were higher in June and July and rapidly decreased (approximately 10 percentage units) from August through October sampling dates. Crude Protein decreased linearly ($P < 0.05$) from June (7.35 %) through October (3.75 %)

Hakkila et al. (1987) reported that cattle grazing desert grassland ranges changed their diet with seasonal advance to maximize diet quality. Stockpiling forage (allowing forage to accumulate during the growing season without grazing for use at a later date) is one management tool that could be utilized to extend the grazing season and decrease the need for harvested forage (Transtrom et al., 2003) which could be economically advantageous (Adams et al., 1994). In a study by Transtrom et al. (2003) data indicated no change in nutrient composition of the forage available for grazing based on hand-

clipped data of stockpiled winter range in western North Dakota. However, the dietary botanical composition varied throughout the fall and winter grazing periods. The data indicated that cows altered dietary botanical composition during different periods of grazing.

Moisture

Precipitation, or the lack of, affects both plant yield and quality (Smoliak, 1956; Dahl, 1963; Rauzi, 1964; Hazell, 1965; Shiflet and Dietz 1974; Hart et al., 1983; Kirby and Parman, 1986; Powell et al., 1986) however, the results have been mixed. Hart et al. (1983) indicated an increase in CP of western wheatgrass and blue grama after abundant spring rainfall. Crude protein of blue grama, however increased after high summer precipitation. Wilson (1983, as sited by Nelson and Moser, 1994) reported digestibility of leaf and stem portions of warm season grasses were highest in water stressed plants. Similar results were seen with increased IVDMD in alfalfa (Snaydon, 1972; Halim et al., 1989). Extended periods of drought generally cause delays in plant maturity, decreased shoot length (resulting in lower forage yield), and increased leaf:stem ratio (Halim et al., 1989; Peterson et al., 1992). Precipitation in May-June is correlated to total yield of perennial vegetation (Smoliak, 1956; Rauzi, 1964). Correlations of r=0.675 (Rauzi, 1964) and r=0.859 (Smoliak, 1956) have been reported on shortgrass prairies. Rauzi (1964) also reported high correlation (r=0.745) between April through August precipitation and annual yield. Hazell (1965) also reported a decrease in herbage production from tall grass prairies due to decreased precipitation in May. Similar results were seen for total production in relation to April-September precipitation. They also

reported high correlation (r=0.755) between precipitation and big bluestem production. Powell et al. (1986) used multiple regression analysis including temperature, precipitation, wind, and freezing dates to predict chemical composition (N, P, K, and Ca) and production of tallgrass prairie hay. The trial began in 1929 and continued through 1951 (25 years). Regression showed high R^2 values for the prediction of production (82 %), N (80 %), P (81 %), K, (81 %), and Ca (91 %).

Decreased forage production was reported (Hazzell, 1965) in the Osage Hills of Oklahoma when low rain fall (14.15 and 4.65 cm [5.66 and 1.86 inches] in 1961 and 1962, respectively) was observed in May of two consecutive years. Dahl (1963) studied weather factors effecting forage yield in eastern Colorado. Soil moisture in the early spring was a major factor contributing to the yield. Lack of spring moisture limits soil moisture storage and can have a tremendous effect on potential forage yield. Shiflet et al., (1974) reported that herbage production could be predicted with fair accuracy (r = 0.58 to 0.78) with either January to September or April to September precipitation.

Holechek et al., (1983) evaluated the effects of drought on yearling heifer diet quality and botanical composition. Diet protein values were lower in 1977 than 1976 at each of the different collection dates. Moisture was reported at 11.4 cm lower in 1977 than 1976. In both years moisture was lower than the 25 year average (53.1 cm annually). They also reported lower weight gains in cattle due to lower dietary CP during drought years.

In a preliminary assessment (Perry, 1976) on the effects of weather on the Northern Great Plains Grasslands it was noted that above ground primary production may

be slightly increased due to early spring and summer rains. With the addition of fertilization along with the moisture, production was higher. They also concluded that increases in spring precipitation would probably result in an increased proportion of higher producing plant species. Summer and late spring moisture would probably elicit a greater response in warm season grasses and forbs compared to cool season grasses primary growth.

Research results on the effect of moisture on grazing livestock diet protein and digestibility indicate that below average moisture decreases diet quality and forage production. However, it appears that moisture has the most effect on forage yield and growth patterns which in turn affects the quality of diets consumed by grazing livestock. Below average moisture reduces forage yield and appears to increase diet protein content. Diet digestibility appears to increase with advancing stages of drought presumably through decrease the rate of plant maturation and the reduction of stem growth increasing the leaf:stem ratio. Low forage yield due to drought can also affect diets through reducing the animals ability to select plants of higher quality and forces them to consume other forages to meet intake requirements. This can be seen in a shift from grass consumption to consumption of more forbs and shrubs which can change diet protein and digestibility. In order to completely assess the effect of moisture on diet quality, long-term (10 to 20 years) research needs to be conducted to determine the effects of total annual precipitation and timing of precipitation on diet quality and botanical composition.

Plant Species

Different plant species differ in nutritive content within the same season (Rodgers

and Box., 1967; Wallace et al., 1972; Kamstra, 1973; Cogswell and Kamstra, 1976). Cogswell and Kamstra (1976) showed blue grama (*Boutelaua gracilis*) and threadleaf sedge (*Carex filifolia*) were more digestible than to needle-and-thread (*Stipa comata*) and prairie sandreed (*Calamovilfa longifolia*) from June to September. Blue grama overall mean CP (8.6%) was higher than the 3 species. Rodgers and Box (1967) studied the seasonal protein content of four southern mixed prairie grasses (buffalograss, *Hierochloe ordata*; blue grama, *Boutelpoua gracilis*; sideoats grama, *Bouteloua curtipendata*; and black grama, *Boutelaua eriopoda*) from December of 1962 through June of 1964. Blue grama was highest in CP throughout the trial whereas sideoats grama was generally lower at any given collection time point. Black grama and buffalograss CP values were intermediate to the other two species. Buffalo grass had the least season fluctuation (3.86 percentage units) from dormant to vegetative stages (5.92, 4.58, and 5.07 percentage units for blue grama, sideoats grama, and black grama, respectively). Wallace et al., (1972) also reported higher CP for blue grama in June when compared to other grasses (needle-and-thread and prairie sandreed) but the protein declined and was lower during the rest of the trial. They also reported CP of forbs were higher as compared to the grasses from June through December. Blue grama and needle-and-thread were similar in DMD but higher than prairie sandreed. Forbs were higher in DMD than all of the grasses tested. Kamstra (1973) evaluated two cool season (western wheatgrass, *Pascopyrum smithlii* and green needlegrass, *Nassella viridula*) and two warm season grasses (little blue stem, *Schizachyrium scoparium* and blue grama, *Bauteloua gracilis*). Western wheatgrass was higher in CP compared to the green needlegrass and little bluestem from

June through mid August and higher than blue grama from early June to mid July, after which blue grama was higher in CP than the other three grasses from mid July through late September. Green needlegrass and little blue stem were similar throughout the sampling period. Digestibility of the cool season grasses was higher than the warm season grasses at each sampling period. The cool season species were similar from early June to mid July then western wheatgrass increased in digestibility whereas green needlegrass decreased from mid July through mid August. In mid to late June blue grama was approximately 8-10 percentage units higher in digestibility compared to little blue stem. However, by early July they were similar and remained similar through mid September.

Grazing Level

The effects of grazing intensity and different grazing systems on botanical and chemical composition of diets have been documented (Cook et al., 1953; Pieper et al., 1959; Vavra et al., 1973; Yates et al., 1982; Kirby and Parman, 1986; Ralphs et al., 1986; Nelson et al., 1989; Walker et al., 1989; McKown et al., 1991; McCollum et al., 1994; Hirschfeld et al., 1996; McCollum and Gillen, 1998; Cullan et al., 1999). The effects of grazing pressure, level and system on diet quality have received mixed results.

Rauzi (1964) reported three times more midgrass was produced on moderately grazed pasture than on lightly grazed pasture. Ralphs et al (1986) studied the relationship of increasing grazing pressure index on diet quality and botanical composition in diets collected from esophageally fistulated sheep and cattle. Data indicated a negative regression in diet quality (as grazing pressure increased) of diets collected from both

sheep and cattle during cool season grazing. Crude protein and IVOMD were both decreased with increasing levels of grazing pressure. During the warm season, grazing sheep diets did not decrease in CP or IVOMD as grazing pressure increased. The researchers attributed this to forage availability not being limiting to the sheep. However, cattle diets did decrease in quality during the same grazing period, because of changes in dietary botanical composition (warm season grasses to sacahuista) during the later part of summer as a result of increased grazing pressure and decreased grass availability. Similar results were reported by Hirschfeld et al. (1996) where cattle diets were higher in CP and digestibility when grazing in a short-duration system than a season-long system. McCollum et al. (1994) compared diet nutrient content collected from 2, 3, and 4-cycle paddocks. Diets collected from the 4-cycle paddocks were higher in CP when compared to the other 2 treatments. No differences were seen between the 2- and 3-cycle paddocks. No differences were seen in IVDMD between treatments but it tended to higher in 3- and 4-cycle treatments. Plant species preference of sheep, grazing typical salt desert range in south-western Millard County, Utah; changed as intensity of grazing increased from moderate grazing intensity to heavy grazing intensity (Pieper et al.1959). Walker et al. (1989) reported a rotational grazing system with high stocking rates did not lower diet quality compared to continuous grazing systems.

Other research has indicated lower diet quality in grazing livestock on short-duration grazing systems compared to continuous systems. Pfister et al. (1994) reported no difference in IVOMD of diets collected from cattle grazing a four-pasture rotation versus a continuously grazed pasture during the dormant season. However, during the

growing season, cow diets differed in digestibility. These trends were not consistent between years. Researchers reported that the higher weight gains of calves grazing the continuous pasture could be attributed to higher digestibility and forage availability. However, digestibility of diets from the continuously grazed pasture was only significantly higher than rotationally grazed pasture in one year of the two year study. McCollum and Gillan (1998) reported flow of organic matter, total nitrogen and microbial nitrogen at the duodenum was lower in cattle grazing in a short-duration (8-paddock) system when compared to a continuous grazing system. Diet nutrient composition and intake was lower in steers grazing the short-duration treatment. Steers also had lower weight gains and there was higher residual standing vegetation at the end of the year for the short-duration treatment.

Rotational or any other grazing systems which increase the number of animals per unit area have been shown to improve livestock distribution within a pasture or paddock and increase the total utilization of the land and forages available (Ralphs, et al., 1986; McKown, et al., 1991). Pfister et al. (1984) reported lower forage utilization in pastures continuously grazed when compared to a four-pasture rotational grazing system. Increasing pasture utilization could force animals to graze plants lower in quality. There also could be a shift in the plant growing cycle forcing it to remain vegetative longer. These could be explain the mixed results from all of the above discussed grazing trials.

There are varying results among researchers as to the effects of grazing on diet quality. There are many other factors such as timing of grazing, intensity of grazing, precipitation, and timing of precipitation that can also contribute to the results obtained.

Grazing generally has been shown to alter diet quality through both changes in diet selectivity and changing the plants' growth cycles. In rotational grazing systems, cattle distribution is increased as well as harvest efficiency. Rotational grazing can keep plants in a vegetative stage longer due to herbage removal thus increasing diet quality. On the other hand increasing the distribution and cattle numbers on a given area could also decrease plant selectivity and force cattle to consume less desirable plants which could lower diet quality. In a continuous system, cattle could graze regrowth from previously grazed plants maintaining diet quality. However, plant maturity of other non-grazed plants would occur at a normal rate. When herbage available for grazing from the regrowth is not meeting the intake requirement animals will be forced to graze other plants that are in advanced stages of maturity and diet quality would be then decreased. The effects of grazing are complicated and it is difficult to sort out the occurring phenomenons. Data are needed in determining changes in diet quality under different grazing management strategies where detailed analysis of moisture, timing of moisture, grazing behavior and diet botanical composition are measured.

Methods for Determining Forage Intake

Forage intake can be measured using either direct or indirect methods in both confined and grazing animals. Determining forage intake through direct measurements is relatively easy in confinement. Harvested forages can be offered to the animal and refusals can be collected. Chemical components of the forage ingested by the animal can be calculated by difference if chemical components are known for the forage offered and

the forage refused . Generally animals are fed individually and several animals can be utilized to account for variation among animals. Forages are fed at adequate levels to allow for *ad libitum* intakes to ensure availability is not limiting (Burns et al., 1994). If digestibility measurements are also taken, then feed offered should not be high enough that the animal has the ability to sort the feed. One way to minimize the affect of sorting is through a feeding period to establish *ad libitum* intakes then reducing the feed offered to a percentage of *ad libitum* intake slightly prior to (at least 2 days) and throughout digestibility measurements (Cochran and Galyean, 1994).

Another approach to determine intake in confined animals is through the use of empirical equations (Burns et al., 1994). These equations use regression techniques to estimate forage intake. In beef cattle the variables included in the model include live weight and daily gain. More complex equations must be utilized for lactating cattle (beef or dairy) where additional variables for milk production, time since calving, and month of lactation (Burns et al., 1994). In both direct methods (if the animal is housed in a controlled environment) and empirical estimates there are no adjustments for outside factors such as environment and animal behavior. Individual animal intake of animals housed together can be established through the use of electronic gates. This approach accounts for some behavior associated with group fed animals. However, animal training is needed and some natural feeding and social behavior may be altered.

Estimation of intake for grazing animals is more difficult than for confined animals. Direct methods used for determination of intake for grazing animals include animal mass differences and herbage mass differences. Indirect methods use fecal output

and diet digestibility or empirical equations. Fecal output can be measured directly (total fecal collection) or indirectly. Indirect methods include daily or pulse dosing of inert markers. Total fecal collection and dosing with inert markers are both labor intensive and animals must be trained to be handled frequently. Handling animals frequently my alter grazing behavior and adds stress the animals. Diet samples can be obtained manually (clipping or plucking) or by the use of fistulated animals. Digestibility of diets can be determined through in vitro or in situ techniques, or internal markers in the plant. The most common internal marker used is lignin. Empirical equations have been developed for grazing animals which estimates daily animal requirements using a back calculation from animal response (Burns et al., 1994).

Forage Quality Effects on Animal Feed Intake and Performance

Feed intake is the primary controlling factor in determining animal production and performance (Allison, 1985; Minson and Wilson, 1994). Forage intake is controlled by the chemical and physical attributes of the forage consumed (Minson and Wilson, 1994; Jung and Allen, 1995; Allen, 1996). Other factors that can alter forage intake include animal body size, physiological status of the animal, supplementation, forage availability, and grazing systems can alter forage intake (Rittenhouse et al., 1970; Allison, 1985).

One way to determine forage quality is through performance of animals. Higher quality forages generally produce improved animal performance assuming forage availability is not limiting. Higher intakes of higher digestible forages generally elicits an

improvement in animal weight gain (Burns et al., 1994; Mertens, 1994). Forages low in energy yet high in bulk, limit animal intake due to the incapacity of the digestive system (primarily the reticulorumen) to hold additional feed. The low concentration of energy in combination with limited intake (due to fill) results in dietary intakes below the animals requirements, resulting in a negative performance response. Johnson et al. (1998) showed an increased OMI in steers grazing native range pastures from July through November and a OMI decline in December. Park et al., (1994) reported a decrease in OMI in ruminally fistulated steers grazing intermediate wheatgrass from May through September. This decrease in intake corresponded with a decrease in particulate passage rate and an increase in gastrointestinal mean retention time. This indicated that the advancing stages of maturity increased reticulorumen fill thus decreasing intake.

Adams et al. (1987) also reported variation in rumen fluid passage, volume, and fermentation was dependent on maturity of the forage consumed. They also reported increased rumen fluid volume with increased forage maturity. However, small variation was observed in OMI for all forage maturities studied. Similar results were also reported by Horn et al. (1979) where forage intake of cattle grazing midland bermudagrasss was positively correlated with IVDMD and negatively correlated with lignin. Organic matter intake was increased in Sandhills upland range pastures rated at good-excellent condition (75%, 83 g/kg $W^{0.75}$) as compared to low-good (58%, 74 g/kg $W^{0.75}$). Intake was also lower in September than June and July when IVOMD and CP values were the lowest. These results differed from results from Funk et al. (1987) where no differences were seen in OMI from early growing season through late dormant season (June to August).

Hirschfeld et al. (1996) reported an increase in forage intake in cattle grazing in a short-duration system as compared to a season-long grazing system. Researchers concluded that cattle consumed a higher quality forage under the short-duration system. Increased cattle weight gains were reported (Vavra et al., 1973) to be due to increased digestibility and intake of cattle grazing lightly grazed pasture. When expressed in weight gain per unit area the, higher gains were observed on heavier used pastures.

Determination and Estimation of Digestibility

Determination of In Vivo Digestibility

Digestibility is simply defined as the portion of a feedstuff or nutrient that is ingested and not recovered in the feces (Cochran and Galyean, 1994). Digestibility is determined by measuring the amount of feed or specific nutrient consumed and measuring the amount excreted in the feces. The difference between the amount fed and the amount excreted is the digested portion. Determining forage digestibility with either direct or indirect methods are time consuming and labor intensive. Intake can be measured by hand or through the use of feed bunks suspended on load cells which will also measure the number of meals and amount of feed consumed in each meal electronically. Carefully measuring feed intake and feed refusals is important. One way to account for feed refusals is to feed at a level below ad libitum intake. This would allow for intake of all feed offered. However, one must consider that passage rate and digestibility may be compromised (Cochran and Galyean, 1994). A method frequently used, once ad libitum intake level is determined, (while attempting to keep the data as

physiologically valid as possible) is offering feed at a level slightly lower (90-95% of ad libitum intake) than ad libitum (Cochran and Galyean, 1994). Using this method, feed refusals are eliminated or at least minimized. This also helps to control or prevent sorting of feed by the animal. Intake can not be controlled or easily measured directly in grazing situations. In these situations researchers must assess the amount of nutrients in the diet consumed. Feed intake can be assessed indirectly through the use of internal markers. Internal markers are inherent dietary constituents that are resistant to digestion (Cochran et al., 1988, Cochran and Galyean, 1994). Cochran et al. (1988) evaluated 4 different internal markers (in vitro ADF, NDF, acid detergent lignin, and ADF extraction followed by cellulase incubation [ADFIC]) and determined ADL and ADFIC were least acceptable internal markers for the diets evaluated.

