EVALUATION OF EASTERN GAMAGRASS USING THE BEEF N.R.C. MODEL

by

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Abstract

Currently there are no model inputs for eastern gamagrass [*Tripsacum dactyloides* (L.) L] for use in the National Research Council's (NRC) beef cattle model. This study was conducted to determine model inputs for two eastern gamagrass varieties for use with the forage database of the NRC model. Cattle producers in the Midwestern US will be able to evaluate eastern gamagrass in a forage system and estimate net energy for maintenance (NEm), metabolizable protein (MP), and degradable intake protein (DIP) balance for various production scenarios. Eastern gamagrass varieties 'Iuka' and 'Pete' were arranged in a split-plot randomized complete block experiment with four replications. Varieties were whole plots with harvest maturities as split plots. Plant tissue was harvested from both varieties at four maturities: vegetative/early elongation, anthesis, seed shattering and dormancy. Various compositional attributes were determined on forage samples to enable the determination of model inputs for predicting energy and protein balances. A slight difference between varieties was found for crude protein concentration ($p<0.05$). Differences ($p<0.05$) due to maturity were found for all analyses used in the model, corroborating the decline in nutritive value of eastern gamagrass with advancing maturity. Neutral detergent fiber (NDF) ranged from 593 g kg^{-1} at the vegetative/early elongation stage of maturity to 731 g kg^{-1} at dormancy. Assumed animal and environmental inputs were used for beef cattle at two stages: non-lactating 160 d prepartum and 60 d peak lactation. Energy and protein balance were estimated using level I of the model. The vegetative/early elongation stage of development supplied the greatest amount of MP. Digestible intake protein declined as plant tissue matured. All but the vegetative/early elongation stage of maturity were limited in DIP for cows in both stages of production. Soybean meal was used as a protein supplement in amounts ranging from 0.8 kg to 1.4 kg to meet nutritional demands of cattle grazing eastern gamagrass at anthesis, seed shattering and dormancy. In the simulations conducted, eastern gamagrass would be an acceptable forage source for beef

cattle provided that appropriate protein supplementation was given when the forage was at more advanced stages of maturity.

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Chapter 1 - Review of Literature

Nutritive Value Defined. Numerous attempts have been made to define forage quality. Allen and Segarra (2001) compiled a list of the following definitions by various authors to better explain forage quality. Smith et al. (1972) concluded that the best measure of feed nutritive value is animal productivity. Mott and Moore (1969) described forage quality as a function of three factors: forage nutritive value (chemical composition, digestibility, and the nature of the digested products); forage consumed (acceptability, rate of passage, and availability); and animal potential (genetics and environmental effects). Raymond (1968) defined forage quality with an equation: Nutrient intake = intake of feed dry matter (DM) x digestibility of feed DM x efficiency of utilization of digested nutrients. Moore (1980) suggested that we rely on ruminant responses to define quality, since the animal, rather than the human, makes the ultimate evaluation of forage quality.

Nutritive Value of Eastern Gamagrass. Eastern gamagrass [*Tripsacum dactyloides* (L.) L.] is a native perennial bunch-type grass that is productive, palatable and highly digestible (Salon and Cherney, 1998). Eastern gamagrass is found mainly in the eastern half of the United States and in some areas of the Southwest (Horner et al., 1985). Some populations of eastern gamagrass spread as far south as the northern portion of South America (Bidlack et al., 1999). Eastern gamagrass in the Kansas Flint Hills grows rapidly during spring and early summer, producing plenty of forage for grazing livestock in the early season (Coblentz et al., 1998). Eastern gamagrass is a warm-season grass that does not go through a dormant period during the summer months like cool-season grasses. This allows for multiple harvests throughout the growing season (Coblentz et al., 1998).

Burns et al. (1991) found eastern gamagrass diet quality generally favored good animal performance when grazed continuously. They indicated high gains could be attributed to ideal canopy morphology, the particle size of the masticate and the digesta kinetics of the grass. Eastern gamagrass masticate collected via esophageal fistulas 3 d after grazing was analyzed for neutral detergent fiber (NDF) and crude protein (CP). Burns found the masticate collected in

North Carolina in July 1984 contained 511 g kg^{-1} NDF and a CP content of 187 g kg^{-1}. In July of the subsequent year, NDF was estimated at 571 g kg^{-1} and CP at 204 g kg^{-1}.

Salon and Cherney (1998) hand-clipped eastern gamagrass in June 1997 and May 1998 in Big Flats, New York, and analyzed forage for NDF, ADF and CP. Data indicate that NDF, ADF and lignin increased throughout the growing season while CP declined. Forage composition also varied from year to year. In 1998 the initial harvest occurred 16 d prior to the first harvest of 1997; however, the later harvest was of higher quality (Table 1.1). Crude protein of forage tissue was 33 g kg^{-1} greater and NDF was 53 g kg^{-1} lower in 1997 than 1998 at nearly the same June harvest date. Although the weather data was not supplied, the differences could likely be attributed to climatic conditions. Nutritive value of eastern gamagrass may vary on the same date from year to year; so long term studies based on harvests at particular maturities may be beneficial for accurately predicting quality.

Coblentz et. al (1998) analyzed eastern gamagrass for NDF, ADF, CP, neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP). Findings were similar to those of Salon and Cherney with regard to decreasing forage quality with advancing maturity (Table 1.2).

Degradable intake protein (DIP) of eastern gamagrass was also studied by Coblentz et al. (1999). Using *Streptomyces griseus* protease at a concentration of 0.066 activity units/ml for 48 h, Coblentz reported a DIP of 58.4%, 59.9%, 50.5% of total plant CP at the vegetative, boot, and seed ripened stages of maturity, respectively.

Modeling the Nutrient Requirements of Beef Cattle. The Beef National Research Council (NRC) developed two models to predict nutrient requirements of beef cattle. The Level 1 and Level 2 models differ due to their complexity. Level 1 uses tabular values and is more simplistic in its design, while Level 2 is more mechanistic and complex in its approach to evaluating diets. For the purpose of this research, Level 1 will be used and discussed in more detail.

One major model input is energy. The NRC subcommitte on beef cattle nutrition defines energy as the potential to do work (1996). The NRC model uses a Total Digestible Nutrients (TDN) value to express energy. Total digestible nutrients are a starting point for calculating digestible energy (DE) and metabolizable energy (ME). Furthermore, net energy for maintenance (NEm) and net energy for gain (NEg) can be calculated using ME values.

The second major model input is protein. In the first stages of model development, protein was expressed on the basis of CP, but now uses a metabolizable protein (MP) system. The new approach separates ruminally degraded protein from ruminally undegraded protein, and the sum of the two equal CP. Since not all protein is degraded in the rumen and utilized by microbes (Beef NRC, 1996), using DIP and undegradable intake protein (UIP) values lends itself to a more valid approach.