Total fecal excretion can be collected in fecal bags for direct measurement of fecal excretion. Frequent emptying of fecal bags is important to reduce the risk of soreness to the animal (Cochran and Galyean, 1994). If total fecal collection is not possible due to the experiment situation, one can measure fecal output through the use of an external marker. These markers can be administered in a single pulse-dose or dosed several times each day (Owens and Hanson, 1992, Cochran and Galyean, 1994). With the use of external markers fecal grab samples are collected and used to measure the concentration of the marker to estimate the quantity of feces excreted (Cochran and Galyean, 1994). Frequent handling of the animals to dose markers and to collect feces can alter grazing behavior and lower intake; therefore, the animals must be well adjusted to frequent handling to minimize the effect of stress on intake (Cochran and Galyean,

1994).

The following calculations can be used to determine digestibility.

(1) $$\% \text{ Nutrient Digestion} = \frac{\text{Nutrient Consumed (wt)} - \text{Nutrient in Feces (wt)}}{\text{Nutrient Consumed (wt)}} \times 100$$

In this calculation the amount of feed refused either is not accounted for or has already been subtracted from the amount of feed offered to the animal. Fecal output is directly measured for this equation as well (Cochran and Galyean, 1994). When intake is known and fecal output is determined via external or internal markers such as rare earths the following equation is used to calculate fecal output.

(2) $$\text{Fecal DM Output (g/d)} = \frac{\text{Marker Dose (g/d)}}{\text{Concentration of Marker in Feces (g/g of DM)}}$$

After fecal output is calculated digestibility is determined using equation (1). If intake is the unknown variable, then the following equation can be used.

(3) $$\% \text{ nutrient digestion} = 100 - 100 \times \frac{\% \text{ Marker in Feed} \times \% \text{ Nutrient in Feces}}{\% \text{ Marker in Feces} \times \% \text{ Nutrient in Feed}}$$

In confined situations, the researcher can also control the environment (day length, and keep the temperature in the thermoneutral zone) and restrain the animal. The environment can alter digestibility and intake of a forage if the temperature is below the thermoneutral zone (Cochran and Galyean, 1994). Photoperiod has been shown to affect intake (Forbes, 1982). When animals are in confined situations, behavior is sometimes altered and can affect voluntary intake and intake patterns (Cochran and Galyean, 1994). Lameness can also be an issue if the animals are confined in small areas for extended periods of time; therefore the researcher should allow time for exercise and use materials

in the stall to aid in the comfort of the animal (Cochran and Galyean, 1994).

Comparison of In Vitro and In Vivo Digestibility

Determination of digestibility of forages grazed by livestock is difficult. Accurately harvesting forages from mixed grass prairie pastures or monoculture pastures which are consumed by grazing livestock is difficult due to animal selectivity. Hand plucking or clipping enough forage to conduct a controlled digestibility study is time consuming and labor intensive. Harvesting equipment could be used to harvest enough forage and reduce labor needs. With the use of hand labor or machinery there is also variability in the diet selected by the animals and clipped samples as discussed earlier. Obtaining estimates of *in vivo* digestibility of grazed forages is important in diet and supplementation formulation. One method for estimating digestibility of forages is through the use of an *in vitro* digestibility procedure. The procedure outlined by Tilley and Terry (1963) indicated that *in vivo* digestibility could be predicted with *in vitro* digestibility of both legumes and grasses with a high degree of accuracy. Since the publication of the original procedur,e modifications have been introduced to increase precision and accuracy (Weiss, 1994). Many studies have shown a strong statistical correlation ($r > 0.9$) between in vivo and in vitro digestibility (Tilley and Terry, 1963; Alexander and McGowan, 1966; McLeod and Minson, 1974; Givens et al., 1989; Ginizi et al., 1990). However, the strong correlation does not mean that IVDMD is equal to *in vivo* digestibility. In order to convert *in vitro* to *in vivo* digestibility a regression equation must be determined from *in vivo* data. The data obtained from an *in vitro* (samples with unknown *in vivo* digestibilities) procedure can be adjusted using those regression

equations to derive an estimated *in vivo* digestibility value (Weiss, 1994). There are three different methods for developing calibration equations (Weiss, 1994). The first way is for each laboratory to determine both *in vitro* and *in vivo* digestibility coefficients for a diverse population of feeds. With this method the data may be limited and appropriate for feeds grown under limited conditions. It is also expensive and labor intensive. The second method uses a set of diverse feeds that have known *in vivo* digestibility as a calibration set. The calibration set is included in the *in vitro* procedure along with the forage samples unknown *in vivo* digestibility. The *in vivo* data are then regressed on the *in vitro* data to generate a regression equation. The *in vitro* data of the unknown samples are entered into the regression equation resulting in an adjustment of *in vitro* values to *in vivo* values. The third method uses indirect calibrations to estimate in vivo digestibility from IVDMD values. This method uses samples of known IVDMD from one laboratory and they are analyzed at another laboratory. An equation is derived to convert *in vitro* data from the second laboratory to estimate *in vitro* data from the original laboratory. The original laboratory must have an accurate *in vitro-in vivo* equation which is then used to convert the estimated *in vitro* data to *in vivo* estimates in the second laboratory (Weiss, 1994). Due to differences between different laboratories and between different *in vitro* runs within a laboratory, each separate *in vitro* run should have its own equations to estimate *in vivo* digestibility (Weiss, 1994). Results indicated that determinations of digestible DM or OM could replace the determination of digestible energy. The following table has been regenerated from Weiss (1994).

Table 1: Sample equations for converting IVDMD values to in vivo OM digestibility. All values are expressed as g/kg, DM basis. In Vivo = a + b*IVDMD

Feed	Intercept	Slope	SEp	Reference
C_3 grasses	124	0.82	22.7	Aerts et al., 1977
C_3 grasses	5.2	1.01	14.6	Terry et al., 1978
C_3 grasses	-136	1.20	18.5	Omed et al., 1989
C_3 grasses	172	0.72	24.0	Moss and Givens, 1990
C_4 grasses	115	0.83	24.0	McLeod and Minson, 1969
C_4 grasses	-125	1.27	37.8	Navaratne et al., 1990
Legumes	-4.1	1.02	16.0	Terry et al., 1978
Legumes	-9.8	1.03	19.4	Omed et al., 1989
C_3 grass & Legume	-48.2	1.08	19.3	Omed et al., 1989
Corn Silage	29.3	0.58	21.1	Aufrere et al., 1992
Concentrates	-26.6	1.10	50.1	Omed et al.. 1989

Urness et al. (1977) reported higher *in vivo* digestibility (determined via total fecal collection) in seven plant species than *in vitro* digestibility in mule deer. The regression equation of all *in vitro* to *in vivo* digestibilities was y=1.28x-23.51 and had a significant correlation coefficient (r=0.84).

In Situ and Mobile Bag Methods for Estimating Protein and Energy Digestibility

In situ or the mobile bag methods are two techniques that are used to determine ruminal degradation or total tract degradation of feeds. The in situ method measures the disappearance of feedstuffs from artificial fiber bags which are suspended in the rumen of an animal. The mobile bag technique uses the same nylon bags as the in situ procedure however, following rumen incubation bags are incubated in a pepsin and hydrochloric acid digestion and then inserted in the duodenum of fistulated animals. This technique can be used to determine digestibility of protein in the rumen, intestine and total tract

(Haugen et al., 2006a). The in situ method only allows for rumen degradability which allows for the separation of DIP and UIP from the total protein and ruminal energy digestibility in the feed. With either of these techniques the diet fed to the fistulated animals being used for the incubations is very important (Weiss, 1994). Feed disappearance from the bags can be affected by the amount of forage and concentrate in the ration. Increasing the amount of concentrate in the diet can decrease the fiber digestibility of forages being tested (Weiss, 1994). Vanzant et al. (1996) compared *in vivo* protein digestibility to in situ digestibility of alfalfa and prairie hay. In situ bags were incubated at 3 different times (a 16-hour single time point, a zero time point and a 16-hour double-point). No difference in protein degradation was observed between the *in vivo* and in situ incubations.

In vitro and in situ techniques were compared to *in vivo* for determination of forage OMD (Gosslink et al., 2004). The researchers indicated that the in situ technique plus crude protein had the highest accuracy in predicting *in vivo* digestibility. Nocek (1988) also indicated that in situ methods for estimating protein and energy digestibility offers a better way to simulate rumen environment within a given feed regimen as compared to artificial rumen simulation models. Usefulness of *in situ* methods may be dependant on standardization of variables associated with the procedure (bag pore size, sample size, feed particle size).

Haugen et al. (2006a) tested the hypothesis that the current 80% values for UIP digestibility used by the NRC (1996) may be high for forages. This study reported that digestibility of UIP of dehydrated alfalfa, sun-cured alfalfa, and lyophilized alfalfa were

46.4 %, 25.6% and 14.7%, respectively. Gustad (2006) reported UIP degradability of diet samples (collected from esophageally fistulated cows) of native range pastures in the Nebraska Sandhills. Diets were collected from June through August. These values agree with values reported by Haugen et al. (2006a) with UIP digestibility from control pastures ranging from 17 to 43% (% of UIP). These results were low compared to the recommendation of both the ARC (85%; 1984) and the NRC (1996). The use of in situ neutral detergent insoluble nitrogen as a method for estimating forage protein UIP degradability has been studied (Mass, et al., 1999; Haugen et al., 2006). Mass et al. (1999) determined that in situ NDIN were adequate for estimating forage UIP. Haugen et al. (2006) studied the use of a single in situ incubation time point for estimating UIP in forages. The single time point which was used was 75% of the TMRT which was derived from estimates from IVDMD plus a 10-hr passage lag. Results from this study indicated that using NDIN at a single in situ incubation could accurately estimate UIP. Rate of protein degradation can also be obtained using this time point when 0- and 96-h incubations are used in addition.

Diet Formulation and Nutrition for Grazing Cattle

As discussed earlier in this review diet digestibility and protein can be altered by precipitation, timing of the precipitation, grazing pressure, forage species and forage maturity. This makes estimating diet quality of grazed forages, especially in mixed grass prairies, difficult. However, knowledge of diet quality is important in the formulation of beef diets. The beef NRC (NRC, 1996) is used by both nutritionists and scientists to

formulate grazing cattle diets and protein or energy supplements. This computer software uses empirical (level 1) and mechanistic (level 2) methods to generate animal requirements and evaluate rations. In order to increase the precision of animal performance, IVOMD values must be either adjusted to in vivo values or converted to DE (Patterson et al., 2006). Small increases or decreases in digestibility when used as a proxy for TDN can greatly alter the predicted animal performance (generally body condition score in beef cows or weight gain in calves) because of the sensitivity of the NRC Model. The TDN proxy is also used to calculate both DMI and NE of the feed (Patterson et al., 2006). In Vitro OMD can be converted to TDN using the equation DE = (1.07 * IVOMD) - 8.13 (Rittenhouse et al., 1971). Patterson et al, (2006) used data from 7 studies in Nebraska and Montana using grazing beef cows that met the criteria of 1) reporting BCS or changes in BCS; 2) defined energy and protein content of grazed forage; and 3) cattle production traits were defined (BW, age, breed, days in lactation, and days pregnant. This trial compared predicted changes in BCS from the NRC model to published BCS. The comparison was made with TDN entered as either IVOMD equal to TDN or IVOMD converted to DE using the equation published by Rittenhouse et al. (1971). Results indicated that when the converted DE values were used in the model no statistical differences (P = 0.44) were indicated between observed BCS changes and predicted changes in BCS. Correlation between predicted and observed BCS was 0.73. When IVOMD was used as TDN, the model overestimated (P = 0.001) the predicted BCS as compared to the observed BCS. Thus indicating that the NRC model was overestimating energy intake. Lardy et al. (2004) also indicated that using IVOMD as a

proxy for TDN the NRC over predicted energy balance of grazing beef cows. The authors concluded that using IVOMD to directly represent TDN results in a greater TDN value than when IVOMD is converted to DE using the equation from Rittenhouse et al. (1971). They also concluded that *in vivo* OMD values should be used when available. Unfortunately because these data are lacking the difficulty in generating *in vivo* OMD data from grazing situations previously discussed. There is a need for *in vivo* OMD values that take into account precipitation, day of the year (forage maturity), and grazing pressure effects.

Not only are TDN values in the NRC model used to predict energy status of grazing livestock, TDN also affects the protein predictions in the model as well. The NRC (1996) uses TDN intake as the determinant of MCP production. Energy intake directly affects energy available for rumen microbes. Low energy intake decreases energy available for the microbes thus decreasing microbial efficiency which decreases microbial CP production. Decreases in MCP decreases the MP available to the host. The NRC (1996) uses the following equations to predict MP:

Microbial efficiency (g/100g TDN intake) = $2.62 + (1.78 * \%TDN) - [9.60 * 10^{-2}) * \%TDN^2] + [1.78 * 10^{-3}) * \%TDN^3] - [(1.054 * 10^{-5}) * \%TDN^4]$

MCP (g/d) = TDN intake (kg/d) * microbial efficiency (g/kg)

MCP (g/d) = DIP intake (g/d)

MP (g/d) = (MCP, g/d *0.80 * 0.80) + (UIP, g/d * 0.80)

Not only does predicted TDN values have an effect on the energy status of the grazing animal, it also has significant impacts on prediction of MP (Lardy et al., 2004; Patterson

et al., 2006). This stresses the importance of determining accurate estimates of forage TDN to use when formulating rations and supplements for grazing cattle.

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Comparison of In Vivo Digestibility to In Vitro Digestibility of Five Forages Fed to Steers and Development of a Calibration Data Set

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Abstract: Determination of *in vivo* digestibility of grazed forages is important in formulating diets and supplements for grazing livestock. The use of a calibration forage sample set could be useful in adjusting *in vitro* digestibility estimates of forage samples to *in vivo* digestibility. The objective of this trial is to develop a calibration set of forages with known *in vivo* digestibilities which can be included in the IVDMD procedure to adjust *in vitro* digestibility estimates of forages to *in vivo* values. Eight crossbred yearling steers (IBW = 323 ∀ 29 kg) were used in a 5x5x8 Latin rectangle design to determine in vivo DMD, OMD, and NDFD of five forages. Five forages (chopped hay) were used and included immature alfalfa (Ialf; *Medicago sativa*), mature alfalfa (Malf), immature smooth bromegrass (Ibrome; *Bromus inermis*), mature smooth bromegrass (Mbrome), and prairie grass hay (Prairie). Twenty one different *in vitro* runs were completed compare of *in vitro* digestibility to *in vivo* digestibility. The Prairie, Mbrome, Ibrome, Malf, and Ialf hays had 7.9, 13.0, 13.7, 14.7, and 16.0% CP and 68.3, 69.6, 66.7, 67.9, and 60.5% NDF, respectively. As quality of the forage increased, DMI increased ($P < 0.01$) (5.2, 5.7 5.8, 6.4, and 6.8 kg/d for Prairie, Mbrome, Ibrome, Malf, and Ialf, respectively). Significant differences ($P < 0.001$) were detected among the individual *in vitro* runs; however, no differences ($P = 0.99$) were detected when slopes were tested.

The average IVDMD from all runs and *in vivo* DMD was correlated (R^2 = 0.831). On average, *in vitro* DMD was 11% higher than *in vivo* DMD. The range in correlation coefficients between the 21 runs was R^2 = 0.5352 to 0.9728. Regression analysis of *in vivo* NDFD plotted against mobile bag NDFD indicated a significant correlation (R^2 = 0.553). Results from this trial indicate that these five forages are excellent for use in *in vitro* runs as standards. Regression equations derived from each run can be used to adjust *in vitro* DMD and OMD values to *in vivo* values.

Keywords: Digestibility, Cattle, Forage

Introduction

The use of calibration data sets for estimating *in vivo* digestibility of forages is not a new technique. High correlations (R^2 = 0.90) between *in vivo* and *in vitro* digestibility (Tilley and Terry, 1963; Genizi et al., 1990) have been reported. Because *in vitro* digestibility does not equal *in vivo* digestibility, equations must be derived to convert *in vitro* data to *in vivo* estimates. Including a set of calibration forage samples within each *in vitro* run which has known *in vivo* digestibilities allows researchers to adjust *in vitro* digestibility of forages to *in vivo* values using regression equations generated from the standards (Weiss, 1994). The use of a calibration data set would prove to be useful in formulating diets and supplements for grazing livestock accurately (Lardy et al., 2004; Patterson et al., 2006) where digestibility trials on pastures would be difficult. Adjusting the *in vitro* results using the equations generated from the standards (with known *in vivo* digestibility) allows researchers to compare estimates from different *in vitro* runs (Weiss,

1994). With these adjustments, forage samples with different species composition can also be compared. Accurate estimates of digestibility are important when balancing rations, determining the true economic value of different feeds, and predicting animal performance (Weiss, 1994). When using the NRC (1996) for formulating rations and supplements for grazing cattle, it is imperative to use digestibilities that are either adjusted to *in vivo* values or actual *in vivo* digestibilities in order to increase the precision and accuracy of the estimated intake and animal performance from the NRC (Patterson et al., 2006). The objective of this experiment was to determine the *in vivo* digestibility of five different forage samples and to test and use these samples as laboratory standards for *in vitro* DM and OM digestibility procedures.