When actual values are used in the NRC model the accuracy of the output will be improved. Inputs necessary in running the NRC model include NDF, lignin, ADICP, NDICP, crude fat, CP, ash, and DIP values. These values can be used to generate the energy values required by the model using a summative equation approach (NRC 2001), expressed as total digestible nutrients (TDN). The TDN values only account for fecal energy loss; therefore, they are a rough estimate of digestion. Calculating TDN in forages is essential as it is the initial step in predicting DE and ME, and ultimately NE values. For the purpose of this research, the current dairy NRC (2001) approach for calculating TDN was used. The specific summative equation (NRC, 2001) is as follows:

$$\mathbf{TDN_{maintenance\,(1x)}\,(\%) = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7}$$

Where:

Total digestible non-fiber carbohydrate (tdNFC)

= 0.98 x (100 – [(NDF – NDICP) + CP + EE +Ash)] x PAF

(Note: PAF = Processing Adjustment Factor)

Total digestible crude protein (tdCP)

= CP x exp[-1.2 x (ADICP/CP)]

Truly digestible fatty acids (tdFA)

= FA (estimated as Ether Extract (EE) – 1); if EE<1, then FA=0

Truly digestible neutral detergent fiber (tdNDF)

=0.75 x (NDFn– Acid Detergent Lignin (ADL) x [1–

$(ADL/NDFn)^{0.667}$] where NDFn = NDF-NDICP

As mentioned previously, TDN is the starting point for predicting digestible energy (DE), which can be used to calculate metabolizable energy (ME). Digestible energy per se has some value for evaluating feedstuffs; however, it overestimates the value of high fiber diets in relation to low fiber diets (Church, 1988). Historically, DE was predicted under the assumption that 1 kg of TDN equaled 4.409 Mcal of DE. However, because nutrients (i.e. fat, protein and carbohydrates) have different gross energy values and their composition varies from one feedstuff to another, it is more realistic to multiply each nutrient by its' own gross energy value. The equation to convert TDN to DE is as follows:

$$DE1x \text{ (Mcal / kg)} = 4.2(tdNFC/100) + 4.2(tdNDF/100) + 5.6(tdCP/100) + 9.4(FA/100) - 0.03$$

Furthermore, ME is the basis for estimating net energy for maintenance and gain (NEm and NEg). Metabolizable energy is a better estimate of nutritive value because it figures in urinary, gaseous and fecal loss, whereas DE uses fecal loss alone. The value of ME is as a starting point for the NE concept (Church, 1988). In Level 1 of the Beef NRC (1996), DE, ME NEm and NEg are predicted automatically by the model based on the TDN values supplied.

Dry matter intake (DMI) is also predicted by the model; however, the predicted DMI of low quality forages can be extremely biased since the standard for this equation is not similar in quality. Several equations have been developed to predict intake of forages. The National Forage Testing Association (1993) determined intake under the assumption that animals will consume feed until they have 1.2% of their body weight consumed in fiber. In the equation, DMI (%BW) = 120 / %NDF, the estimate is driven by fiber concentration in the forage. An alternative approach to predicting forage intake in beef cows was suggested by Hibberd (Vanzant, 1996) based on historical observation of forage intake from various research experiments (Table 1.3). In the approach, forage quality, physiological state and supplementation are the main factors that affect estimated forage intake by mature beef cows. Because the table was developed for application with mature beef cows and, in application, mature cow size can vary substantially, intake predictions are expressed as a percent of mature body weight.

Nutritive Value in C_4 versus C_3 Plants. Grasses can be divided into two major groups based on differences in their internal leaf anatomy and ecological adaptation. These two groups are classified as C_3 and C_4 grasses (Jones, 1985).

One biological difference lies in the photosynthetic pathway of C_3 and C_4 plants. The first stable product of photosynthetic carbon fixation in a C_3 grass is a 3-carbon compound, and a 4-carbon compound in C_4 grasses, hence the names C_3 and C_4 (Jones, 1985). Many of the world's most important crops, including warm-season range and forage grasses, are of the C_4 type. Because of their advantage under certain environmental conditions, C_4 plants are adapted to growth during warm and relatively dry periods and are better suited in areas with low fertility soils. Nitrogen and water use efficiency is greater for C_4 plants versus C_3 plants (Waller and Lewis, 1979).

Another notable difference between the two groups is in their internal leaf anatomy. Particularly, C_4 grasses have a higher frequency of vascular bundles, a higher proportion of thick-walled vascular and sclerenchyma tissues, and a specialized sheath of chlorenchyma cells surrounding each bundle (Wilson, 1994). Wilson (1994) also noted 50% of the leaf's reserve carbohydrates and proteins are contained inside these specialized cells of the bundle sheath. Because the walls of the bundle sheath digest slowly, the nutrients contained by these are not readily available by rumen microbes (Wilson, 1994). Wilson (1994) also noted that chewed tropical grass leaves, also of the C_4 type, do not easily split their epidermis, therefore, the resulting particles are composed of many vascular bundles in width. In contrast, C_3 grasses have a thin-walled epidermis. The C_4 grasses generally are of lower nutritional value than C_3 grasses; however, they are higher yielding due to their physiological and water use efficiency. Grasses of the C_4 type dominate rangelands and pasturelands in Kansas. This is due to their ability to tolerate warmer, drier conditions as previously stated.

Nutritive Value and Animal Selectivity. Research has determined that humans cannot accurately select a forage sample that is representative of the diet chosen by an animal. Umoh (1977) found that cattle select a diet approximately 2% higher in crude protein than that clipped by researchers. Such research suggests the need to increase CP values from clipped samples when intended for use in the NRC (1996) model. That is, in order to accurately predict gains with the NRC (1996) model, it is necessary to adjust the CP on the basis of selectivity.

Nutritive Value and Carbohydrate Composition. Forage dry matter can be separated into two categories; namely, the cell contents and the cell wall. Neutral detergent solubles (NDS), or cell contents, are accumulated in the plant and are readily available for use in metabolism or are translocated to other plant parts (Smith, 1981). The digestibility of cell contents has been reported as approximately 980 g k^{-1} (Van Soest, 1967). The carbohydrates in the NDS include sugars, short oligosaccharides, organic acids, starch, pectin and soluble fiber. These components can be extracted using the Detergent Fiber System of Analysis of Goehring and Van Soest (1970). This class of carbohydrates is very diverse. Sugars and starch can be digested by mammalian enzymes while soluble fiber cannot. The NDS fraction is rapidly/intermediately and extensively digested in the rumen, but the fermentation characteristics of most neutral detergent soluble fiber (NDSF) components differ from those of sugar and starch (Mertens, 1992). The percentage of neutral detergent soluble carbohydrate (NDSC) in C_4 grasses is quite low, and this gives way to higher percentages of structural carbohydrates (SC). Many types of forage in the Great Plains are warm-season C_4 grasses and are characterized as having lower NDSC and higher cell wall and lignin values than cool-season C_3 grasses.

The plant cell wall comprises 20-80% of forage dry weight and is composed primarily of SC (hemicellulose and cellulose) and lignin. Forage quality is directly related to the amount of SC in plants. Plants with high cell wall content have a lower digestibility that result in depressed intake by ruminants. Structural carbohydrates represent the fiber portion of a plant. As stated earlier, fiber has a substantial effect on feed quality. Fiber in a monogastric animal's diet largely represents indigestible matter in a feedstuff because of limited fermentative capacity. However, fiber is looked at quite differently in terms of ruminant nutrition.

Ruminants sustain a symbiotic relationship with a microbial population in the rumen allowing them to extensively utilize fiber in their diet. Fiber is rich in glucose polymers that contain numerous $\beta(1\rightarrow4)$ linkages. Mammalian enzymes cannot break these linkages. Microbes produce cellulase, the enzyme necessary to break the $\beta(1\rightarrow4)$ link, allowing the glucose residues to be utilized by the animal. Fiber, in terms of ruminant nutrition, is closely related to the inherent digestibility of a feedstuff, affects the degree of mastication required to reduce particle size, and the space required in the digestive tract while the feed is being processed (Mertens, 1973).

Nutritive Value and Protein Composition. Protein is an essential nutrient in the diet of a ruminant; however, it is often the most lacking (Kellums and Church, 1998). Crude protein values are commonly used as a rough estimate of the ability of a forage to supply protein, and, as such, is an indicator of forage quality. Both the animal and the rumen microbial population require protein or nitrogen (N) to persist. While the host requires preformed amino acids to thrive, microbes require an adequate supply of N for optimal protein synthesis and fiber digestion and, hence, growth. Crude protein is a quick and easy indicator of the forage to meet such needs; however, it does not always lend itself to an accurate estimate. First, CP provides no indication of protein quality. Like carbohydrates, protein can be partitioned into fractions based on differing ruminal availability. These fractions are indicators of the amount of forage protein that will either be utilized in the rumen by microbes or will escape rumen degradation. The protein that bypasses the rumen may be digestible in the small intestine where it will be absorbed in the form of amino acids (Church, 1988). One advantage ruminants have over non-ruminants is their ability to convert non-protein nitrogen (NPN) to microbial protein (Church, 1988). Non-protein nitrogen is an inexpensive N source; however, it is advised that NPN not comprise more than one-third of the CP intake (Church, 1988). Non-protein nitrogen is a good source of readily available N for microbial growth.

Solubility of protein is positively correlated with protein degradability. As with carbohydrates, the soluble components of protein may be (but not always) attacked more rapidly and digested more completely than insoluble compounds, due mainly to microbial access. For example, some N is tied up in the specialized bundle sheath as mentioned previously, and is likely to bypass the rumen.

The amount of protein entering the small intestine is the sum of microbial crude protein (MCP) and dietary protein escaping the rumen intact. When production demands are high, MCP alone will not meet animal protein requirements (Church, 1988). From 0-80% of protein will escape degradation and reach the small intestine (Table 1.4; Church, 1988). With such a wide range of bypass proteins in feedstuffs, the need for partitioning protein into DIP and UIP becomes evident.

Nutritive Value Measurements. As previously mentioned, Moore (1980) described nutritive value in terms of animal performance: for example, average daily gain by growing steers, or average daily milk production by cows. Experiments using live animals can be

extremely costly and labor intensive, so researchers have developed laboratory techniques to estimate quality.

The Proximate Analysis System, developed in the late 1800's at the Weende Experiment Station in Germany (Kellems and Church, 1998), has been used for many years to determine the quality of forage tissue. This method is based on several wet chemistry assays including moisture, crude protein, crude fiber, crude fat, ash and nitrogen free extract (NFE). A major concern with this system is in the NFE. Nitrogen free extract is determined by difference (using the other fractions), so this fraction is a repository for errors that may have resulted from the other assays. Total digestible nutrient (TDN) values have been used to approximate the energy value of a feed. The sum of the digestible portions of protein, fat, fiber, and NFE are used in determining TDN.

As science and technology evolved, more accurate and less labor-intensive techniques to measure quality were developed. Van Soest (1967) developed the neutral and acid detergent fiber system of analysis to more accurately predict the digestibility of components associated with forages. The LECO Nitrogen (N) Analyzer, St. Joseph, Michigan, is often used in place of the Kjeldahl procedure of N analysis. This method can be more efficient and safer in determining sample N.

Tilley and Terry (1963) developed the in vitro dry matter digestibility technique that simulates rumen fermentation. Using forage tissue, ruminal inoculum, an anaerobic environment, agitation and appropriate temperature, sample digestion can be determined in the laboratory. This allows the digestibility of large numbers of samples to be determined.

All of these laboratory assays have been useful in estimating quality; however, farmers and ranchers must understand that in the final analysis, these are only predictors of quality. True quality lies in the ability of a forage to support maintenance and production functions.

Tables

Table 1.1 Influence of harvest date on forage quality parameters (g kg^{-1}) of eastern gamagrass, first cutting.[1]

Harvest Date	NDF[2]	ADF	Lignin	CP
1997				
June 13	693[a]	312[a]	33[a]	163[a]
June 20	773[b]	381[b]	62[b]	164[a]
June 27	770[b]	396[b]	68[b]	159[b]
1998				
May 29	709[a]	319[a]	23[a]	135[a]
June 4	721[b]	338[b]	26[a]	131[a]
June 12	746[b]	355[c]	30[b]	130[a]

[1]Taken from Salon and Cherney (1998).

[2]NDF=neutral detergent fiber, ADF=acid detergent fiber, CP=crude protein.

[a,b,c] Least squares means in the same column and year with different superscripts differ ($P<0.05$).

Table 1.2 Carbohydrate and nitrogen composition of whole-plant tissue of eastern gamagrass ($g\ kg^{-1}$)[1].

Forage[2]	NDF	ADF	CP	NDICP	ADICP
GGB	694[c]	353[c]	176[a]	94[a]	13.1
GGA	731[b]	396[b]	135[b]	69[b]	12.5
GGM	780[a]	448[a]	94[c]	58[bc]	13.8

[1]Taken from Coblentz et al. (1998).
2GGB=gamagrass harvested at boot stage, GGA=gamagrass harvested at anthesis stage, GGM= gamagrass harvested at physiological maturity.
[a,b,c] Means in a column with different superscripts differ ($P<0.05$).

Table 1.3 Forage Intake Guide for Beef Cows[a]

Diet	Dry	Lactating
	DMI, %BW[b]	
Forage - low (<7% CP)[c]		
No Supplement	1.5	2
Protein Supplement	1.8	2.2
Energy Supplement	1.5	2
(When energy supplements exceed approximately 0.5% BW, a 0.5:1 substitution of supplement forage will occur above this threshold).		
Forage - medium (7-11% CP)		
No Supplement	2	2.3
Protein Supplement	2.2	2.5
Energy Supplement	2	2.3
(When energy supplements exceed approximately 0.5% BW, a 0.5:1 substitution of supplement forage will occur above this threshold).		
Forage - low (12% ≥ CP)		
No Supplement	2.5	2.7
Protein Supplement	2.5	2.7
Energy Supplement	2.5	2.7
(1 unit energy supplement will decrease forage intake by about 1 unit)		

[a]Adapted from tabular guidelines developed by C.A. Hibberd and presented in Vanzant (1996).

[b]Use average mature weight for cows.

[c]Protein values added to provide rough estimate of low, moderate, and high quality forages.

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Table 1.4 Estimates of ruminal escapes of protein from common feedstuffs from animal trials[1].