Materials and Methods

Animals and Feeding

This experiment used eight crossbred yearling steers (Initial BW = 323 kg) in a five period, five treatment cross-over designed trial. Steers were randomly assigned to treatment within each period. Diets included five different chopped hays including immature alfalfa (Ialf), mature alfalfa (Malf), immature smooth bromegrass (Ibrome), mature smooth bromegrass (Mbrome), and prairie grass hay (Prairie). The prairie hay consisted of a mixture of warm and cool season grass species. All hay was chopped prior to the initiation of the trial through a tub grinder using a 10-mm screen. Chopped hay was mixed and stored on concrete in an enclosed building to minimize spoiling and contamination. Collection periods consisted of a 16-d adaption period followed by a 5-d

collection period. During the first 10-d of the adaption period, steers were fed at ad libitum intake level. Feed refusals were collected and weighed daily during the adaptation period. During the last 6-d of the adaptation period and during the collection period steers were fed at 95% of their individual ad libitum intake. During d 16 through d 20, feed and feed refusals were collected (0.5 kg), weighed, and a sub-sample was taken for laboratory analysis when necessary. Sub-samples were composited by week.

Steers were fed once daily at 0800 hr immediately following feed refusal collection. Daily feed refusals were composited on a weighted average by week. Diet and feed refusal samples were dried in a 60°C forced-air oven for 48 h. Dry matters were calculated and recorded. Samples were ground through a 2-mm screen in a Wiley mill. Approximately one half of the 2-mm ground samples was then ground through a 1-mm screen in a Wiley mill. Samples were later analyzed in the laboratory for CP, DM, OM, IVDMD, NDF, and ADF. Dry matter and OM were determined following the AOAC standard procedure (1996). Acid detergent fiber was determined following procedures outlined by Goering and Van Soest (1970). Neutral detergent fiber was determined using the ANKOM$^{220/220}$ fiber analyzer modified through the removal of acetone and alpha-amylase (Van Soest et al., 1991). Nitrogen was determined by the combustion method (AOAC, 1996) using a nitrogen analyzer (Leco FP-528, St Joseph, MI). Nitrogen was coverted to CP using the equation % CP = % N * 6.25.

Fecal Collection

Steers were fitted with fecal bags on d 16 at 1700 hr. Fecal bags were emptied

twice daily at 0700 and 1700 hr. Fecal collection began on d 16 and ended on d 21. Feces were weighed and sub-sampled for later analysis. Fecal sub-samples were dried in a 60° C forced-air oven for 48 hours. They were then ground through a 2-mm screen using a Wiley mill. Ground sub-samples were composited by collection period. Composite samples were ground through a 2-mm screen using a Wiley Mill and then analyzed for CP, DM, OM, and NDF.

In Vitro and In Vivo Digestibility

In vivo digestibility was determined using the steer intake and fecal excretion. Nutrient (DM, OM, and NDF) digestibility was determined using the equation:

$$\% \text{ nutrient digestibility} = \frac{\text{nutrient consumed (wt) - nutrient excreted (wt)}}{\text{nutrient consumed, (wt)}} \times 100$$

True DMD was calculated using the equation: $\text{True DMD } \% = \frac{\text{(DMI, kg - Fecal NDF, g)}}{\text{DMI, kg}}$

Metabolic losses were calculated by subtracting in vivo DMD from true DMD.

In vitro DMD was estimated using a modified version of the *in vitro* procedure described by Tilly and Terry (1964). Hay samples were ground through a 1-mm screen. The original procedure was modified with the addition of 1g urea L^{-1} of McDougall's buffer. Equal volumes of rumen fluid was collected from two steers (BW = 250 kg) for each of the *in vitro* runs. Steers were fed a smooth bromegrass hay diet once daily at 1.5% of BW at 0700 hr.

In comparing *in vivo* and *in vitro* analyses, the hay samples were included in 21 separate *in vitro* runs. The 21 different runs were performed by a total of 6 different

technicians. Rumen fluid was collected from steers fed either a 100% smooth bromegrass hay diet once daily at 1.5% of BW or a mixed diet consisting of 70% smooth bromegrass hay and 30% concentrate diet twice daily at 1.5% of BW. Of the 21 runs, 9 of them were run with rumen fluid collected from steers fed a Bromegrass hay diet while the other 12 runs used rumen fluid collected from steers fed a mixed diet.

In Situ and Mobile Bag Incubations

In situ incubations were conducted using two ruminally and duodenally fistulated steers (BW = 250 kg). Dacron bags (Ankom Inc, Fairport, NY) measuring 5 x 10 cm with 50 Φm pore size. Bags were heat sealed containing 1.25 g of air-dried hay sample ground through a 2-mm screen. Donor animals were fed once daily a bromegrass hay ration at 1.5% BW. Triplicate bags were incubated at each time point and replicated within each of the two steers. Time points for incubation were 0, 25, 30, and 96 h. The 25 and 30-h times were calculated at 75% total mean retention time (TMRT) which yielded a 25-h incubation for the Malf, Mbrome, and prairie hay and the 30-h incubation for the Ialf and Ibrome hays. The 75% TMRT was determined by calculation of rates of passage (kp) of each forage using the following equation: kp = 0.07*IVDMD (%) - 0.20. The kp was used to determine the mean retention time (MRT = 1/kp). A 10 h lag was added to the MRT to yield the total mean retention time (Haugen et al., 2006a). Following ruminal incubation, bags were washed in a washing machine for 0.25 h using a 1 min agitation and 2 min spin cycle. The washing cycle was repeated a total of 5 times (Haugen et al., 2006a). Following washing bags were refluxed in neutral detergent fiber solution in order to remove any microbial contamination and to determine the NDIN

following the procedure outlined by Mass et al., (1999). The 0-hr bags were not suspended in the rumen; however, they were washed and refluxed following the same procedure. Following reflux, the bags were dried in a 60°C forced air oven for 48 h. They were weighed directly out of the oven after equilibration in a desicator for 5 minutes. Following the hot weight, bags were allowed to air equilibrate for 3 h and were weighed again. Residue remaining in the bags were analyzed for neutral detergent insoluble N (NDIN) using the combustion method (AOAC, 1996) in a combustion analyzer (Leco FP-528, St. Joseph, MI)

The rate of NDF ruminal degradation (kd, % h^{-1}) was calculated using a first order disappearance model using the equation: kd (% h^{-1}) = [LN(% of B remaining at X) - LN (% of B remaining at Y)] / (X - Y) h. Variable X and Y are time points in hours incubated. The original (0-h) NDF minus the 96-h NDF represent the potentially degradable fraction (B), whereas the C fraction represents the 96-h NDF.

A second set of bags (75% TMRT) was incubated in the rumen following the same procedure and donor animals described for the in situ incubation. Each hay was replicated in 3 bags/steer. Following rumen incubation, bags were incubated in a pepsin and HCl (1 g L^{-1} pepsin and 0.01 M HCl; 62.5 ml/bag) solution at 37°C for 3 h to simulate abomasal digestion. Bags were then randomly sorted for duodenal insertion with 7-8 bags/d. Bags were inserted into the duodenal fistula of each steer on two consecutive d. Seven bags were inserted on d 1 and eight bags on d 2. Steers were fed a smooth bromegrass hay diet at 1.5% BW at 0700 h daily. Bags were inserted beginning at 1700 at a rate of 1 bag every 5 minutes to prevent blockages in the intestine of the

animal (Haugen et al., 2006a). Bags were collected in the feces beginning 12 -24 h after insertion and frozen until all bags were collected. Any bags retained for longer than 24h were not included in the analysis. Following collection of all bags, they were machine washed and refluxed in neutral detergent fiber solution following the same procedure as the in situ incubation. Bags were weighed and analyzed for NDIN in the same manner as previously described for the in situ bags. This analysis was used to calculate the digestibility of the UIP.

Statistical Analysis

Dietary chemical composition (*in vitro* and *in vivo*) data were analyzed using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effects of period and hay and random effect of animal. The REG procedure of SAS (SAS Inst. Inc., Cary, NC) was used to test the regression of *in vivo* to *in vitro* digestibility as well as testing slopes of regression equations. *In vivo* digestibility was predicted from *in vitro* digestibility values. A protected F-test was used to evaluate treatment mean differences. Least square means were separated using Least Significant Difference method when an overall significant treatment ($P < 0.05$) F-test was detected. The IVDMD values from each of the separate runs was regressed against the *in vivo* DMD. The slope of each regression line were compared for equal slopes. Run differences were also tested (SAS Inst. Inc., Cary, NC). Mobile bag and in situ data were analyzed as a fixed block design with animal as blocks using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the fixed effect of hay and random

animal effect. All trial procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Results and Discussion

Diet and Intake

Crude protein content of the diets ranged from 7.5 to 17.6% (Table 1). There was a wide range in IVDMD between the different hays as well (52.8, 52.8, 53.9, 59.1, and 63.9 for Malf, Prairie, Mbrome, Ibrome, and Ialf, respectively). As expected, increasing maturity of forages decreased IVDMD. This has been reported in numerous reports where increasing levels of maturity decreases certain chemical components such as IVDMD, CP, and increases others including fiber and lignin (Kamstra et al., 1968; Wallace et al., 1972; Kamstra, 1973; Cogswell and Kamstra, 1976; Powell, et al., 1983; McCollum et al., 1985; McCollum and Galyean, 1985; Lardy et al., 1997; Johnson, et al., 1998).

Apparent DMD (Table 2) was highest ($P < 0.001$) for both the immature hays and lowest for the prairie hay. The Malf and Mbrome hay did not differ ($P > 0.05$) from each other, however, they were different ($P < 0.50$) from the other three hays. Metabolic losses were also different ($P < 0.01$) with the highest metabolic loss for the Prairie hay (18.5%) and lowest for the Ibrome hay (13.7%).

As digestibility of the hay increased, so did forage intake ($P < 0.001$; Table 2). Intakes were the highest when steers were fed either of the alfalfa hays and lowest when fed Mbrome and prairie hay with Ibrome as an intermediate. There were no differences

in DMI within the three grass hays or within the two alfalfa hays. Intake of both of the alfalfa hays was higher than for the three grass hays. This could be explained by increased reticulorumen fill when grass hays were fed. The higher intake observed when the Ialf hay was fed could be explained by a combination of both decreased reticulorumen fill and increased digestibility, which increased the rate of passage. Physical bulk found with lower digestible forages decreases forage intake because of the lower passage rate of particles from the reticulorumen (Weiss, 1994). Physical bulk is the first limiting factor affecting forage intake. Intake generally increases when low quality forages are fed in a pellet (Weiss, 1994), which suggests that fill and slower flow of feed from the reticulorumen decreases intake. Digestibility of forages decreases with maturation of the forage plant. Horn et al. (1979) showed a positive correlation between IVDMD and forage intake. Adams et al. (1987) reported rumen fluid passage, volume and fermentation was dependent on forage maturity. Similar intake results were reported by Park et al. (1994) and Hirschfeld et al. (1996) where OMI decreased with advancing stages of maturity.

In Vivo versus In Vitro Digestibility

When runs were tested against each other there was a significant difference ($P < 0.001$) among the 21 different runs (Figure 1). This indicates that within a single laboratory, variation occurs between different runs performed using the same procedure. This variation between the *in vitro* runs using the same forage samples make the comparison of separate *in vitro* runs containing the same samples impossible. No differences ($P = 0.99$) were detected between the slopes of the 21 different regression

lines. Regression equations and R^2 for each run are listed in Table 4. The R^2 ranged from 0.5352 to 0.9728. When all 21 runs were averaged together (Figure 2) there was a significant ($R^2 = 0.8305$) correlation between *in vivo* and *in vitro* digestibility of the five forages. *In vitro* digestibility was 6.4 percentage units higher on average than *in vivo* DMD, or an 11% difference between *in vivo* and *in vitro*.

A different equation should be generated to adjust each separate *in vitro* run because in there were equations were different in each separate run. These standards should be included in each run and the equation generated should only be used to adjust samples with unknown digestibilities in the respective runs. McLeod and Minson (1969a,b, 1974, 1976.) suggested that in order to accurately predict *in vivo* digestibility of feed samples, *in vitro* data should be corrected by a standard set of feeds with known *in vivo* digestibilities. The correction will account for variation between runs due to differences in rumen fluid inoculum. Tilley and Terry (1963) also suggested that at least two feeds should be used as standards to predict *in vivo* digestibility more accurately. Weiss (1994) concluded that a universal equation can not be used and that each *in vitro* run should be adjusted accordingly because of variation in analytical techniques and variation caused by donor animals. It was also stressed that high correlations between *in vitro* and *in vivo* digestibilities does not make them equal and equations must be derived in order to convert *in vitro* data to *in vivo* data.

Genizi et al. (1990) reported that regression equations differed between three different laboratories using the same samples with known *in vivo* digestibilities. They also reported that within a single laboratory the equations between runs differed even in

one laboratory where two water baths were used and the technicians and inoclum were the same. The residual standard error was 0.0002 higher for uncorrected data compared to corrected data. Genizi et al. (1990) concluded that no information suggests that the use of regression equations will reduce the variation between *in vivo* and *in vitro* estimates. They did suggest that if similar feeds consistently vary between *in vivo* and *in vitro* digestibility then adjustment equations should be used.

The standard deviation of the 21 *in vitro* runs ranged from 2.63 to 3.61 percentage units with an average of 3.27 percentage units. Data from each *in vitro* run were entered into the corresponding regression equation to convert *in vitro* data to *in vivo* values. The standard deviation of the adjusted data ranged from 0.86 to 2.43 percentage units with an average of 1.78 percentage units. The decrease in the standard deviation units indicates that the regression equations adjusted the data closer together among runs and closer to the in vivo values; thus, making the estimated digestibility more accurate and precise. Increasing the number of tubes per standard within a single *in vitro* run improved the R^2 of the regression equation (Table 5) when two tubes per standard were compared to five tubes per standard. The R^2 for regression equations increased from 0.7248 to 0.7602 for DMD and from 0.7249 to 0.7752 for OMD between two and five tubes per run. When the number of tubes increased from two to three or four tubes per run the R^2 values were greater for the higher number of tubes. These results indicate that the precision of the regression equations is increased with increasing the number of tubes per standard within a single *in vitro* run.

In Situ and Mobile Bag

Undegradable intake protein digestibility of the five forages ranged from 34 to 62.4% (% of UIP) (Table 1). These results agree with several other published UIP digestibilities for forages (Gustad, 2006; Haugen et al., 2006a;). Haugen et al. (2006a) reported that UIP digestibilities of dehydrate alfalfa hay, sun-cured or freeze-dried were 46.4, 25.6, and 14.7% (% UIP), respectively. The researchers also reported UIP digestibility of clipped smooth bromegrass in June and July was 70% and 46% whereas, it was of 28% and 47% for birdsfoot trefoil (*Lotis cormiculatus*) in June and July, respectively. Gustad (2006) determined UIP digestibility of diet samples collected from esophageally-fistulated cows grazing Sandhills range pastures with varying levels of grazing pressure. In control pastures (stocking rates = recommended rates for the area), UIP digestibility ranged from 15.9% in early August to 44.9% in mid June and appeared to decrease with advancing stages of forage maturity. When stocking rate was double the recommended rate, UIP digestibility ranged from 16.9% to 33.6% (% UIP) Digestibility of UIP appeared to decrease from mid June through mid July; however, it increased in late July and early August (23.8 and 25.0%, in late July and early August, respectively). Similar trends were observed in the double stocked treatment with the addition of supplement (protein and energy); UIP digestibility ranged from 11.6% to 30.5% (% UIP). When the authors expressed UIP and total track indigestible protein (TTIDP) as a percent of dry matter, no differences were observed between treatment or collection time. The authors also found UIP and TTIDP (% DM) ranged from 1.59 to 2.53 and 1.27 to 2.18, respectively. Results from these experiments indicate that the UIP digestibility is lower

than the current equations used by both the ARC (85%) and the 1996 beef NRC (80%) for forages.

The rate (kd, % h^{-1}) of NDF in situ digestibility was significantly different ($P < 0.0001$) among the different hays (Table 3). Both of the alfalfa hays had higher rates of NDF digestibility (7.1 and 7.5 % h^{-1} for Malf and Ialf, respectively) than the three grass hays. Within the three grass hays Ibrome was higher ($P < 0.0001$) compared to Mbrome and Prairie hay (4.8, 3.3, and 3.6 % h^{-1} for Ibrome, Mbrome, and Prairie, respectively). Gustad et al. (2006) reported rates of NDF digestibilities ranging between 4.44 to 6.61 % h^{-1} in diets collected from native Sandhills range pastures with differing levels of grazing pressure. They also reported no differences ($P > 0.05$) between collection time points (Mid June through early August) or grazing levels (recommended stocking rate or double recommended stocking rates).

Rate of ruminal CP degradation differed ($P = 0.025$) among the five hay samples (Table 3). Prairie hay degradation was lower ($P < 0.05$) than the other four hays. No differences ($P > 0.05$) were found among the two brome hays and the two alfalfa hays. The rate of degradation ranged from 4.2 to 10.4 % h^{-1} . Similar rates were observed (Haugen et al., 2006a) in diet and clipped samples of alfalfa, birdsfoot trefoil, kura clover, and bromegrass measured between 10 h and 75 % TMRT. Their rates were lowest (7.98 % h^{-1}) for diet samples of birdsfoot trefoil and highest in clipped samples of kura clover. The average rate of CP degradation was 9.44 % h^{-1} for the clipped samples and 8.65 % h^{-1} for the diet samples.