Feedstuff	In vivo N escape, %	Escape, %	
		Mean	SD
Protein Supplements			
Blood Meal	54, 71, 82	69	14
Corn Gluten Meal	55, 46-61, 62, 57	55	6.3
Cottonseed Meal	24-61, 27-33, 35-57	40	15.7
Rapeseed Meal	23	23	
Soybean Meal	10, 27, 29, 61, 35, 18, 17, 15, 18, 20, 24, 25, 26, 22, 21, 24 46, 23, 43, 14	26	12
Energy Feeds			
Corn, flaked	50	50	
Corn, ground	73, 58, 47	59	13
Corn, whole	52	52	
Sorghum grain	49, 20, 38, 64, 58, 52, 69, 65	52	16.3
Roughages			
Alfalfa hay	30, 41, 21, 28, 20-24	27	17.7
Corn silage	27	27	

[1]Taken from Church (1988) and NRC (1985).

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Chapter 2 - Research Study

Introduction

With approximately 7 million hectares of rangeland and tame pastureland in Kansas, forage utilization is of great significance to beef cattle operations in this region of the country. Feed costs alone can account for greater than one half the annual costs associated with maintaining a beef cow in production. This fact has stimulated in interest to develop forage systems that can reduce feed costs. Accurate predictions of nutrient balance enable producers to accurately evaluate the suitability of various forages to meet animal requirements. The National Research Council's (NRC) beef model was developed to predict animal requirements and nutrient balance of beef cattle under varying conditions; therefore, the NRC model should be helpful in describing the effects that changes in forage composition would have on animal performance.

This study was conducted to determine model inputs for two eastern gamagrass varieties in order to expand the forage database available for the NRC beef Model; and run model simulations using two hypothetical production stages for beef cows (a non-lactating cow 160 d prepartum and a cow 60 d postpartum at peak lactation) in order to evaluate the effectiveness of eastern gamagrass in meeting the nutritional needs of beef cattle in a forage-beef system. Results of this study will allow cattle producers of Kansas and the Midwestern US greater ease in evaluating the potential use of eastern gamagrass within their particular production constraints.

Materials and Methods

Experimental Site and Weather. The eastern gamagrass used in this study was grown on a Wymore silty clay loam (fine, smectitic, mesic Aquertic Argiudoll) soil and harvested during the 2002 growing season at the Kansas State University Agronomy Farm in Manhattan, located at 39˚ 13' latitude and 96˚ 36' longitude. The elevation of Manhattan reaches 319.5m above sea level. The 2002 growing season was unusually dry.

Sporadic precipitation coupled with temperatures reaching 37˚C made for a dry growing season (Tables A.1-A.7).

Plot Establishment and Sampling Procedures. Two varieties of eastern gamagrass, 'Pete' and 'Iuka' were selected for use in the study, as they are commonly utilized throughout central to eastern Kansas, as well as other areas of the Midwest. Plots were grown in pure stands of eastern gamagrass in 10 rows. Prior to the beginning of the experiment, on April 17, 2002, plots were burned and nitrogen fertilizer was applied on May 14, 2002 at 66.6 kg N per hectare. For each variety, plots were sub-divided into four sub-plots represented by maturity. Gamagrass was harvested May 30, June 17, August 6, and October 15 representing four maturities: vegetative/early elongation, anthesis, seed shattering and dormancy. Two kilograms of plant tissue were collected at each sampling time using a sickle-bar mower with a blade height of 5 cm. Plant tissue was immediately placed in a forced air oven and dried at 55˚ C for 72 hours. All samples were ground in a hammer mill (Meadows Mills, Inc., North Wilkesboro, NC) to reduce particle size. A sub-sample representative of the entire sample of plant tissue was taken and ground to pass a 1 mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ).

Chemical Analyses. Standard Association of Analytical Communities (AOAC) procedures (1990) were used to measure DM, ash and ether extract (EE). Van Soest's detergent fiber analysis system (1963, 1973) was used to assay for NDF and ADF using an ANKOM Fiber Analyzer (Macedon, NY). Lignin was determined using the acid detergent lignin procedure as described by Van Soest (1963, 1973).

Crude Protein (N x 6.25) was determined using a LECO Nitrogen Analyzer (Model FP-2000, Serial # 3273, LECO Corp., St. Joseph, Missouri). Neutral detergent and acid detergent insoluble nitrogen were determined and converted to protein values by multiplying N x 6.25 (Van Soest et al., 1991; Licitra et al., 1996).

Enzymatic Protein Degradation Methods. Protease enzyme from *Streptomyces griseus*, type XIV (5.3 units/mg protein, Sigma Chemical Co., St. Louis, Missouri) was used for estimating the ruminal degradability of protein (Kohn and Allen, 1995). The enzyme concentration used was 0.066 activity units/ml when incubating forage tissue (Coblentz, 1999). A fresh batch of *Streptomyces griseus* enzyme solution was prepared in a borate-phosphate buffer, then forage samples containing 15 mg of feed N were

incubated in 40 ml of buffer for one hour and, subsequently, 10 ml of protease enzyme solution was added. The incubation time used for determining N disappearance was 48 hours. Samples were filtered by gravity and undegradable intake protein (UIP) was determined using a LECO N analyzer. Degradable intake protein (DIP) was calculated by difference.

Model Inputs. A non-lactating beef cow 160 d prepartum and a beef cow in peak lactation (60 d postpartum) were chosen to estimate the ability of eastern gamagrass to meet a beef cow's nutritional needs. Table 2.1 shows the various animal and environmental inputs for the NRC beef model. Intake was determined using a forage intake table that was adapted from a table originally developed by Hibberd (Vanzant, 1996).

Statistical Analysis. The experimental design was a split-plot with whole plot treatments arranged in randomized complete blocks with four replications. Whole plots represented variety and were divided into sub-plots represented by maturity. Terms in the model were block, variety and maturity. Data were analyzed using the MIXED procedure of SAS and means were separated at $p \leq 0.05$.

Results and Discussion

Forage composition values are an excellent tool for evaluating diet adequacy and (or) performance. When composition data are available, a better understanding of intake, digestibility and efficiency are afforded. Two eastern gamagrass varieties at four maturities were analyzed for composition. Crude protein (CP), neutral detergent insoluble crude protein (NDICP), acid detergent insoluble crude protein (ADICP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ether extract (EE) and degradable intake protein (DIP) assays were performed for use in the NRC beef (1996) model. For use in the NRC model, CP values were increased 2% to reflect animal selectivity (Table 2.3). Umoh (1977) found that cattle select a diet approximately 2% higher in CP than that clipped by researchers. Such research suggests the need to increase CP values from clipped samples when intended for use in the NRC (1996) model. That is, in order to accurately predict gains with the NRC (1996) model, it is necessary to adjust the CP on the basis of animal selectivity. Crude protein was

significantly different ($p=0.0496$) between varieties (Table 2.3). Higher CP values were observed for the vegetative/early elongation stage of development and declined throughout the growing season. Crude protein for 'Iuka' ranged from 121 g kg^{-1} to 41 g kg^{-1} and 'Pete' from 112 g kg^{-1} to 36 g kg^{-1} for the vegetative/early elongation and dormant stages, respectively. Coblentz et al. (1998) reported the same trend for CP over time; however, they reported higher concentrations of CP at similar maturities. Salon and Cherney (1998) reported widespread CP concentrations when comparing gamagrass in subsequent growing seasons. Their CP concentrations were also higher than values found in our study. These differences could be attributed to varying levels of nitrogen fertilization. Crude protein of 'Iuka' was higher than 'Pete' at all maturities. At the vegetative/early elongation stage of development 'Iuka' had an adjusted crude protein (ACP) value of 141.8 g kg^{-1} and 'Pete' 132.3 g kg^{-1}. Dormant 'Iuka' had an ACP of 61 g kg^{-1} and 'Pete' 56 g kg^{-1}. Significant differences existed in CP at all maturities ($p<0.0001$) (Table 2.3). No other differences were found between varieties for chemical or enzymatic analyses. No variety by maturity interactions ($p<0.05$) were found for any of the analyses; however, differences were found among maturity for all assays ($p<0.05$) (Tables 2.3 and 2.4).

Differences in NDICP and ADICP were found among all maturities. The mean NDICP at vegetative/early elongation was 58.07 g kg^{-1} and decreased with advancing maturity to 15.15 g kg^{-1} at dormancy. Values of ADICP at vegetative/early elongation, anthesis, seed shattering and dormancy were 6.1 g kg^{-1}, 4.02 g kg^{-1}, 4.09 g kg^{-1} and 5.62 g kg^{-1}, respectively.

Differences between maturities were observed for DIP. Degradable intake protein for eastern gamagrass tissue in the vegetative/early elongation stage of development was highest among the maturities (Table 2.3). Degradable intake protein of plant tissue in the vegetative/early elongation, anthesis, seed shattering and dormant stages were 57.8 g kg^{-1}, 50.54 g kg^{-1}, 52.31 g kg^{-1}, and 56.27 g kg^{-1}. A slight increase in DIP of dormant tissue was noted. These values were similar to findings of Coblentz et al. (1999), with the exception of the physiologically mature tissue. Coblentz reported mature tissue having a DIP of 50.5% of total CP. The increase in DIP toward the end of the growing season could be attributed to warm temperatures the first of October and a substantial amount of

precipitation causing the plants to put on new tillers or to stimulate leaf growth from already developed tillers before the October 15 harvest date.

The plant cell wall is composed primarily of structural carbohydrates (hemi-cellulose and cellulose) and lignin. These components were isolated using the Detergent Fiber System of Analysis of Goering and Van Soest (1970). The NDF fraction represents hemicellulose, cellulose and lignin in a feedstuff, the ADF fraction represents cellulose and lignin, and the ADL fraction represents lignin. Negative correlations exist between NDF and dry matter intake and ADF and digestibility. Plants with high cell wall content have a lower digestibility that result in depressed intake. The NDF values ranged from 593 g kg^{-1} at the vegetative/early elongation stage of maturity and increased with advancing maturity to 731 g kg^{-1} at dormancy (Table 2.4). The ADF fraction increased from 317.2 g kg^{-1} at vegetative/early elongation to 397.8 g kg^{-1} at dormancy (Table 2.4). Lignin isolated using the ADL procedure in the vegetative/early elongation, anthesis, seed shattering and dormant stages were 30.9 g kg^{-1}, 35.4 g kg^{-1}, 51.8 g kg^{-1}, and 50.9 g kg^{-1}, respectively (Table 2.4). These data were consistent with that reported by Coblentz et al. (1998). Deinum and Van Soest (1969) found decreases in nutritive value of plants, as rising temperatures stimulate lignification of supporting tissue, thus decreasing digestibility of forage tissue.

Lipids were isolated using the EE analysis. Ether extract of vegetative/early elongation plant material was 18.6 g kg^{-1}. Plant material at anthesis, seed shattering and dormancy was 17.1 g kg^{-1}, 20.8 g kg^{-1}, and 21.1 g kg^{-1} (Table 2.4).

Calculating TDN in forages is essential as it is the initial step in predicting DE and ME, and ultimately NE values. For the purpose of this research, the current dairy NRC (2001) approach for calculating TDN was used. The specific summative equation used (NRC, 2001) is as follows:

$$\mathbf{TDN_{maintenance\ (1x)}\ (\%) = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7}$$

Where:

Total digestible non-fiber carbohydrate (tdNFC)

$= 0.98 \times (100 - [(NDF - NDICP) + CP + EE + Ash)] \times PAF$

(Note: PAF = Processing Adjustment Factor)

Total digestible crude protein (tdCP)
= CP x exp[-1.2 x (ADICP/CP)]

Truly digestible fatty acids (tdFA)
= FA (estimated as Ether Extract (EE) – 1); if EE<1, then FA=0

Truly digestible neutral detergent fiber (tdNDF)
=0.75 x (NDFn– Acid Detergent Lignin (ADL) x [1–
(ADL/NDFn)$^{0.667}$] where NDFn = NDF-NDICP

No differences were found between varieties for TDN. No variety by maturity interactions ($p<0.05$) were found. Significant differences existed between maturities. Total digestible nutrients declined with advancing maturity. The TDN value ranged from 594.9 g kg^{-1} at vegetative/early elongation to 548.7 g kg^{-1} at dormancy (Table 2.4). Total digestible nutrients is the starting point for predicting DE, which can be used to calculate ME, which are needed to make predictions with the NRC beef model (1996)

Model simulations were run using two beef cows with different levels of nutritional demand (non-lactating 160 d prepartum and 60 d postpartum peak lactation) in order to evaluate the effectiveness of eastern gamagrass in a forage-beef cattle system. Forage composition values and animal and environmental descriptions were entered into the NRC model and yielded the following results. The net energy supplied by both varieties of eastern gamagrass at vegetative/early elongation, anthesis, and seed shattering were sufficient to meet the energy demands of the non-lactating cow. Energy was deficient in the physiologically mature tissue and would require energy supplementation. Only the most immature tissue supplied adequate protein to meet nutritional requirements of the non-lactating cow with a DIP surplus of 247 g d^{-1} grazing 'Iuka' and 195.8 g d^{-1} grazing 'Pete' (Table 2.5). Degradable intake protein was inadequate for proper rumen function at anthesis, seed shattering and dormancy. Additional DIP required for the non-lactating cow grazing 'Iuka' at these maturities are 141.8 g d^{-1}, 214.9 g d^{-1} and 217 g d^{-1}. Degradable intake protein is deficient by 188.4 g d^{-1}, 257.9 g d^{-1} and 177.9 g d^{-1} for the

non-lactating cow grazing ‘Pete’; therefore, protein supplementation is necessary (Table 2.7). A surplus of 772 g d^{-1} metabolizable protein (MP) was observed in ‘Iuka’ and 710 g d^{-1} in ‘Pete’. Metabolizable protein values for all other maturities are not reliable. When DIP supply is insufficient, microbial yield is suppressed.

Nutritional requirements increase with lactation. Protein requirements are increased due to the high amount of protein contained in milk solids (Church, 1988). Only the most immature tissue of both ‘Iuka’ and ‘Pete’ eastern gamagrass contained sufficient energy and protein to meet nutritional demands. Degradable intake protein was inadequate for proper rumen function at anthesis, seed shattering, and dormancy of both varieties. The amounts of protein still required in the diet at these maturities were 163 g d^{-1}, 247.2 g d^{-1}, and 289.3 g d^{-1} for a lactating cow grazing ‘Iuka’. Additional requirements of DIP needed with ‘Pete’ at these maturities are 216.6 g d^{-1}, 296.5 g d^{-1} and 237.2 g d^{-1}, respectively. At these three maturities, the amount of protein supplied in a diet of eastern gamagrass is inadequate to support maintenance (Table 2.5); therefore, supplementation is required.