Total tract NDFD determined via the mobile bag procedure (Figure 3) was highly

correlated (r = 0.744) to *in vivo* NDFD. Ruminal NDFD was also correlated (r = 0.508) to in vivo NDFD. When total tract NDFD was regressed against in vivo NDFD the equation indicated a strong relationship (R^2 = 0.553) between in vivo and mobile bag NDFD. The relationship between in vivo NDFD and rumen NDFD was not as strong (R^2 = 0.261). These results indicate that the mobile bag procedure using a total tract incubation for estimating NDF digestibility could be used as an estimate for *in vivo* NDFD. The mobile bag technique can also be used for determining protein fractions and the digestibility of the UIP fraction. This would be beneficial for nutritionist and researchers when formulating rations and estimating MP.

Implications

Results from this trial indicate that the five forages (Malf, Mbrome, Ialf, Ibrome and Prairie) could be included in IVDMD determination procedures as standards. Within each separate IVDMD run, regression equations can be generated to adjust the data to *in vivo* digestibility values. This adjustment also could enable researchers to compare separate *in vitro* runs in situations where sample numbers are too large to perform a single run or in the case where multiple *in vitro* runs are used to increase replication.

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Table 1: Chemical composition of the hays fed to steers.

			Diet		
Variable	**Prairie**	**Malt**	**Mbrome**	**Ialt**	**Ibrome**
CP, %	7.9	16.3	7.5	17.6	9.3
UIP, % of CP	27.9	14.9	37.2	10.1	22.6
TTIDP, % of CP [1]	16.6	8.0	15.3	5.0	14.8
UIPD,% of CP [2]	40.1	62.4	58.9	46.0	34.0
UIP, % of OM	2.53	2.78	3.91	2.10	1.92
TTIDP, % of OM [1]	1.45	1.45	1.58	1.00	1.20
IVDMD, %	52.8	52.9	53.9	63.9	59.1
NDF, %	68.3	67.9	69.6	60.5	66.7
ADF, %	43.4	43.7	43.7	35.2	40.0

[1] Total Tract Indigestible Protein
[2] Lower Tract UIP Digestibility

Table 2: *In Vivo* and *In Vitro* digestibility of five different hays fed to yearling steers.

	Diet					Statistics	
Variable	**Prairie**	**Malf**	**Mbrome**	**Ialf**	**Ibrome**	**SEM**	**P-value**
In Vivo							
DMI, kg[1]	5.2[b]	6.4[ac]	5.7[b]	6.8[c]	5.8[ab]	0.6	<0.01
DMD, %	44.4[b]	48.3[ab]	49.8[a]	61.7[d]	55.5[c]	1.6	<0.01
OMD, %	48.6[c]	51.5[bc]	54.5[b]	63.9[a]	59.2[a]	1.4	<0.01
NDFD, %	47.1[b]	47.0[b]	45.2[b]	53.7[a]	57.0[a]	2.3	<0.01
True DMD, %[2]	61.3[c]	64.6[a]	64.8[a]	75.8[d]	69.1[b]	1.1	<0.01
Metab Loss, %[3]	18.7[c]	16.5[bcd]	14.6[ab]	14.7[ad]	13.7[a]	0.8	<0.01
In Vitro							
DMD, %	52.8[c]	52.9[c]	53.9[ac]	63.9[b]	59.1[ab]	1.6	0.02
OMD, %	49.8[c]	54.5[c]	57.9[ac]	64.2[b]	62.4[ab]	2.0	0.03
NDFD, %	43.8[b]	43.4[b]	48.6[ab]	51.5[a]	54.0[a]	1.6	0.03

[1] DM basis
[2] Means true dry matter digestibility = (DMI - Fecal NDF) / DMI
[3] Means metabolic losses = True DMD - DMD
[abcd] Least square means within row without common superscripts differ (P<0.05)

Table 3: Rate of ruminal degradation of five hays incubated at 0 and 75 % total mean retention time in two ruminally fistulated steers

	Diet					Statistics	
Variable	**Prairie**	**Malf**	**Mbrome**	**Ibrome**	**Ialf**	**SEM**	**P-value**
NDF kd, %/hr^{-1} [1]	3.6[b]	7.1[a]	3.3[b]	4.8	7.5[a]	0.2	<0.01
CP kd, %/hr^{-1} [1]	8.2[a]	10.4[a]	4.2[a]	7.6[b]	8.6[a]	0.8	0.02

[1] Means true dry matter digestibility = (DMI - Fecal NDF) / DMI
[abcdef] Least square means within row without common superscripts differ (P<0.001)

Table 4: Regression equations and correlation between *in vitro* and *in vivo* digestibility for each of the 21 different in vitro runs.

Run	Regression Equation	R^2
1	$y^a = 1.0625x - 10.157$	0.6936
2	y = 1.4638x - 29.149	0.9728
3	y = 0.8894x - 0.2181	0.6708
4	y = 0.9487x - 5.0014	0.7516
5	y = 0.7739x + 6.3929	0.6245
6	y = 1.0496x - 14.577	0.7104
7	y = 0.9545x + 0.534	0.6683
8	y = 1.01001x - 2.4536	0.8222
9	y = 0.8313x + 4.7293	0.6414
10	y = 1.0827x - 8.989	0.9723
11	y = 1.0158x - 3.944	0.8367
12	y = 1.2546x - 18.997	0.7720
13	y = 1.3713x - 27.817	0.7471
14	y = 1.4169x - 29.331	0.5352
15	y = 0.9396x - 4.9711	0.7611
16	y = 0.9549x - 3.1735	0.7491
17	y = 1.0491x - 8.8189	0.7696
18	y = 1.1266x - 14.804	0.8437
19	y = 0.9456x - 9.1987	0.6874
20	y = 1.1538x - 14.568	0.8948
21	y = 1.088x -13.859	0.5766
All Runs[1]	y = 1.1626x - 15.584	0.8305

[1] Analysis of the 21 individual combined together
[a] y predicted *in vivo* digestibility
[b] x means non-adjusted *in vitro* digestibility

Table 5: Regression equations and R2 values for different number of tubes per standard within a single in vitro run.

Number of Tubes	Regression Equation	R^2
DMD [1]		
2	$y^a = 0.9303x^b + 0.5207$	0.7248
3	y = 0.9951x - 3.3778	0.7088
4	y = 0.9357x - 0.1108	0.7385
5	y = 0.9165x + 1.0275	0.7602
OMD [2]		
2	y = 0.9538x - 5.346	0.7249
3	y = 1.0232x -9.4986	0.7487
4	y = 0.9467x - 5.2607	0.7485
5	y = 0.9432x + 5.1267	0.7752

[1] Regression equations from DMD of *in vivo* and *in vitro* digestibility of five different hay samples
[2] Regression equations from OMD of *in vivo* and *in vitro* digestibility of five different hay samples
[a] y predicted *in vivo* digestibility
[b] x means non-adjusted in vitro digestibility

Figure 1: Regression analysis of *in vivo* vs. *in vitro* digestibility. No significant difference between slopes (P=0.99) was found. There was a significant difference between run (P=0.04).

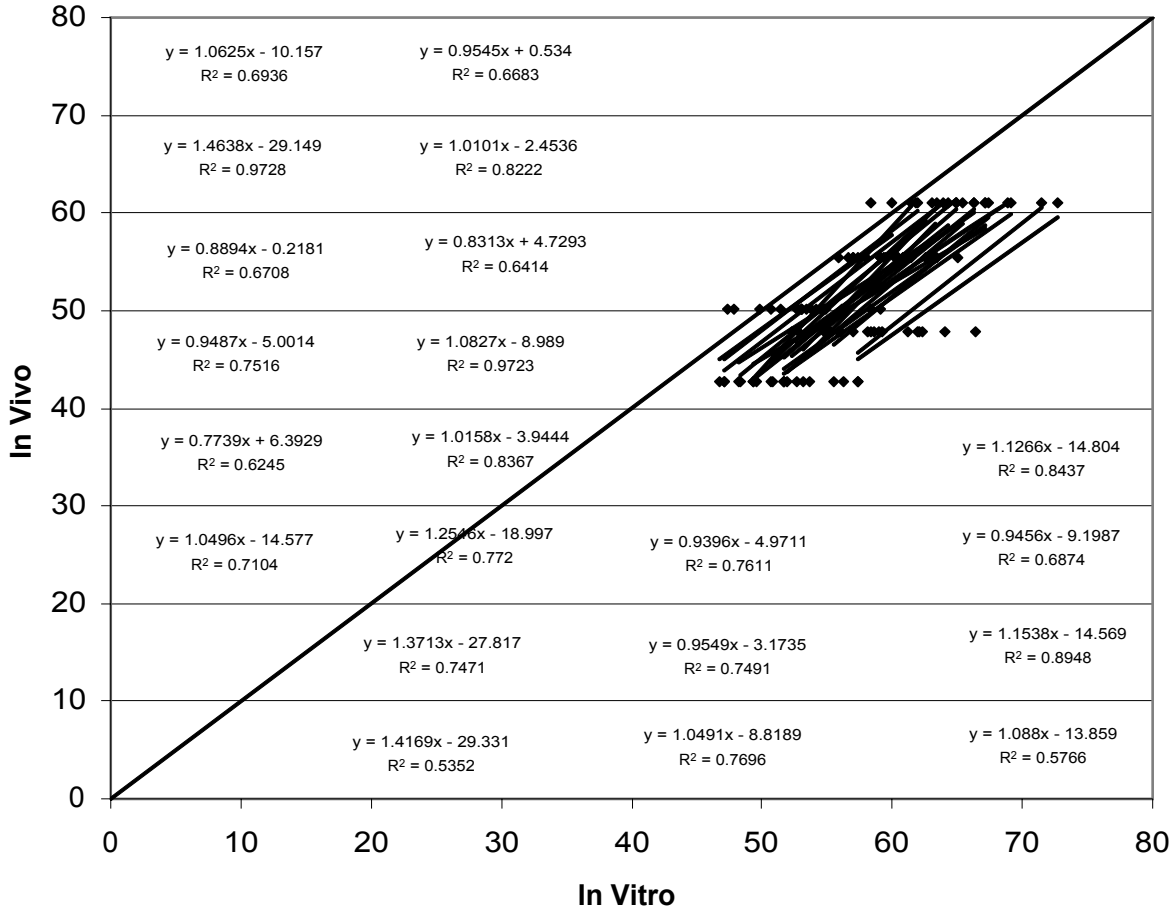

y = 1.0625x - 10.157
R² = 0.6936
y = 0.9545x + 0.534
R² = 0.6683
y = 1.4638x - 29.149
R² = 0.9728
y = 1.0101x - 2.4536
R² = 0.8222
y = 0.8894x - 0.2181
R² = 0.6708
y = 0.8313x + 4.7293
R² = 0.6414
y = 0.9487x - 5.0014
R² = 0.7516
y = 1.0827x - 8.989
R² = 0.9723
y = 0.7739x + 6.3929
R² = 0.6245
y = 1.0158x - 3.9444
R² = 0.8367
y = 1.1266x - 14.804
R² = 0.8437
y = 1.0496x - 14.577
R² = 0.7104
y = 1.2546x - 18.997
R² = 0.772
y = 0.9396x - 4.9711
R² = 0.7611
y = 0.9456x - 9.1987
R² = 0.6874
y = 1.3713x - 27.817
R² = 0.7471
y = 0.9549x - 3.1735
R² = 0.7491
y = 1.1538x - 14.569
R² = 0.8948
y = 1.4169x - 29.331
R² = 0.5352
y = 1.0491x - 8.8189
R² = 0.7696
y = 1.088x - 13.859
R² = 0.5766
In Vivo
In Vitro
0
10
20
30
40
50
60
70
80

Figure 2: Regression analysis of the average of all 21 *in vitro* runs. On average *in vitro* DMD is 6.4 percentage units higher than *in vivo* digestibility. This equates to an 11% difference between *in vivo* and *in vitro* digestibilities.

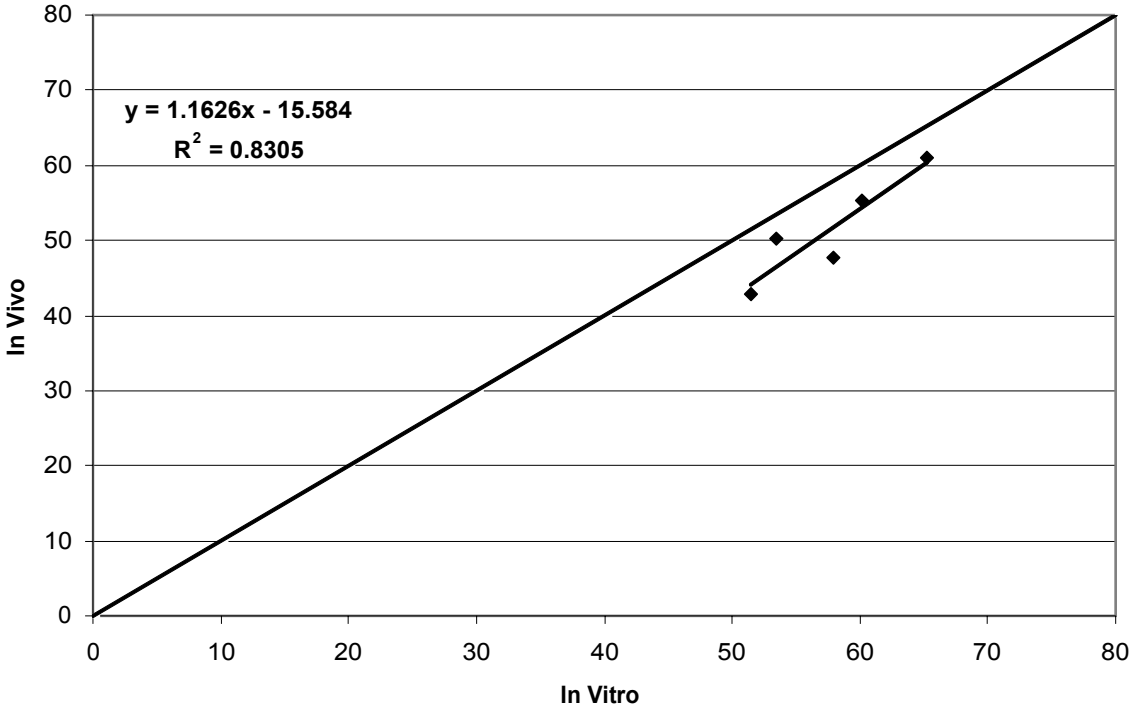
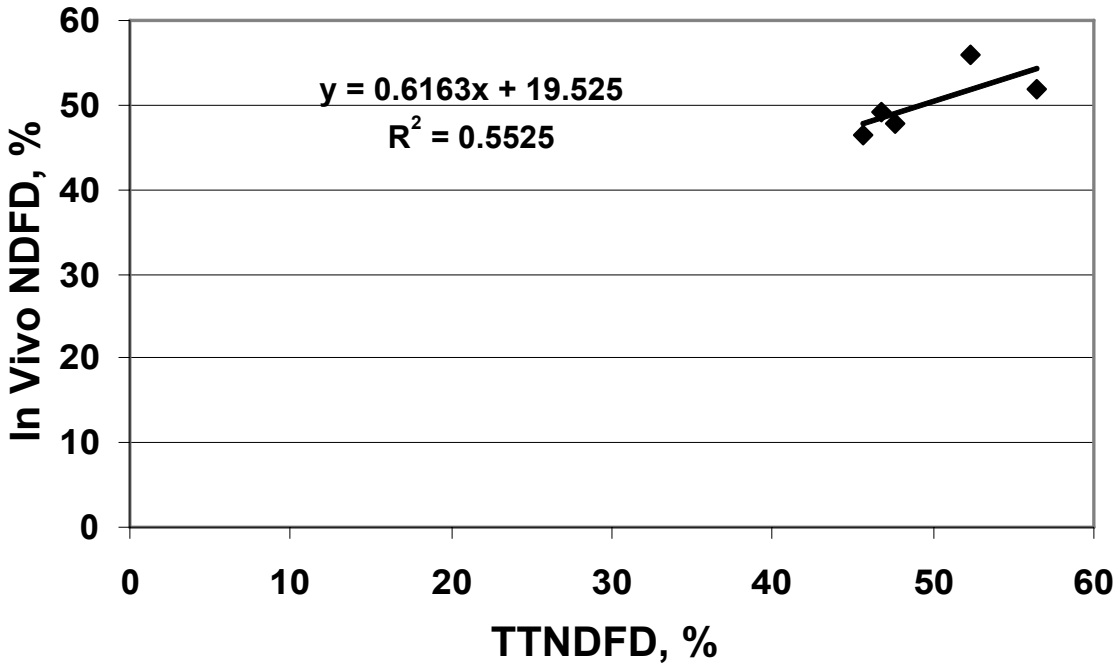

y = 1.1626x - 15.584
R2 = 0.8305
In Vivo
In Vitro
0
10
20
30
40
50
60
70
80

Figure 3: Regression analysis of in vivo NDF digestibility and Total Tract NDF digestibility determined from the mobile bag procedure. Strong relationship (R2 = 0.553) between in vivo and total tract NDFD was detected.


y = 0.6163x + 19.525
R2 = 0.5525
In Vivo NDFD, %
TTNDFD, %
0
10
20
30
40
50
60

Prediction of Year Round Protein and In Vivo Digestibility of Diets Consumed by Cattle Grazing Native Nebraska Sandhills Range Pastures

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Abstract: Feed accounts for the majority of the variable costs with beef production. Formulating supplements for grazing cattle to accurately meet their nutrient requirements with economical feedstuffs is challenging due to the limited data on diet quality of pastures. The objective of this trial was to develop a prediction model which will estimate diet digestibility and protein while accounting for precipitation, time of the year and grazing pressure. Monthly diet samples were collected from esophageally fistulated cows from May 2003 through November 2005. Samples were freeze dried, ground and composited for CP, UIP, UIP digestibility, IVOMD, and NDF analysis. Diet samples were highest ($P < 0.0001$) in CP and digestibility during April and May, declined throughout the remaining summer and remained relatively constant through the dormant season. A significant year*grazing effect ($P = 0.035$) was detected for CP where CP was lower at high levels of grazing during 2005 compared to all other levels of grazing in the three years. No other month*grazing or year*month*grazing interactions ($P > 0.05$) were detected for diet CP and no month*grazing or year*month*grazing interactions ($P > 0.05$) were detected for diet digestibility. As stocking rate increased, OMD and DMD decreased ($P < 0.0001$). Diets collected in 2005 were lower ($P < 0.0001$) in OMD and

DMD compared to 2003 and 2004, with 2003 being the highest in digestibility and 2004 intermediate. Prediction equations models generated to estimate diet CP and OMD were significant ($P < 0.012$ and R^2 ranging between 0.3371 and 0.630). Predicted OMD values were highly correlated ($r = 0.7996$) to observed OMD and there were no statistical differences ($P = 0.9999$) between predicted and observed OMD. Predicted CP values were also correlated ($r = 0.8107$) to observed CP and no difference ($P = 0.1615$) was observed between observed and predicted CP values. Prediction equations generated from these data can be used to estimate diet CP and *in vivo* OMD of diets consumed by cattle grazing Sandhills range pastures.

Introduction

Feed inputs account for the majority of the variable costs associated with beef production. Use of year-round grazing systems can reduce the need to feed harvested or purchased forages (Adams et al., 1994) and increase profit potential for beef producers. Forages can be harvested during periods when the quality is higher and the forage quality may be preserved until time of feeding. However, when grazing native range year-round, diet quality varies throughout the year in response to weather patterns, grazing pressure and other variable (Lardy et al., 1997; Patterson et al., 2000). Lower diet quality during the dormant months may increase the need for protein and energy supplements during these periods to meet the animal's requirement (Lardy et al., 1997). Reports of digestibilities of diets collected by grazing cattle are limited. Lardy et al. (1997) demonstrated that diet dry matter digestibility of cattle on Sandhills upland range was the highest in June and July and

decreased through the dormant season. However, these digestibility estimates are relative differences and *in vivo* digestibility was not estimated nor was IVDMD was not adjusted to DE therefor, may not be as accurate for use in the NRC Model. Prediction of diet quality of grazing cattle can be difficult because of the interacting effects of grazing, moisture, animal selectivity, plant maturity, and diversity of plant communities (Weir and Torell, 1959; Cook, 1964; Kamstra et al., 1968; Wallace et al., 1972; Kamstra, 1973; Powell et al., 1986; Walker et al., 1989; McKown et al., 1991; McCollum et al., 1994; Lardy et al., 1997). Accurate estimates of diet energy and protein are very important in formulating supplements and prediction of animal performance in grazing situations (Weiss, 1994; Lardy et al., 2004; Patterson et al., 2006).

In vitro OMD procedures have been shown to be highly correlated to in vivo digestibility (Weiss, 1994). However, when using the beef NRC (1996) to predict animal performance, the direct conversion of IVOMD equal to TDN is not as precise as using an equation (Rittenhouse et al., 1971) to convert IVOMD to DE, where DE is equal to TDN (Lardy et al., 2004; Patterson et al., 2006). Lardy et al. (2004) and Patterson et al. (2006) evaluated the accuracy of using the NRC model to predict grazing cattle response. They used the conversions of IVOMD equal to TDN or IVOMD to DE using the equation described by Rittenhouse (1971) to predict animal performance of grazing cattle. Both Lardy et al. (2004) and Patterson et al. (2006) reported that the accuracy of predicting animal performance based on the conversions to DE as an estimate of TDN was improved trials where actual changes in BCS were known. Researchers concluded that the DE conversion was a better estimate for TDN. However, *in vivo* data should be used

when available. Unfortunately *in vivo* data are very limited and difficult to develop. The objectives of this trial were to: 1) evaluate yearly diet digestibility and protein in the Sandhills; 2) convert in vitro DMD and OMD to in vivo DMD and OMD to use for TDN estimation; and 3) develop a model to predict diet energy and protein with the inputs of day, precipitation, and grazing pressure.

Materials and Methods

Diet Collection

Masticate samples were collected from the University of Nebraska Gudmundsen Sandhills Laboratory (GSL) located 20 km northeast of Whitman, Nebraska. GSL is located in the west-central region of the Nebraska Sandhills. The ranch consists of approximately 4695 ha of native upland rangeland. Average annual precipitation for the area is 46-51 cm. Diet samples were collected beginning in May 2003 and collection was continued through November 2005. The plant growth patterns (April through March) were used to separate the data collected into three separate years. Cumulative precipitation data began on October first of the previous year and was accumulated until one week prior to the collection date. Therefore, the yearly moisture calendar was from October first of the previous through September 30th of the current. Masticate samples were collected monthly during the dormant (October, November, December, January, February, and March) season and every three weeks during the growing (April, May, June, July, August, and September) season to account for rapid changes due to plant growth. Masticate samples were collected using mature, multiparous beef cows fitted

with esophageal fistulae. Six cows were fasted overnight prior to collection. On the morning of collections, cows were randomly separated into two groups consisting of three cows each. Each group of cows collected samples from two different pastures beginning at 0700 hr for a total of four pastures sampled per collection time point. Cows were hauled to the collection sites in a trailer. Once at the collection site esophageal fistulae plugs were removed and bags (with screen bottoms) were hung on the neck of each cow and secured in place via a nylon belly strap tied behind the front shoulder. A bungee cord was used to attach the belly band to the nylon collection bag so that bags remained in place and cow movement was not restricted. Cows were allowed to graze for 15 to 45 minutes (until a significant sized sample was collected, approximately 1 kg of sample). Following grazing, bags were removed and the masticate samples were sub-sampled and excess saliva was hand squeezed from the sample. Sub-samples were frozen and were later freeze dried. Following freeze drying samples were ground through a Wiley Mill using a 2-mm screen. Sub-samples were then composited by collection date and pasture. Composite samples were mixed and a portion was ground through a Wiley Mill using a 1-mm screen. Samples were analyzed for N, undegradable intake protein (UIP), degradable intake protein (DIP), IVDMD, IVOMD and neutral detergent fiber (NDF).

One pasture remained constant throughout the trial and was not grazed and was sampled during every collection time. The other three pastures varied between each collection time and were selected based on the amount of grazing pressure at the time of collection. These three pastures varied from a high levels of grazing pressure to a low

level of grazing pressure at each time point. They also varied in location throughout the ranch based on where the different cow herds were grazing at the time of collection. Stocking rate was used in determining the main effects of month, year, grazing level and their respective interactions. Study pastures were generally in good to excellent range condition. Recommended stocking rate for pastures in good to excellent conditions in the GSL area is 1.2 AUM/ha. Stocking rate was calculated for each pasture at the time of collection. If the stocking rate was equal to or greater then the recommended stocking rate for the area (1.2 AUM/ha) the stocking rate was considered to be high (High). While stocking rates between 0.1 and 1.1 AUM/ha, grazing pressure was considered medium (Med). Stocking rate was considered zero (None) at stocking rates less than 0.1 AUM/ha.

Grazing pressure was used in for regression analyses for forage quality prediction. Grazing pressure (AU/unit forage over a period of time) was determined based on the actual grazing (AUM/ha) history of the pasture and forage yield up to the time when the sample was collected.

Forage Yield Prediction

Forage yield was determined using standing crop data (clipped on August 15th of each year) from GSL from 1998 through 2006 and the Barta Brothers Ranch from 1999 through 2006. Cumulative precipitation was recorded from each of the locations during these dates. Cumulative moisture was recorded from October 1 of the previous year to 15 days prior to the sampling date. Amount of precipitation was collected and recorded throughout the trial using the weather station located on the ranch site for GSL and the

weather station located at Rose, Nebraska for the Barta Brothers Ranch. Forage yield was regressed against the cumulative precipitation to generate an equation to adjust forage yield for precipitation. The resulting regression equation is $y = 71.056x + 412.47$ ($R^2 = 0.3575$) where x is the cumulative moisture and y is forage yield. After annual forage production was calculated, the total forage production was adjusted to the different days of the year in order to account for forage growth patterns. Forage yield was calculated for each day of the year based on forage growth curves generated by the NRCS for the Nebraska Sandhills region. The total forage yield was then adjusted using the equation $y = 1.953E07x^4 - 1.692E05x^3 + 0.0498x^2 - 5.244x + 178.284$ ($R^2 = 0.99$) where x is day and y is cumulative forage production percentage for day of the year. April 1 was entered as d 1, the beginning of plant growth.

Validation Data Set

Masticate samples were collected from three additional locations to be used for validating the prediction model. One location was at GSL (GSL2) in a separate set of 1-ha pastures not used in the main data set. Prior to the initiation of this trial this upland range site had not been grazed for 7 years and was in good to excellent condition. These pastures were stocked at 3 different levels which included the recommended stocking rate (1.2 AUM/ha,), double the recommended stocking rate (2.5 AUM/ha) and double stocked plus supplement (2.27 kg/hd/d DDGS). Cattle used in this experiment rotationally grazed the 1-ha paddocks. Masticate samples were collected following the same procedure previously described at the mid point of each grazing event. Masticate samples were collected from mid June through mid August in 2005 and 2006.

The second location was near Imperial, Nebraska at a commercial ranch. Diets were collected from non-grazed pastures using three esophageally fistulated cows. Vegetation at this location was a mixed grass prairie consisting predominately warm season grass species with a smaller portion of cool season grass species. This location is on the southern edge of the Sandhills and the edge of the Plains regions. The plains region is flat-lying land which is above the valley regions. Cows were transported to the location two days prior to collection. They were allowed to graze a pasture near the collection sites in order to acclimate the cows to the forages in the area. Collection protocol was the same as previously described. Masticate samples were collected from May through September in 2003 and from May through November in 2004.

The third location was at the University of Nebraska Barta Brothers Ranch (BBR) near Rose, Nebraska. The Barta Brothers Ranch is located near the eastern edge of the Sandhills. Four mature, multiparous esophageally-fistulated cows were used to collect masticate samples periodically through the summer grazing season (May 15 to October 15) of 2005. Masticate samples were collected from 4-pasture deferred rotational grazing systems and 8-pasture management intensive grazing systems. Cows were maintained in pastures near the handling facilities. All samples collected from the validation locations were analyzed for CP, IVDMD, IVOMD, and NDF. All masticate samples were handled following the same procedure as described above.

In Vitro to Predict In Vivo Digestibility

In vitro DMD and OMD were measured using a modified version of the in vitro procedure described by Tilly and Terry (1964). Samples were ground through a 1-mm

screen. The original procedure was modified with the addition of 1g urea L^{-1} of McDougall's buffer. Rumen fluid was collected from two steers (BW = 250 kg) for each of the in vitro runs. Steers were fed a bromegrass hay diet once daily at 1.5% of BW. A standard set of samples (five hays) with known in vivo digestibilities were included in each in vitro run (Geisert et al., 2006). Regression equations were determined for each separate run and in vitro DMD and OMD of the masticate samples were adjusted using those equations (Geisert et al., 2006; Weiss, 1994). Due to the large number of samples, four separate in vitro runs were conducted. Samples were run in triplicate tubes over three separate *in vitro* runs, and standards were included with five tubes in each run.

In Situ and Mobile Bag Incubations

In situ and mobile bag procedures were performed on samples collected in 2004 and 2005. In situ incubation was conducted using two ruminally and duodenally fistulated steers (BW = 250 kg). Dacron bags (Ankom Inc, Fairport, NY) measuring 5 x 10 cm with 50 Φm pore size were filled with 1.25 g of air-dried hay sample ground through a 2-mm screen and heat sealed. Donor animals were fed once daily a bromegrass hay ration at 1.5% BW. Triplicate bags were incubated at each time point and replicated within each of the two steers. Time points for incubation were 0, 25, 30, and 96 h. The 25 and 30 h times were calculated at 75% TMRT plus a 10 h lag with the 25 h incubation for samples collected in April through September and the 30 h incubation for samples collected January through March and October through December. Following ruminal incubation, bags were washed in a washing machine for 0.25 h using a 1-min agitation and 2-min spin. The washing cycle was repeated a total of 5 times. Bags were refluxed

in neutral detergent fiber solution following washing in order to remove any microbial contamination and to determine NDIN. The 0-hr bags were not suspended in the rumen, however, they were washed and refluxed following the same procedure. Bags were dried in a 60°C forced air oven for 48-h following reflux. They were weighed out of the oven after setting in a desicator for 5-min (hot weight). Following the hot weight, bags were allowed to air equilibrate for 3 h and were weighed again. Residue remaining in the bags were analyzed for N using the combustion method (AOAC, 1996) in a combustion analyzer (Leco FP-528, St. Joseph, MI)

A second set of bags (75% TMRT) were incubated in the rumen following the same procedure and the same donor animals described for the in situ incubation. Each masticate was replicated in 2 bags/steer. Following ruminal incubation, bags were incubated in a pepsin and HCl (1 g L^{-1} pepsin and 0.01 M Hcl; 62.5 ml/bag) solution at 37°C for 3 h to simulate abomasal digestion. Bags were then randomly sorted for duodenal insertion with 7 or 8 bags/d. Bags were inserted into the duodenal fistula of each steer over two d. Seven bags were inserted on d 1 and 8 bags on d 2. Steers were fed a bromegrass hay diet at 1.5% BW at 0700 h daily. Bags were inserted beginning at 1700 at a rate of 1 bag every 5 min to prevent blockages in the intestine of the animal. Bags were collected in the feces beginning 12 h after insertion and frozen until all bags were collected. Following collection of all bags they were machine washed and refluxed in NDF solution following the same procedure as the in situ incubation. Bags were weighed and analyzed for N in the same manner as previously described for the in situ bags.

Model Prediction

Dietary OMD and CP predictions models were generated using the regression procedures in SAS (SAS Inst. Inc., Cary, NC) and the forward, backward, and stepwise options for model selection. Variables included in the model included the linear, quadratic, and cubic effects of precipitation, grazing pressure and Julian day. In building the prediction model of OMD, the data were separated into three categories by Julian day to account for the difference in the plant growth curves and to accurately separate the variables significantly impacting OMD at each given point in time. The three categories were early growing season (Julian d 1-76), late growing season (Julian d 77-183) and dormant season (Julian d 184-365).

Statistical Analysis

Diet chemical analyses were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The main effects of month, fixed effects of year and grazing level, and their respective interactions were analyzed using the Mixed procedures of SAS (SAS Inst. Inc., Cary, NC). A protected F-test was used to evaluate moisture, grazing pressure, and day of year differences. Least square means were separated using Least Significant Difference method when a significant ($P < 0.05$) F-test was detected. The diet quality prediction model and forage yield model were analyzed using the GLM and REG procedure of SAS (SAS Inst. Inc., Cary, NC). Mobile bag and in situ data were analyzed as a completely randomized design using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). All trial procedures were approved by the University of Nebraska Institutional Animal Care and Use Committee.

Results and Discussion

Precipitation and Grazing Level

Yearly precipitation (Table 1) ranged from a total of 32.0 to 46.7 cm. Average annual precipitation for this area ranges from 46 to 51 cm annually. During the third year annual precipitation was near the average for the area. However, 2003 and 2004 were drought years for the Sandhills with totals of only 32.0 and 38.1 cm in 2003 and 2004, respectively.

Diet Protein, Fiber, and Digestibility Analysis

No year*grazing, month*grazing, or year*month*grazing interactions ($P > 0.05$) were detected for diet digestibility. As expected, IVOMD and IVDMD were highest ($P < 0.001$; Table 2) during spring and early summer (April through June). Digestibilities decreased over the course of the rest of the summer and remained fairly constant throughout the winter months. Significant year and grazing level effects ($P < 0.001$) were detected (Figure 1 and Figure 2). Similar results were observed (Lardy et al., 1997) at GSL in earlier studies. Lardy et al. (1997) used multiple regression equations to estimate diet protein and digestibility. Estimated protein and energy values for upland Sandhills range pastures were highest in May and June, decreased through the summer and early fall, then remained relatively constant during the winter and early spring (Lardy et al., 1997). The slight increases in August and September likely were the result of cool season grass growth. Lardy et al. (1997) reported IVDMD values ranging from 59.2 to

68.1% during the growing season and from 48.9 to 55.7% for diets collected during the dormant season. Lardy et al. (1997) results (52.0%) were on average very similar to adjusted IVDMD values (53.3%) from this trial for diets collected during the dormant season. During the growing season, adjusted IVDMD values for the current trial (average IVDMD, 58.4%) were on average 5.5 percentage units lower than IVDMD reported (63.8 %) by Lardy et al. (1997). These lower values during the growing season are due to the adjustment equation which decreases the IVDMD values. Adjustments of the current IVOMD data were greater in samples collected during the growing seasons than those collected during the dormant season. Results from Lardy et al. (1997) were not adjusted to *in vivo* digestibility and may not be as accurate in predicting animal response and formulating rations as data generated from this trial especially during growing season grazing periods. Patterson et al. (2006) adjusted IVOMD data to DE using the equation published by Rittenhouse et al. (1971) which was entered into the NRC (1996) model as TDN. Researchers compared the ability of the NRC to predict animal response (BCS) using IVOMD = TDN and DE = TDN. Patterson et al. (2006) concluded that using the adjusted TDN based on the Rittenhouse et al. (1971) equation, the NRC model (1996) was more accurate at predicting changes in BCS.