Maturities of ‘Iuka’ and ‘Pete’ eastern gamagrass varied in the amount of protein supplementation needed to balance requirements for the non-lactating and lactating cow (Table 2.9). Soybean meal (SBM), a natural protein, was chosen to balance DIP, and dry matter intake (DMI) was adjusted accordingly (Table 2.9). Dry matter intake is also predicted by the model; however, the predicted DMI of low quality forages can be extremely biased since the standard for this equation is not similar in quality. In our study, the approach to predicting forage intake in beef cows suggested by Hibberd (Vanzat, 1996) based on historical observation of forage intake from various research experiments was utilized (Table 2.2).

Soybean meal was added in increments of 0.05 kg until the DIP balance was sufficient for proper rumen function (Table 2.7). Supplementation ranged from 0.80 kg SBM to 1.40 kg SBM. The maximum amount of SBM required for a non-lactating cow 160 d prepartum was 1.25 kg at the dormant stage of ‘Pete'. A lactating cow 60 d postpartum grazing ‘Iuka’ required less supplementation than ‘Pete’ for anthesis and seed shattering, while ‘Pete’ required less supplementation at dormancy (Table 2.9). Due to the high demands of lactating cattle, the maximum amount of supplementation required

was at seed shattering for ‘Pete’ and dormancy for ‘Iuka’ and equaled 1.40 kg (Table 2.9).

Through protein supplementation, DIP was balanced in ‘Iuka’ and ‘Pete’ at the anthesis and seed shattering maturities and satisfied nutritional demands. Digestible intake protein was balanced for plant tissue in the dormant stage; however, the energy supplied in the diet was inadequate (Table 2.8). An energy supplement could be supplied; although, energy demands of lactating cows often may not be met during periods of high production. However, when production demands decrease the animal compensates for lost energy. If supplying an energy supplement, the DIP supplied will need to be adjusted to balance for the energy increase.

Tables

Table 2.1 Animal and environmental descriptions used in the Beef NRC to evaluate forages.

Description	Inputs		Units
	Dry Cow	Lactating Cow	
Animal Description			
Animal Type	3	2	Type
Age	60	60	mo
Sex	4	4	cow
Body Weight	500	500	kg
Condition Score	5	5	1=emaciated to 9=very fat
Mature Weight	500	500	kg
Breeding System	2	2	way cross
Dam's Breed	11	11	Hereford
Sire's Breed	1	1	Angus
Days Pregnant	120	0	d
Days in Milk	0	60	d
Lactation Number	0	4	
Peak Milk Production	0	7.5	kg
Time of Lactation Peak	0	8.5	weeks
Duration of Lactation	0	30	weeks
Milk Fat	0	4	%
Milk Protein	0	3.4	%
Milk Solids not Fat	0	8.3	%
Age at Puberty	15	15	mo
Calving Interval	12	12	mo
Expected Calf Birth Weights	39	39	kg
Management Description			
Additive	1	1	none
On Pasture?	0	0	no
Diet Nem Adjuster	100	100	%
Diet Neg Adjuster	100	100	%
Diet Microbial Yield	11	11	%
Environmental Description			
Wind Speed	8	8	kph
Previous Temperature	10	10	degrees C
Current Temperature	10	10	degrees C
Night Cooling	2	2	yes
Hair Depth	1	1	cm
Hide	2	2	average
Hair Coat	1	1	clean and dry
Heat Stress	1	1	none

Table 2.2 Forage Intake Guide for Beef Cows[a]

Diet	Dry	Lactating
	DMI, %BW[b]	
Forage - low (<7% CP)[c]		
No Supplement	1.5	2
Protein Supplement	1.8	2.2
Energy Supplement	1.5	2
(When energy supplements exceed approximately 0.5% BW, a 0.5:1 substitution of supplement forage will occur above this threshold).		
Forage - medium (7-11% CP)		
No Supplement	2	2.3
Protein Supplement	2.2	2.5
Energy Supplement	2	2.3
(When energy supplements exceed approximately 0.5% BW, a 0.5:1 substitution of supplement forage will occur above this threshold).		
Forage - low (12% $\geq$ CP)		
No Supplement	2.5	2.7
Protein Supplement	2.5	2.7
Energy Supplement	2.5	2.7
(1 unit energy supplement will decrease forage intake by about 1 unit)		

[a]Adapted from tabular guidelines developed by C.A. Hibberd and presented in Vanzant (1996).
[b]Use average mature weight for cows.
[c]Protein values added to provide rough estimate of low, moderate, and high quality forages.

Table 2.3 Protein analysis used for evaluating cattle performance with the NRC Model (g kg^{-1})

[*]Forage	[1]DIP	[2]UIP	[3]NDICP	[4]ADICP	[A,6]CP	[A,5]AdjustedCP
	CP basis					
Iuka						
VE	57.25[a]	42.75[b]	55.94[a]	6.26[a]	121.8a	141.8[a]
A	50.38[b]	49.62[a]	33.04[b]	4.20[b]	77.6b	97.6[b]
SS	52.92[b]	47.08[a]	23.85[c]	4.32[b]	55.3c	75.3[c]
D	53.98[a]	46.02[b]	14.39[d]	5.57[a]	41.4d	61.4[d]
Pete						
VE	58.36[a]	41.64[b]	60.20[a]	5.94[a]	112.3a	132.3[a]
A	50.69[b]	49.3[a]	36.38[b]	3.86[b]	70.7b	90.7[b]
SS	51.69[b]	48.31[a]	26.63[c]	3.88[b]	48.8c	68.8[c]
D	58.55[a]	41.44[b]	15.92[d]	5.68[a]	36.9d	56.9[d]
LSD						
variety	…	…	…	…	3.80	…
maturity	3.69	3.69	3.60	0.06	5.40	…
Mean						
VE	57.80	42.20	58.07	6.10	117.02	137.02
A	50.54	49.46	34.71	4.03	74.11	94.11
SS	52.31	47.70	25.24	4.10	52.04	72.04
D	56.27	43.73	15.15	5.62	39.13	59.13

[*]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[a, b, c, d]Means within a column with different superscripts differ ($P \leq 0.05$).

[A]Differences were found between varieties ($P \leq 0.05$).