Similar results in diet quality patterns were observed (Cogswell and Kamstra, 1976; Johnson et al., 1998) where CP and digestibility decreased from June through September (Cogswell and Kamstra, 1976) and December (Johnson et al., 1998). It has been well documented that advancing stages of plant maturity decrease diet protein and digestibility and increases diet fiber and lignin (Kamstra et al., 1968; Wallace et al1972;

McCollum and Galyean, 1985; Johnson et al., 1998) .

A significant (P <0.001) year effect was detected for both IVDMD and IVOMD (Figure 1). Organic matter digestibility was lower in 2004 and 2005 than in 2003. Dry matter digestibility was different among all three years with DMD highest in 2003, lowest in 2005 and intermediate in 2004. The decrease in diet digestibility among years likely is related to annual precipitation. In 2003 and 2004, when the annual precipitation was well below the average for the region, plant maturity could have been delayed, allowing animals to graze plants in a vegetative stage of plant growth for a longer period of time. The lower precipitation in 2003 could have increased IVDMD and IVOMD compared to 2004. Wilson et al. (1983, as cited by Nelson and Moser, 1994) reported higher digestibility of leaf and stem portions in water-stressed plants. Extended periods of drought have been reported to delay plant maturity (Halim et al., 1989; Peterson et al., 1992). They also reported decreased shoot length in drought stressed plants which increases the leaf:stem ratio also resulting in an increase in forage digestiblity.

Increasing stocking rate significantly (P < 0.02) decreased OMD and DMD of masticate samples, where high grazing was different compared to none-grazed pastures (Figure 2). Medium stocking rate was intermediate and not different from either high or none-grazed pastures. Increasing grazing likely decreased the amount of higher digestible plants or plant parts available for grazing which decreased the digestibility of the diets collected. Rauzi (1964) reported a negative regression in diet quality during cool-season grazing (both IVOMD and CP) as grazing pressure increased in esophageally-fistulated sheep and cattle diets. During warm-season grazing, CP and

IVOMD decreased in diets collected by cattle with increasing grazing pressure. However, sheep diets during the same grazing season did not differ in CP or IVOMD. Researchers concluded that similarity in the diets collected from sheep was because the ability of sheep to select diets of higher quality was not affected by increasing levels of grazing pressure.

Plant species preference by livestock likely changes as grazing intensity increases because of decreased availability of highly preferred plant species (Pieper et al., 1959). Other research (McCollum et al., 1994; Hirschfeld et al., 1996) has shown increased CP and digestibility of diets in grazing animals as grazing systems are shifted from a continuous grazing to a rotational grazing system and when the number of cycles within a rotational system increases. Increased diet quality in these trials could be attributed to increasing the time that the preferred plant species are in the vegetative growth stage. A year-by-stocking rate effect (Figure 4) was detected (P = 0.035) for CP content of masticate samples from range pastures. Diets collected from high stocking rate pastures during 2005 were lower (7.1% CP) in CP compared to the other two stocking rates (average of 8.5%) over the three years. No difference was detected between the other two stocking rates within year. This effect could be attributed to below average precipitation in both 2003 and 2004. During those years of drought, CP concentration in plant tissue was greater because of lower plant tissue yields (Weir and Torell, 1959, and Gregorini et al., 2006). Cows could also have selected plants such as shrubs and forbs during those times of below average forage yields which could have contributed to increased protein content of diets collected from pastures with high levels of grazing

(Taylor et al., 1980). During 2005 when precipitation was average, forage yield was increased thus decreasing the concentration of protein in the plants. Cows could have consumed more grass species and fewer forb and shrub species during 2005 as well. In this trial, increased grazing likely reduced the forage available for grazing thus decreasing the protein content of the diets collected in 2005.

Monthly CP (Table 2) values were highest ($P < 0.001$) during May (peak of cool-season plant vegetative growth) and remained high during June and July (during warm-season plant vegetative growth). Crude protein values decreased through the remainder of the growing season and then remained relatively constant during the dormant season (Figure 4). Lardy et al. (1997) reported similar results for CP values of upland Sandhills ranges pastures with the highest protein values in May and June with a sharp decrease throughout the growing season and relatively stable during the dormant season. On average, the CP content of masticate samples collected during the growing season were similar for the current trial (10.1 % CP) and the trial reported by Lardy et al. (1997) (10.0 % CP). Crude protein values of diets collected during the dormant months for the current trial were higher (7.2 % CP) than those reported (5.4 % CP) by Lardy et al. (1997). This could be due to more data collected in the current trial and more months included in the data set (6 months vs 4 months). White (1983) reported CP of vegetative tillers was higher (25% CP) than floral tillers (5.9% CP) indicating that mature plants have lower CP values than growing plants. Johnson et al. (1998) showed a linear decrease in dietary CP of diet samples collected from mid June through December.

No grazing-by-year or grazing-by-month interactions ($P > 0.301$) were detected

for NDF content of the diet samples. Results from NDF data followed similar trends as IVOMD and IVDMD. Diet samples in 2005 were significantly higher ($P = 0.0173$) in NDF than diet samples collected in 2003 and 2004 (Figure 3). Neutral detergent fiber of diets collected in 2003 and 2004 were not different. The higher NDF content in 2005 could be explained the higher precipitation in 2005. Lower precipitation in 2003 and 2004 delayed plant maturity keeping the plants in a vegetative stage of growth longer thus, decreasing fiber content.

The NDF content differed ($P < 0.0001$) among months (Table 2). Diets were lowest in fiber in May, slighly increased in June, decreased in July and August then increased throughout the dormant season. The decrease in May could be due to the vegetative growth of cool-season species and the increase in June could be due to the maturation of cool season species. By July and July warm-season grasses were in vegetative growth stages and the continued lower fiber into August could be due to cool-season growth and some continued vegetative growth of warm-season grasses. Neutral detergent fiber of dormant season diet samples were not different among months ($P > 0.05$). These results match results in diet digestibility. The lower NDF content during the early growing season correspond with the increase of diet digestibility during the same time point. Cogswell and Kamstra (1976) showed fiber content of four different range grass species was lower in mid June and increased through mid September. Rao et al. (1973) reported decreased NDF of diet samples in June and in August through September. The decreased NDF in late summer was due to cool-season plant growth. The lower fiber content of diet samples in June was due to vegetative growth of plants consumed.

Protein Fractionation and Digestibility

When expressed as a percent of dietary CP, diet UIP, DIP, and total tract indigestible protein (TTIDP) showed a month by grazing interaction (Figure 5) (P = 0.02, 0.02, and 0.03 for UIP, DIP, and TTIDP, respectively) (Table 3). However, when the protein (% CP) fraction was expressed as a percent of dietary OM the month by grazing interaction was not significant (P < 0.50) for diet UIP, DIP, and TTIDP. Undegradable intake protein was the lowest in May and increased through December (Table 3). Seasonal protein factions are shown in Figure 6.

When expressed as a percent of OM, significant year (P < 0.05) and grazing (P = 0.04) effects were observed for UIP and significant effects of year (P < 0.05) were observed for TTIDP (Table 4). Undegradable intake protein was higher in 2005 as compared to 2004 (2.91 and 2.65 %, respectively). Increasing stocking rates from none to high significantly (P = 0.04) increased UIP. Johnson et al. (1998) reported a linear decrease in UIP, % of CP (mid June through December) of diet samples collected from native range in western North Dakota. Gustad et al. (2006) reported no difference in UIP values when expressed on a percent of DM of diet samples of upland range pastures between different grazing levels. Digestibile UIP (% of DM) ranged from 1.2 % to 3.0 % with an overall average of 2.2 % of dietary DM.

No month by grazing interactions were detected (P > 0.40) for undegradable intake protein digestibility (UIPD). Undegradable intake protein digestibility tended to differ among months (P = 0.06). The overall average UIPD was 38.4% of the dietary UIP. When expressed as a percent of CP, significant year and grazing effects (Table 4)

were observed in this trial for UIPD. In 2004, UIPD values were higher than in 2005 (40.1 and 36.7%, respectively). Increasing grazing pressure from none to high increased the digestibility of the UIP with moderately grazed pastures as an intermediate. When UIPD was expressed as a percent of dietary OM (Table 4) year effects were significant ($P < 0.01$) whereas grazing level was not ($P = 0.24$). There was, however, a numerical increase in UIPD with increasing levels of grazing. Digestibility of the UIP was still lower in 2005 then in 2004.

Undegradable intake protein digestibility values of upland Sandhills range pastures were reported (Gustad et al., 2006) to decrease when grazing pressure increased from the recommended stocking rate to two times the recommended stocking rates. When supplementation (protein and energy) was added to the double stocked paddocks, UIPD also decreased (Gustad et al., 2006). The UIPD in that study ranged from 11.6 % to 44.9 % with an overall average of 26.4 % percent of the dietary UIP. In the control paddocks, UIPD ranged from 15.9 % in early August to 44.9 % in mid June and appeared to decrease with advancing stages of forage maturity. When stocking rates were doubled, digestibility of UIP appeared to decrease from mid June through mid July; however, it increased in late July and early August. Similar trends were observed with the addition of supplements to the double stocking rate treatment.

Rate of ruminal protein degradation was different ($P < 0.0001$) among months with the highest rate of degradation for samples collected in April and May. The rate of protein degradation decreased from May through July, slightly increased in August then decreased throughout the dormant season and increasing through April. Increasing grazing pressure

decreased (P = 0.02) the rate of ruminal protein degradation (2.14, 3.10, and 7.08 % h^{-1} for high, medium, and none, respectively) (Table 4). Dietary protein of samples collected in 2004 degraded at a slower rate (P < 0.001) compared to those collected in 2005 (Table 4). Gustad et al. (2006) showed a tendency for the rate of protein degradation to decrease with doubling grazing pressure in upland Sandhills pastures.

The beef NRC (1996) assumes 80% digestibility of UIP; therefore, one can not simply enter the calculated CP into the NRC. UIP digestibility and CP must be adjusted to account for this assumption when entering protein values into the NRC (Table 3). Digestibility of UIP can be adjusted using the equation: Adjusted UIP = DUIP / 0.80. Then by back calculation CP can be determined using the equation; Adjusted CP = DIP (OM basis) + Adjusted UIP (OM basis)

Model Prediction

The crude protein model (Table 5) included Julian d as the only significant variable (P < 0.0001, R^2 = 0.6330). The equation for predicting CP of range diets in the Nebraska Sandhills is: CP = 0.27321*JD - 0.00456*JD2 + 2.86E^{-5}*JD3 - 8.00949E^{-9}*JD4 + 8.34511E^{-11}*JD5 + 7.88021, where JD = Julian day, JD2 = Julian day*Julian day, JD3 = Julian day*Julian day*Julian day, JD4 = Julian day*Julian day*Julian day*Julian day, and JD5 = Julian day*Julian day*Julian day*Julian day*Julian day. Predicted CP of the validation diet samples were correlated (r = 0.69) to the observed CP of the samples. The predicted CP (Figure 7) values peak in May and June and decrease throughout the growing season and remain relatively constant during the dormant season. No difference (P = 0.1615) was observed between the predicted CP and the observed CP. The predicted

values were on average 0.27 percentage units lower than the observed values.

Significant variables in the OMD prediction equations varied among the three different seasonal categories (Table 5) (P values ranged from <0.001 to 0.012, R^2 ranged from 0.3371 to 0.5490). Predicted OMD values were not significantly different (P > 0.99) from the observed OMD values. When evaluating the prediction of the control pasture (no grazing pressure) the model predicted similar results as seen in the observed OMD results (Figure 8). In 2003, lower moisture increased diet OMD, most likely due to delayed plant maturity. In 2005, when moisture was higher than both 2003 and 2004 and more indicative of average annual precipitation, diet OMD was lowest, with OMD in 2004 intermediate and 2003 highest. To evaluate the model prediction for the effect of grazing pressure we isolated 2005 (Figure 8). The comparison was made between high grazing pressure (32 AUD/T) and no grazing. Diet OMD was lower at any time point throughout the year when grazing pressure was high compared to no grazing.

When the predicted CP was regressed (Figure 9) against the observed CP from the three validation data sets (Barta, GSL2, and Imperial) the R^2 values ranged from 0.537 to 0.66 (Table 6). Predicted CP was correlated (r = 0.55) with the observed CP from the different locations. There were no differences (P = 0.51) between the observed and predicted CP values from the validation data sets. When evaluating the regression (Figure 10) of predicted versus the observed OMD from the validation data sets the R2 values ranged from 0.41 to 0.73 among the three different locations (Table 6). No differences (P = 0.55) were observed between the predicted and observed OMD from the validation data set.

Conclusion

Using the five standard forage samples with known *in vivo* digestibility effectively adjusted *in vitro* digestibility of forages to *in vivo* digestibility. This is useful in determining an accurate estimate of TDN to be used in the NRC model when formulating supplements or predicting animal response in cattle grazing native Sandhills range pastures. The CP and OMD values generated from the prediction equations were highly correlated to *in vivo* values. Prediction model equations will work relatively well in predicting dietary CP and energy when collection of actual diets are not attainable. This will prove to be a very useful tool for cattle producers, nutritionist, and researchers to accurately predict diet nutritional components (CP and energy) to use in diet formulation and predicting animal response. These equations take into account some of the major contributing factors in the variation in diet quality.

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Table 1: Monthly and yearly cumulative precipitation for Gudmundsen Sandhills Laboratory. Cumulative precipitation for the current year begins in October 1st of the previous year

		Year	
Month 2003*	**2004**	**2005**	
January	3.6(1.4)	1.3(0.5)	2.8(1.1)
February	4.8(1.9)	1.3(0.5)	4.1(1.6)
March	7.6(3.0)	2.5(1.0)	4.1(1.6)
April	10.4(4.1)	5.3(2.1)	5.3(2.1)
May	14.5(5.7)	13.0(5.1)	16.3(6.4)
June	19.6(7.7)	15.2(6.0)	25.1(9.9)
July	24.9(9.8)	19.3(7.6)	37.3(14.7)
August	27.7(10.9)	29.5(11.6)	39.3(15.5)
September	29.7(11.7)	30.5(12.0)	46.2(18.2)
October	32.0(12.6)	38.1(15.0)	46.7(18.4)
November	32.0(12.6)	38.1(15.0)	46.7(18.4)
December	32.0(12.6)	38.1(15.0)	46.7(18.4)
Total	**32.0(12.6)**	**38.1(15.0)**	**46.7(18.4)**

* Precipitation is presented in cm(in).

Table 2: Monthly digestibility and crude protein values of masticate samples collected from the Gudmundsen Sandhills Laboratory

	Month												Statistics[4]		
Variable	**Jan**	**Feb**	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**SEM**	**P-value**	**LSD**
IVOMD[1],%	54.2	54.6	52.6	59.5	65.8	62.6	55.9	55.2	51.4	53.0	51.4	53.9	1.7	<0.01	4.2
IVDMD[2],%	48.0	48.8	47.7	53.2	59.7	58.6	50.2	49.8	46.0	47.1	45.7	47.8	1.5	<0.01	5.4
CP, %	6.9	6.2	7.4	8.0	12.4	10.8	11.5	8.9	8.8	7.9	7.6	7.0	0.7	<0.01	0.87
NDF, %	83.3	82.5	83.0	77.1	68.3	70.1	65.6	64.5	69.3	74.0	74.7	77.6	2.1	<0.01	6.1
kd[3], $\%h^{-1}$	1.35	0.87	2.11	5.05	6.50	3.57	3.03	4.82	3.24	3.50	1.90	1.32	0.85	<0.01	1.9
Pool, %[5]	1.95	1.29	2.62	5.27	8.34	5.90	5.95	3.42	3.65	2.95	2.78	1.42	0.45	<0.01	1.3

[1]IVOMD means in vitro organic matter digestibility.
[2]IVDMD means in vitro dry matter digestibility.
[3]kd means the rate of ruminal protein degradation expressed as $\% h^{-1}$.
[4]No year*month, year*grazing, month*grazing, or year*month*grazing interactions were detected ($P > 0.05$).
[5]Pool means residue protein content following NDF analysis.