[1]Degradable Intake Protein

[2]Undegradable Intake Protein

[3]Neutral Detergent Insoluble CP

[4]Acid Detergent Insoluble CP

[5]Crude Protein + 2% (Umoh, 1977)

[6]Crude Protein

Table 2.4 ADF, NDF, EE, TDN, ADL and ASH content for evaluating cattle performance with the NRC Model (g kg^{-1})

[*]Forage	[1]ADF	[2]NDF	[3]EE	[4]TDN	[5]ADL	Ash
	DM Basis					
Iuka						
VE	317.6[a]	592.7[a]	18.4[b]	595.6[a]	31.0[b]	11.09[a]
A	343.6[b]	653.2[b]	16.9[b]	581.7[a]	38.1[b]	8.81[b]
SS	368.7[c]	666.0[c]	21.2[a]	566.4[b]	51.8[a]	7.77[c]
D	392.7[d]	730.0[d]	21.8[a]	558.8[c]	49.7[a]	6.71[c]
Pete						
VE	316.8[a]	593.0[a]	18.9[b]	594.3[a]	30.8[b]	11.27[a]
A	341.3[b]	645.6[b]	17.4[b]	594.4[a]	32.8[b]	8.71[b]
SS	372.0[c]	667.4[c]	20.5[a]	565.0[b]	51.7[a]	7.77[c]
D	402.9[d]	731.7[d]	20.5[a]	53.86[c]	50.7[a]	8.35[c]
LSD						
variety	…	…	…	…	…	…
Mean						
VE	317.20	592.80	18.60	594.90	30.9	11.17
A	342.50	649.40	17.10	588.00	35.4	8.76
SS	370.30	666.70	20.80	565.70	51.8	7.77
D	397.80	730.80	21.10	548.70	50.9	7.53
LSD						
maturity	10.70	14.40	1.57	16.21	6.29	0.83

[*]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[a, b, c, d]Means within a column with different superscripts differ ($P \leq 0.05$).

[1]Acid Detergent Fiber

[2]Neutral Detergent Fiber

[3]Ether Extract

[4]Total Digestable Nutrients

[5]Acid Detergent Lignin

Table 2.5 NRC Model output for a non-lactating 160 d prepartum beef cow consuming two gamagrass varieties at four maturities with no supplementation.

	Non-lactating Beef Cow							
Variety	Iuka				Pete			
Maturity[1]	VE	A	SS	D	VE	A	SS	D
NE Supplied, Mcal/d	16.7	12.3	11.5	8.4	16.5	12.5	11.4	7.3
NE required, Mcal/d	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
NE balance, Mcal/d	6.9	2.5	1.7	-1.4	6.7	2.7	1.6	-2.5
DIP Supplied, g/d	1086	508	419	255	1032	475	373	255
DIP Required, g/d	839	650	634	472	836	663	631	433
DIP Balance, g/d	247	-141.8	-214.9	-217	195.8	-188.4	-257.9	-177.9
[2]MP Supplied, g/d	1185	817	704	476	1124	794	683	422
MP Required, g/d	413	413	413	413	413	413	413	413
[2]MP Balance, g/d	772	403	291	62	710	381	270	8

[1]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[2]Values are not reliable. When DIP supply is insufficient, microbial yield is suppressed.

Table 2.6 NRC Model output for a lactating beef cow 60 d postpartum consuming two gamagrass varieties at four maturities with no supplementation.

	Lactating Beef Cow							
Variety	Iuka				Pete			
Maturity[1]	VE	A	SS	D	VE	A	SS	D
NE Supplied, Mcal/d	18.1	14.1	13.2	11.2	17.8	14.4	13.1	9.7
NE required, Mcal/d	16.6	16.6	16.6	16.6	16.6	16.6	16.6	16.6
NE balance, Mcal/d	1.5	-2.4	-3.3	-5.3	1.3	-2.1	-3.4	-6.9
DIP Supplied, g/d	1172	585	482	340	1114	546	429	340
DIP Required, g/d	906	748	729	629	903	763	726	577
DIP Balance, g/d	266.5	-163	-247.2	-289.3	211.5	-216.6	-296.5	-237.2
[2]MP Supplied, g/d	1280	939	810	634	1214	913	785	562
MP Required, g/d	794	794	794	794	794	794	794	794
[2]MP Balance, g/d	486	145	16	-160	419	119	-9	-232

[1]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[2]Values are not reliable. When DIP supply is insufficient, microbial yield is suppressed.

Table 2.7 NRC Model output for a non-lactating beef cow 160 d prepartum consuming two gamagrass varieties at four maturities with supplementation when appropriate.

	Non-lactating Beef Cow							
Variety	Iuka				Pete			
Maturity[1]	[2]VE	[3]A	[4]SS	[5]D	[2]VE	[6]A	[7]SS	[8]D
NE Supplied, Mcal/d	16.7	14.9	14.8	12	16.5	15.7	15.1	10.7
NE required, Mcal/d	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
NE balance, Mcal/d	6.9	5.1	5	2.7	6.7	5.8	5.3	0.9
DIP Supplied, g/d	1086	786	802	679	1032	814	816	614
DIP Required, g/d	839	780	794	672	836	813	810	607
DIP Balance, g/d	247	6.4	7.1	6.3	195.8	1.6	6	6.9
MP Supplied, g/d	1185	1037	983	799	1124	1053	1000	695
MP Required, g/d	413	413	413	413	413	413	413	413
MP Balance, g/d	772	624	570	386	710	639	586	281

[1]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[2]no supplementaion required

[3]0.70 kg soybean meal supplemented

[4]1.05 kg soybean meal supplemented

[5]1.15 kg soybean meal supplemented

[6]0.90 kg soybean meal supplemented

[7]1.25 kg soybean meal supplemented

[8]0.95 kg soybean meal supplemented

Table 2.8 NRC Model output for a lactating beef cow 60 d postpartum consuming two gamagrass varieties at four maturities with supplementation when appropriate.

	Lactating Beef Cow							
Variety	Iuka				Pete			
Maturity[1]	[2]VE	[3]A	[4]SS	[5]D	[2]VE	[6]A	[5]SS	[7]D
NE Supplied, Mcal/d	18.1	17	16.9	15.2	17.8	17.9	17.2	13
NE required, Mcal/d	16.6	16.6	16.6	16.6	16.6	16.6	16.6	16.6
NE balance, Mcal/d	1.5	0.4	0.3	-1.3	1.3	1.3	0.6	-3.5
DIP Supplied, g/d	1172	895	913	828	1114	934	921	747
DIP Required, g/d	906	887	903	821	903	926	918	741
DIP Balance, g/d	266.5	8.4	9.7	6.5	211.5	8.1	2.4	5.9
MP Supplied, g/d	1280	1180	1119	976	1214	1201	1132	847
MP Required, g/d	794	794	794	794	794	794	794	794
MP Balance, g/d	486	386	325	182	419	407	338	53

[1]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[2]no supplementaion required

[3]0.80 kg soybean meal supplemented

[4]1.20 kg soybean meal supplemented

[5]1.40 kg soybean meal supplemented

[6]1.05 kg soybean meal supplemented

[7]1.15 kg soybean meal supplemented

Table 2.9 Kilograms of soybean meal used as supplementation for dry and lactating cows consuming two gamagrass varieties at four maturities.

Forage	Dry Beef Cow		Lactating Beef Cow	
[1]Maturity	[2]Forage, DMI	[3]SBM, kg	[2]Forage, DMI	SBM, kg
Iuka				
VE	12.5	…	13.5	…
A	10, (11)	0.7	11.5, (12.5)	0.8
SS	10, (11)	1.05	11.5, (12.5)	1.2
D	7.5, (9)	1.15	10, (11)	1.4
Pete				
VE	12.5	…	13.5	…
A	10, (11)	0.9	11.5, (12.5)	1.05
SS	10, (11)	1.25	11.5, (12.5)	1.4
D	7.5, (9)	0.95	10, (11)	1.15

[1]VE= vegetative/early elongation; A=anthesis; SS= seed shattering; D= dormancy

[2]Supplemented intake are in parentheses.