Table 3: Protein fraction data and digestibility of UIP of diet samples collected in 2004 and 2005 from native range pastures at the Gudmundsen Sandhills Laboratory

	Month													Statistics		
Variable	**Jan**	**Feb**	**March**	**April**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**SEM**	**Month**	**M*G[5]**	**LSD[6]**
% of CP																
UIP,%	39.6	44.8	41.8	23.5	17.8	21.0	30.1	34.3	33.7	37.0	39.1	51.0	3.0	<0.01	0.02	6.5
DIP,%	60.4	55.2	58.2	76.5	82.2	79.0	69.9	65.7	66.3	63.0	60.9	49.0	2.8	<0.01	0.03	6.5
TTIDP[2], %	23.4	28.9	24.4	15.2	10.4	12.9	17.3	20.4	22.0	23.5	35.4	32.7	1.1	<0.01	0.03	4.6
UIPD[1], %	42.6	37.7	44.0	35.0	39.3	38.1	41.7	41.7	34.3	34.3	36.6	35.6	3.0	<0.01	0.44	7.4
% of OM																
CP, %	5.6	4.9	5.9	8.8	12.0	9.7	10.2	7.2	7.5	6.4	6.2	5.7	0.6	<0.01	0.73	1.61
UIP %	2.8	2.8	2.9	2.3	2.1	2.5	4.0	3.2	2.9	3.2	2.5	2.9	0.3	<0.01	0.57	0.66
DIP[3], %	3.1	2.6	3.2	6.6	9.5	7.2	6.7	4.8	4.6	3.5	3.6	2.5	0.4	<0.01	0.49	0.51
TTIDP,%	1.6	1.7	1.6	1.4	1.2	1.5	2.3	1.7	1.9	1.9	1.5	1.8	0.1	<0.01	0.85	0.46
DUIP[4], %	1.2	1.1	1.3	0.9	0.9	1.0	1.7	1.5	1.0	1.3	1.0	1.1	0.1	<0.01	0.68	0.33
NRC Adjust																
Adjust UIP[7]	1.5	1.4	1.6	1.1	1.1	1.3	2.1	1.9	1.3	1.6	1.3	1.4	--	--	--	--
Adjust CP[8]	4.6	4.0	4.8	7.7	10.6	8.5	8.8	6.7	5.9	5.1	4.9	3.9	--	--	--	--

[1]UIPD means digestibility of the UIP, expressed as a percent of the UIP.
[2]TTIDP means total tract indigestible protein.
[3]DIP means degradable intake protein.
[4]DUIP means digestibility of UIP calculated as DUIP = UIP - TTIDP.
[5]M*G means month by grazing interaction.
[6]LSD means the least square difference for the main effect of month.
[7]Adjust UIP means adjusted for NRC estimated UIP digestibility of 80 % where; Adjusted UIP = DUIP (% OM)/0.80.
[8]Adjust CP means CP adjusted for NRC estimated UIP digestiblility of 80% where; Adjust CP(% OM)=DIP(% OM) + Adjust UIP(%OM).

Table 4: Year (yr) and grazing (gr) effect on protein fraction of diet samples collected from the Gudmundsen Sandhills Laboratory.

Variable	year		grazing			Statistics[7]		
	2004	2005	High	Med	None	SEM	yr	gr
% of CP								
UIP[1], %	32.8[a]	36.2[b]	38.8[a]	36.1[a]	28.5	1.9	0.02	<0.01
DIP[2], %	67.2[a]	63.8[b]	61.2[a]	63.9[a]	71.5	2.6	0.02	<0.01
TTIDP[3],%	19.6[a]	23.1[b]	23.9[a]	22.3[a]	17.9[a]	0.6	<0.01	<0.01
UIPD[4], %	40.1[a]	36.7[b]	40.0	38.6	36.9	2.3	0.03	0.32
% of OM								
UIP[1] , %	2.65[a]	2.91[b]	2.94[a]	2.88[ab]	2.53[b]	0.20	0.05	0.04
DIP, %[8]	5.31[a]	4.16[b]	4.09[a]	4.70[b]	5.42[c]	0.19	<0.01	<0.01
TTIDP[3], %	1.50[a]	1.79[b]	1.94	1.71	1.53	0.02	<0.01	0.16
DUIP[4], %	1.2	1.1	1.2[a]	1.2[a]	1.0	0.08	0.65	0.04
kd, $\%h^{-1}$ [5]	2.62[a]	3.59[b]	2.14[ab]	3.10[ac]	4.08[bc]	0.63	0.02	<0.01
Pool. %[6]	4.32	3.27	3.30	3.68	4.41	0.20	<0.01	<0.01

[a,b,c] Least square means within row and variable without common superscripts differ ($P < 0.05$).
[1] UIP means undegradable intake protein.
[2] DIP means degradable intake protein.
[3] TTIDP means total tract indigestible protein.
[4] Means UIP digestibility.
[5] Means rate of protein degradation expressed as percent per hr, $\% h^{-1}$.
[6] Pool means residue protein content following NDF analysis.
[7] No year*month, year*grazing, month*grazing, or year*month*grazing interactions were detected ($P > 0.05$).
[8] DIP means degradable intake protein, expressed as a percentage of dietary OM.

Table 5: Organic matter digestibility and CP prediction equations for diets consumed by cattle grazing native Sandhills Range pastures

Variable	Equation	R^2Model	P-value
CP	$0.273*JD^{a} -4.56E^{-3}*JD2^{b} +2.86E^{-5}*JD3^{c} -8.01E^{-8}*JD4^{d} +8.345E^{-11}*JD5^{e} +7.88$	0.630	<0.001
OMD			
Early Growing[f]	$3.2825*M^{i} - 5.7359E^{-4}*JD2 - 2.0086E^{-1}*M2^{j} - 1.67E^{-3}*GP2^{k} +5\ 4.47846$		0.4590
			0.0120
Late Growing[g]	$-0.4268*GP^{l} -0.76643*M -0.06015*JD +0.01070*GP2 +73.98686$	0.3371	0.0025
Dormant[h]	$-0.14294*GP -7.77112*M + 0.1923*M2 + 0.00271*GP2 + 126.15238$	0.5490	<0.001

[a] Means Julian day.
[b] Means Julian day*Julian day.
[c] Means Julian day*Julian day*Julian day.
[d] Means Julian day*Julian day*Julian day*Julian day.
[e] Means Julian day*Julian day*Julian day*Julian day*Julian day.
[f] Means growing season beginning April 1 (Julian D 1) through June 15 (Julian D 76).
[g] Means growing season beginning June 16 (Julian D 77) through September 30 (Julian D 183).
[h] Means dormant season beginning October 1 (Julian D 184) through March 31 (Julian D 365).
[i] Means cumulative moisture.
[j] Means cumulative moisture*cumulative moisture.
[k] Means grazing pressure*grazing pressure.
[l] Means grazing pressure.

Table 6: Regression equations comparing predicted CP and OMD for three different validation data sets.

Location	Equation	R^2
CP		
Barta	$y^a = 0.6105x^b + 4.0296$	0.5370
GSL2	y = 1.2991x - 5.2337	0.5502
Imperial	y = 0.9325x + 2.6576	0.6619
OMD		
Barta	y = 1.162x - 10.042	0.7271
GSL2	v = 1.3907x - 21.519	0.4055
Imperial	y = 0.656x + 20.565	0.5293

[a] Means the observed variable (OMD or CP).
[b] Means the predicted variable (OMD or CP).

Figure 1: Year effects (P < 0.001) of *in vitro* OMD and DMD of diet samples collected at the Gudmundsen Sandhills Laboratory. Least square means without common superscripts differ (P < 0.05).

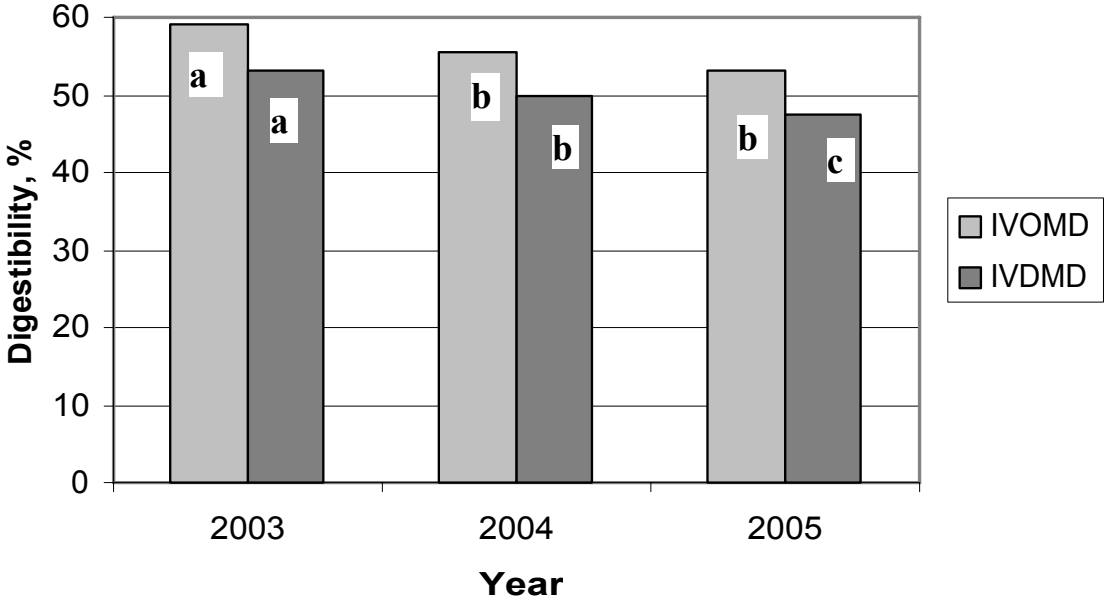

60
50
40
30
20
10
0
Digestibility, %
a
a
b
b
b
c
2003
2004
2005
Year
IVOMD
IVDMD

Figure 2: Grazing effect on in vitro OMD (P = 0.0144) and DMD (P = 0.0097) of diet samples collected at the Gudmundsen Sandhills Laboratory. Within variable without common superscripts differ significantly (P < 0.05).

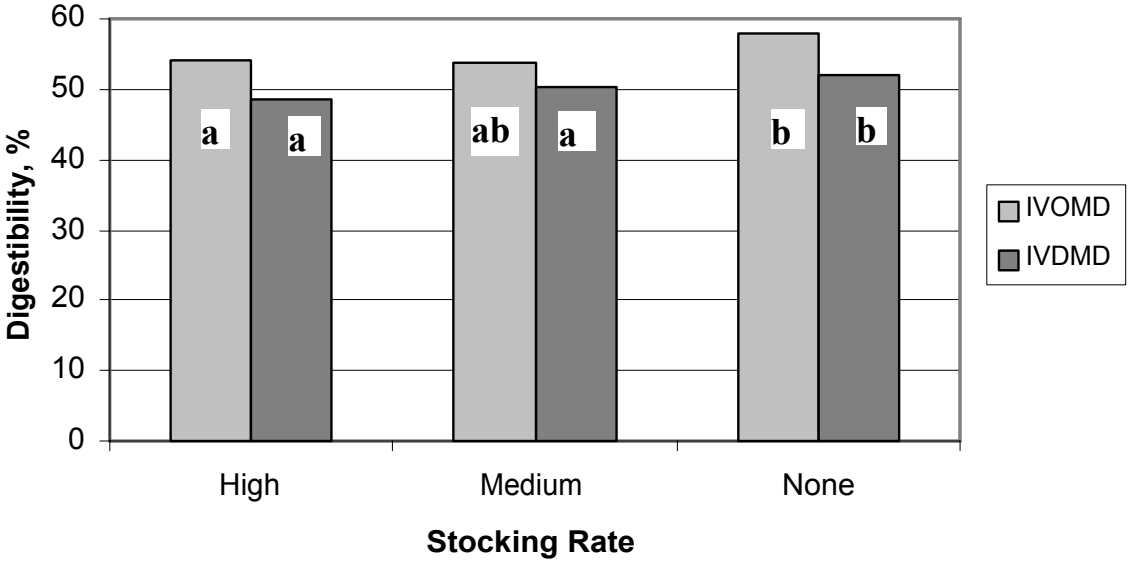

60
50
40
30
20
10
0
Digestibility, %
a
a
ab
a
b
b
IVOMD
IVDMD
High
Medium
None
Stocking Rate

Figure 3: Year effect (P = 0.0173) on NDF content of diet samples collected at the Gudmundsen Sandhills Laboratory. Least square means without common superscripts differ (P < 0.05).

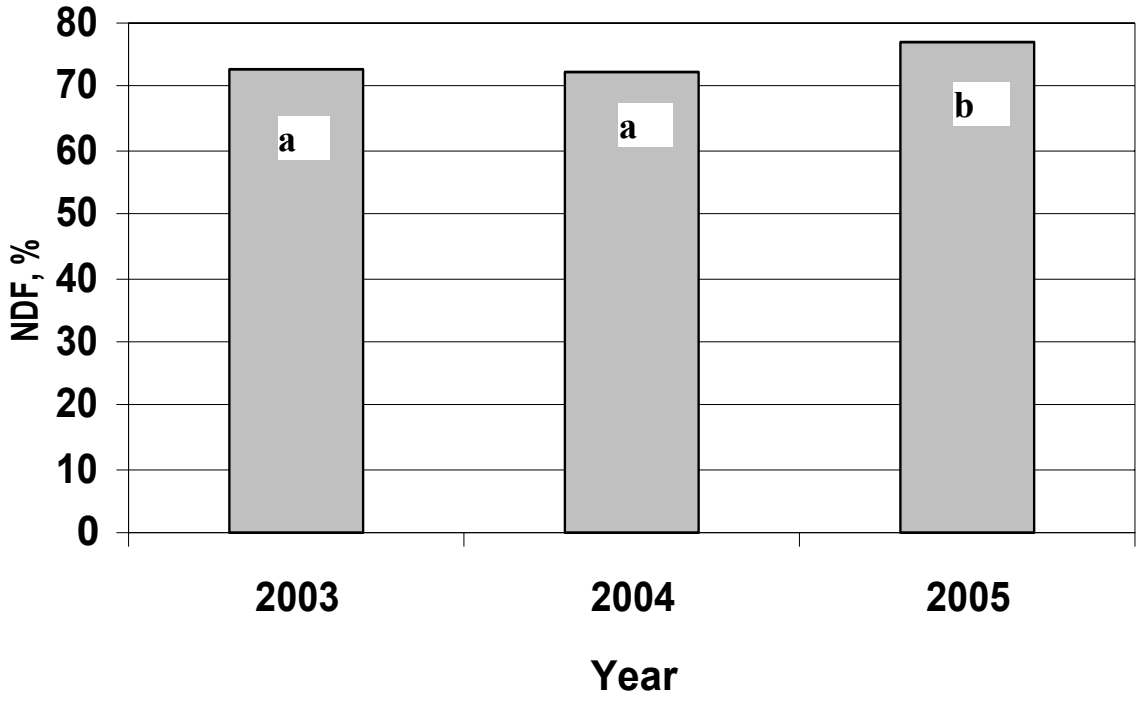

80
70
60
50
40
30
20
10
0
NDF, %
a
a
b
2003
2004
2005
Year

Figure 4: Year by grazing level interaction (P = 0.035) of crude protein from diets collected from upland Sandhills range pastures. High grazing levels in 2005 were lower than Medium and None grazing levels in 2005 and grazing level None in 2004 . No differences were noted between any of the other levels. Least square means without common superscripts do not differ (P < 0.05).

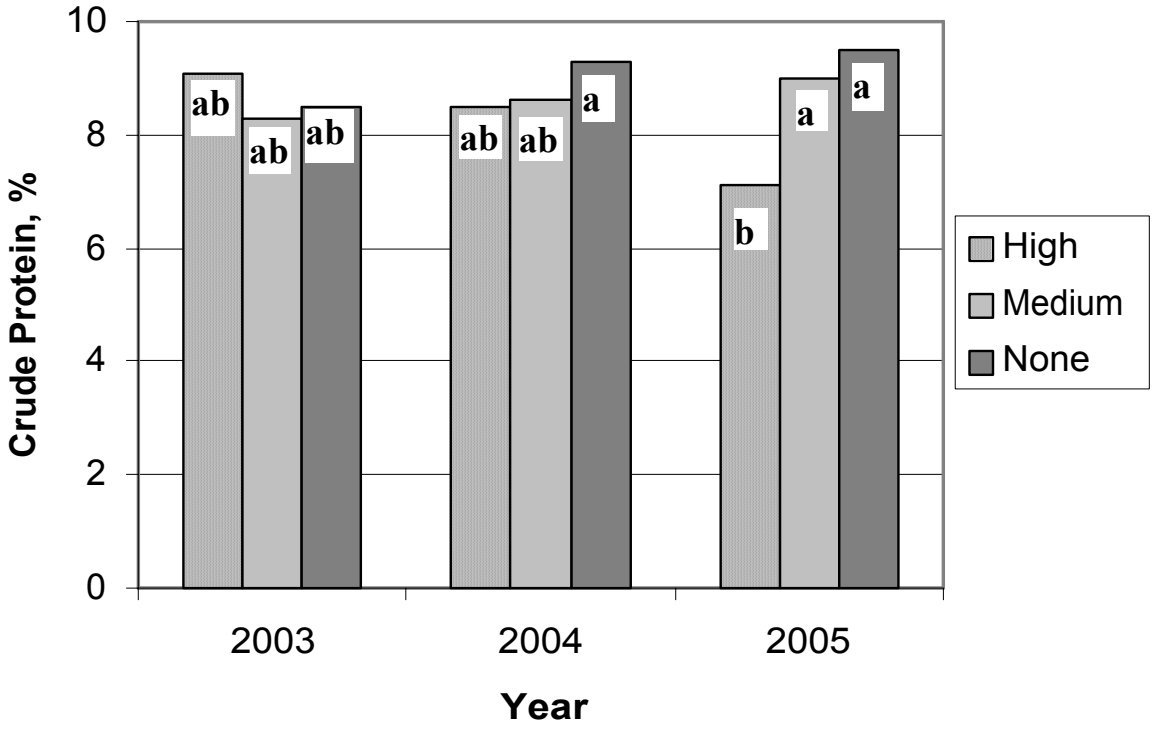

10
8
6
4
2
0
Crude Protein, %
ab
ab
ab
ab
ab
a
b
a
a
2003
2004
2005
Year
High
Medium
None

Figure 5: Month by grazing interaction of UIP of masticate samples collected in 2004 and 2005 from Gudmundsen Sandhills Laboratory. Grazing levels are High (stocking rate < 1.2 AUM/ha), Med (SR = 0.1 - 1.1 AUM/ha) and None (SR = 0 AUM/ha).

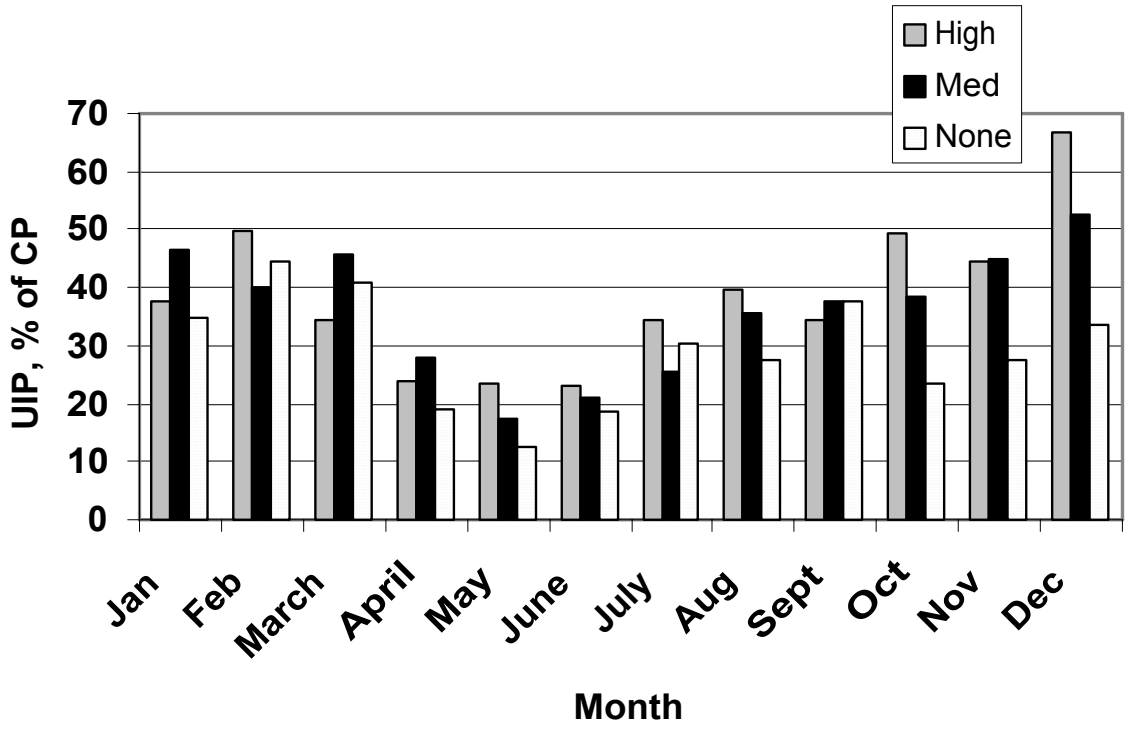

High
Med
None
70
60
50
40
30
20
10
0
UIP, % of CP
Jan
Feb
March
April
May
June
July
Aug
Sept
Oct
Nov
Dec
Month

Figure 6: Monthly protein content (% OM) of diets samples collected from esophageally-fistulated cows at the Gudmundsen Sandhills Laboratory.

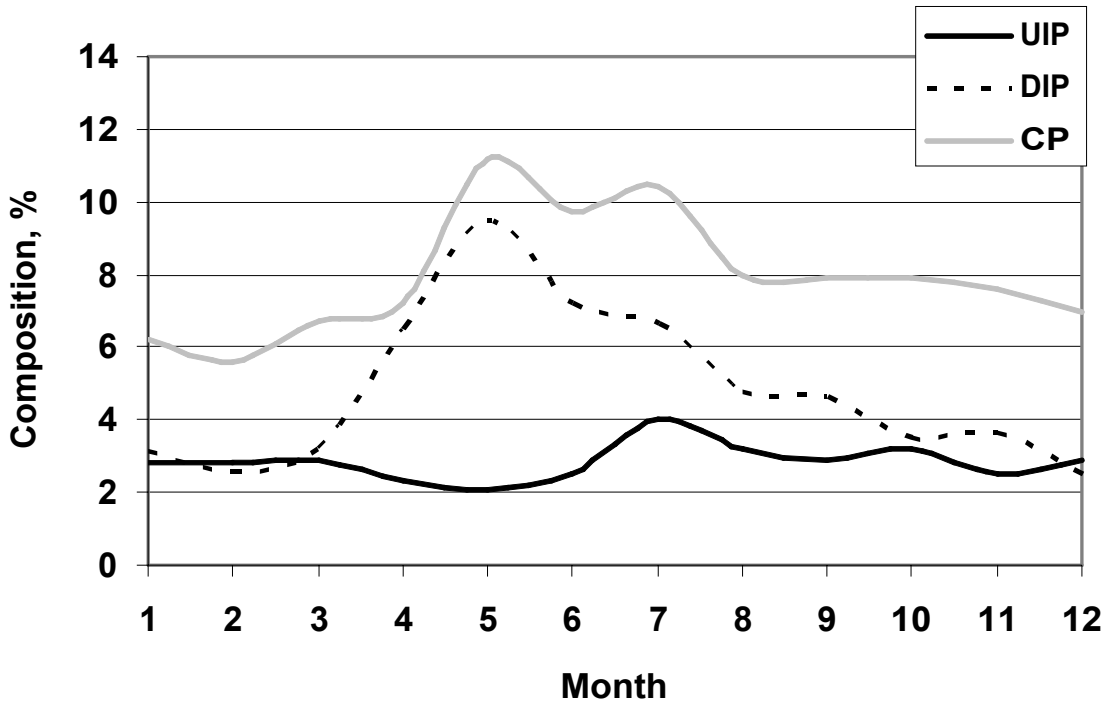

UIP
DIP
CP
14
12
10
8
6
4
2
0
Composition, %
1
2
3
4
5
6
7
8
9
10
11
12
Month

Figure 7: Predicted CP of diets of grazing cattle in the Nebraska Sandhills.

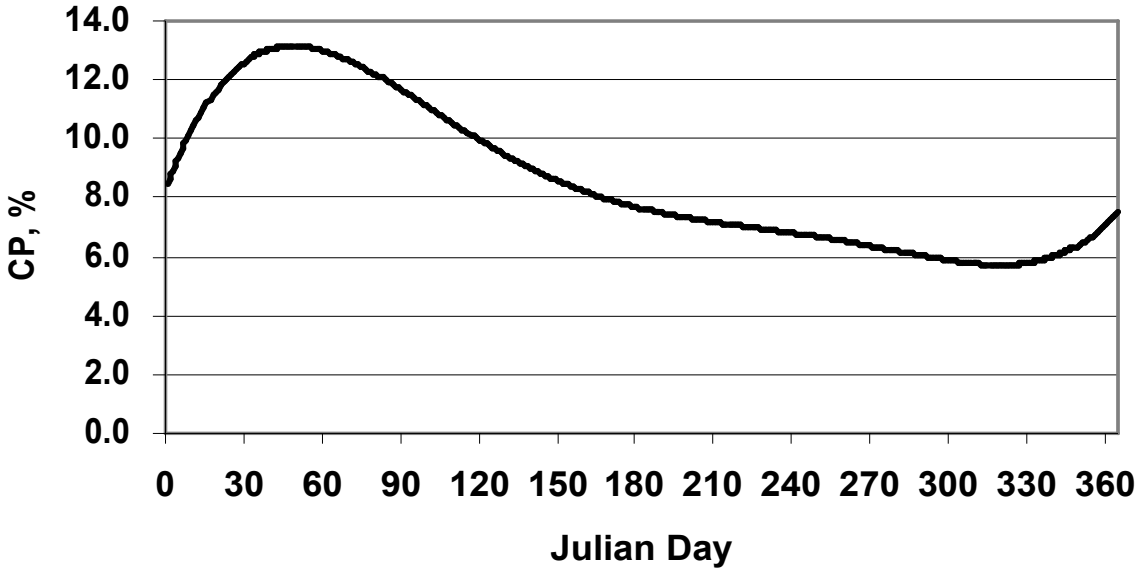

14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
CP, %
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Day

Figure 8: Seasonal predicted dietary OMD for the control pasture (un-grazed) during three consecutive years.

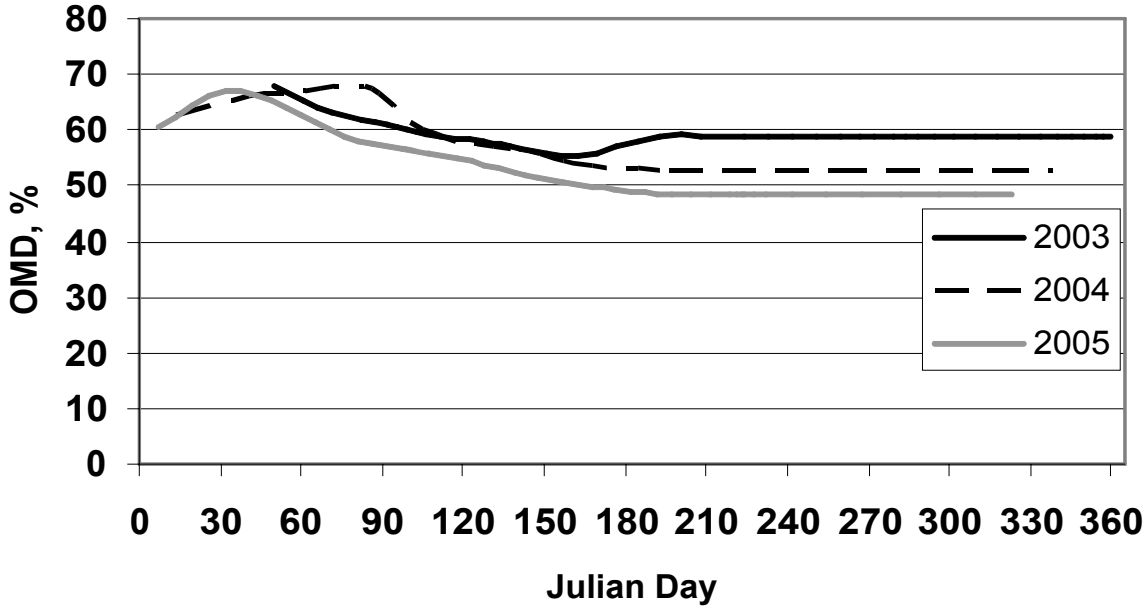

80
70
60
50
40
30
20
10
0
OMD, %
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Day
2003
2004
2005

Figure 9: Grazing pressure effect on predicted dietary OMD values. High grazing pressure assumed at 32 AUD/T of forage produced.

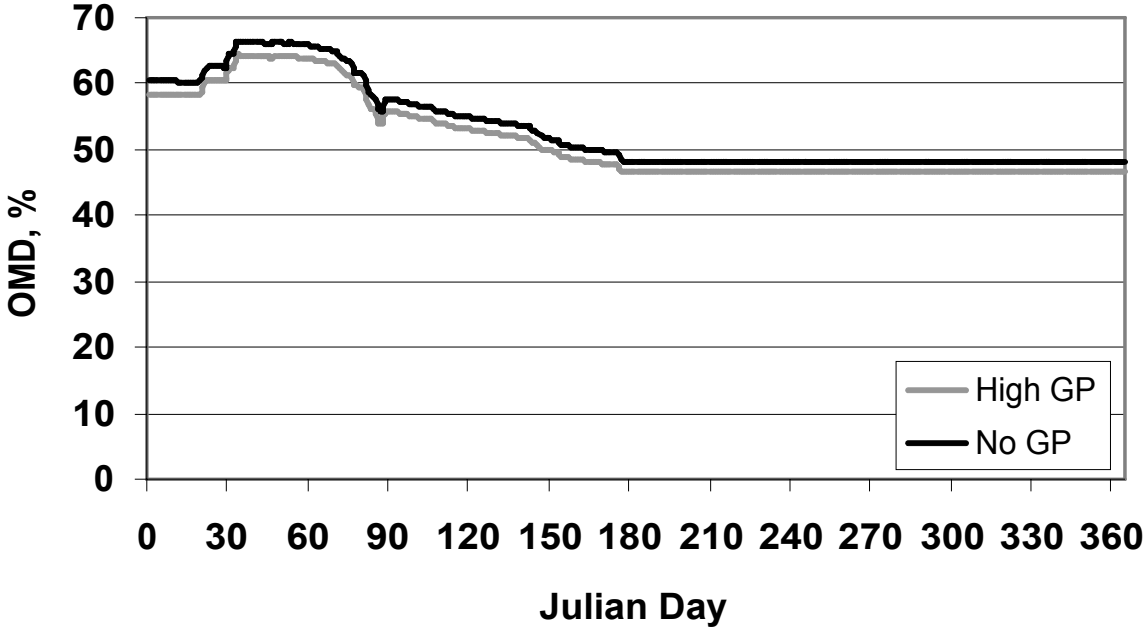

70
60
50
40
30
20
10
0
OMD, %
0
30
60
90
120
150
180
210
240
270
300
330
360
Julian Day
High GP
No GP

Figure 9: Validation of predicted CP at three different sampling locations. Barta data are represented by black diamonds and a solid black regression line, GSL2 is represented by grey circles and a solid gray regression line, and Imperial is represented by grey triangles and a dashed grey regression line.

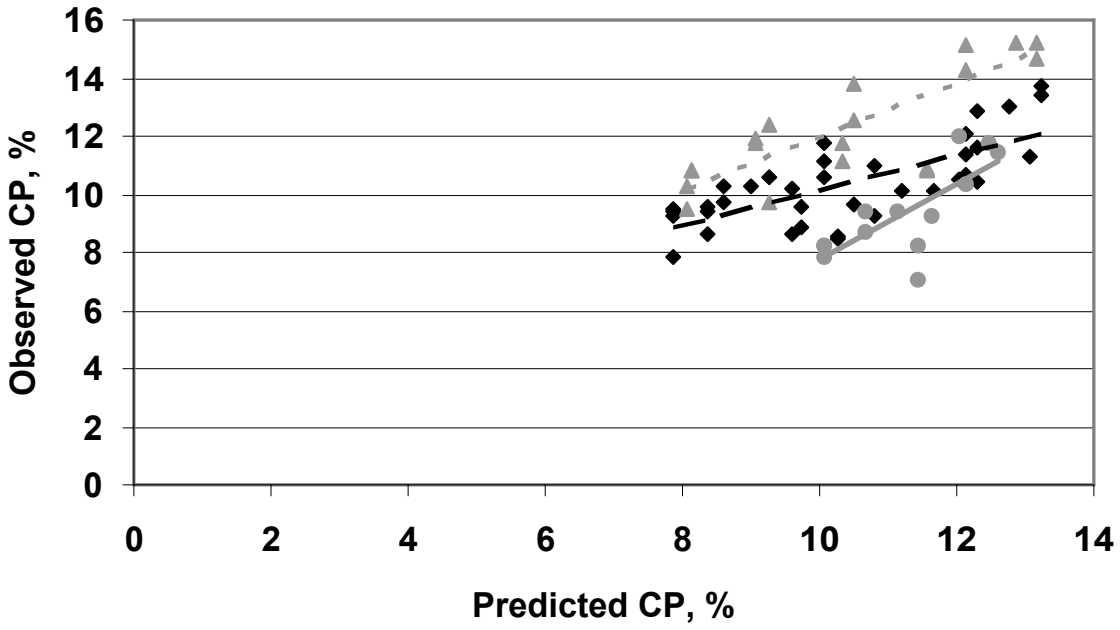
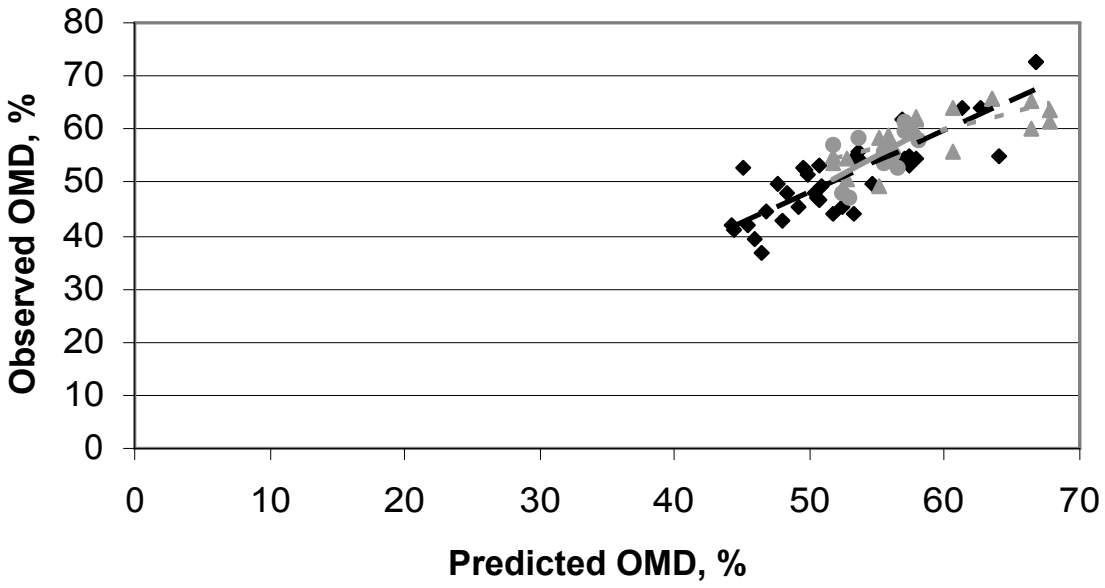

16
14
12
10
8
6
4
2
0
Observed CP, %
0
2
4
6
8
10
12
14
Predicted CP, %

Figure 10: Validation of predicted OMD at three different sampling locations. Barta data are represented by black diamonds and a solid black regression line, GSL2 is represented by grey circles and a solid gray regression line, and Imperial is represented by grey triangles and a dashed grey regression line.


80
70
60
50
40
30
20
10
0
Observed OMD, %
0
10
20
30
40
50
60
70
Predicted OMD, %