[3]Soybean Meal

Conclusions

Differences were found between eastern gamagrass varieties, 'Iuka' and 'Pete', for crude protein (p=0.0496). This difference was small and required protein supplementation varied with model simulations. Maturity differed significantly for all laboratory analyses, thus corroborating that quality declines as the plant develops. Eastern gamagrass in the vegetative/early elongation stage of maturity is adequate to meet nutritional demands of dry and lacatating beef cows. Eastern gamagrass in anthesis, seed shattering and dormancy are not nutritionally adequate to meet animal demands and therefore require protein supplementation.

The NRC beef model is a user-friendly approach in allowing producers to accurately predict nutrient requirements of beef cattle under unique production settings. With accurate animal and environmental descriptions, the NRC beef model can predict energy and protein balances of eastern gamagrass for use in Kansas and Midwestern US forage systems while supplying the producer with information on supplementation when needed. The NRC model is cost effective in that it allows the producer to determine the least amount of supplementation necessary to balance both protein and energy demands.

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Appendix A - Seasonal Data

Table A.1 Temperature and precipitation readings for April 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
Apr-17	19.37	32.15	12.78	25.76	0.00
Apr-18	8.61	26.79	18.18	17.70	12.56
Apr-19	8.11	13.34	5.24	10.72	0.00
Apr-20	7.91	13.36	5.46	10.63	24.42
Apr-21	1.13	22.37	21.24	11.75	0.00
Apr-22	6.46	26.95	20.48	16.71	2.76
Apr-23	10.10	23.91	13.81	17.01	0.00
Apr-24	-0.96	17.61	16.65	8.33	0.00
Apr-25	8.91	15.01	6.09	11.96	0.00
Apr-26	7.47	22.11	14.64	14.79	10.28
Apr-27	3.70	20.91	17.21	12.31	7.65
Apr-28	8.14	23.20	15.06	15.67	0.00
Apr-29	9.79	24.21	14.42	17.00	0.00
Apr-30	10.99	24.02	13.03	17.50	0.00
Average	7.84	21.85	13.88	…	…
Total	…	…	…	…	57.66
30-yr AVG	6.17	19.94	13.77		70.87

Table A.2 Temperature and precipitation readings for May 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
May-1	4.07	16.60	12.53	10.34	0.00
May-2	4.24	19.87	15.63	12.05	0.00
May-3	5.69	20.53	14.84	13.11	0.00
May-4	10.42	25.31	14.89	17.86	0.00
May-5	15.00	29.03	14.03	22.01	26.09
May-6	14.72	29.47	14.75	22.09	6.12
May-7	14.29	27.73	13.44	21.01	0.00
May-8	5.69	29.35	23.66	17.52	0.00
May-9	5.71	16.34	10.64	11.02	0.00
May-10	9.76	21.48	11.72	15.62	30.76
May-11	9.91	27.66	17.75	18.78	28.25
May-12	2.78	17.23	14.45	10.00	0.00
May-13	4.45	22.78	18.34	13.61	0.00
May-14	15.56	26.67	11.11	21.11	0.00
May-15	15.56	27.23	11.67	21.39	2.97
May-16	6.67	25.56	18.89	16.11	6.32
May-17	2.78	18.89	16.11	10.84	0.00
May-18	11.11	19.45	8.33	15.28	0.00
May-19	9.01	19.00	9.99	14.00	2.81
May-20	7.78	22.78	15.00	15.28	0.00
May-21	13.89	23.89	10.00	18.89	0.00
May-22	17.78	25.56	7.78	21.67	0.00
May-23	17.78	23.89	6.11	20.84	0.00
May-24	4.45	16.67	12.22	10.56	16.46
May-25	11.67	22.23	10.56	16.95	0.00
May-26	12.23	26.67	14.45	19.45	16.99
May-27	15.56	29.45	13.89	22.50	0.00
May-28	15.21	28.70	13.49	21.96	0.00
May-29	13.34	30.56	17.22	21.95	0.00
May-30	18.89	32.78	13.89	25.84	0.00
May-31	19.41	33.94	14.52	26.68	0.00
Average	10.82	24.43	13.61	…	…
Total	…	…	…	…	136.76
30-yr AVG	11.94	25.17	13.23		100.58

Table A.3 Temperature and precipitation readings for June 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
Jun-1	21.11	35.00	13.89	28.06	0.00
Jun-2	21.18	33.90	12.72	27.54	0.00
Jun-3	16.67	35.00	18.34	25.84	0.00
Jun-4	13.34	28.89	15.56	21.11	5.21
Jun-5	11.67	26.12	14.45	18.89	0.00
Jun-6	13.77	28.63	14.86	21.20	0.00
Jun-7	16.44	30.37	13.93	23.41	0.00
Jun-8	19.43	31.46	12.03	25.44	0.00
Jun-9	21.81	28.70	6.89	25.25	0.00
Jun-10	23.27	31.68	8.41	27.48	0.00
Jun-11	20.89	32.88	11.99	26.89	0.00
Jun-12	19.67	31.76	12.09	25.72	0.00
Jun-13	13.07	25.96	12.88	19.51	2.72
Jun-14	10.88	26.64	15.75	18.76	0.00
Jun-15	10.10	28.52	18.42	19.31	0.00
Jun-16	12.33	28.37	16.04	20.35	0.00
Jun-17	15.66	30.02	14.36	22.84	0.00
Jun-18	20.88	30.02	9.14	25.45	0.00
Jun-19	20.82	32.61	11.78	26.71	0.00
Jun-20	20.08	32.76	12.69	26.42	0.00
Jun-21	21.50	33.01	11.52	27.26	0.00
Jun-22	21.32	32.91	11.59	27.12	0.00
Jun-23	18.64	33.19	14.56	25.91	0.00
Jun-24	17.90	33.01	15.10	25.46	0.00
Jun-25	19.99	33.81	13.82	26.90	0.00
Jun-26	20.25	34.95	14.70	27.60	0.00
Jun-27	19.69	32.84	13.15	26.27	0.00
Jun-28	20.58	34.48	13.90	27.53	0.00
Jun-29	22.24	33.57	11.33	27.90	0.00
Jun-30	23.24	31.89	8.64	27.57	0.00
Average	18.28	31.43	13.15	…	…
Total	…	…	…	…	7.94
30 yr AVG	17.33	30.11	12.78		134.37

Table A.4 Temperature and precipitation readings for July 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
Jul-1	21.18	28.86	7.68	25.02	0.00
Jul-2	20.11	29.31	9.20	24.71	0.00
Jul-3	18.98	29.44	10.46	24.21	0.00
Jul-4	19.05	28.45	9.41	23.75	0.00
Jul-5	18.83	29.69	10.86	24.26	0.00
Jul-6	18.35	31.98	13.63	25.16	0.00
Jul-7	19.91	32.62	12.71	26.26	0.00
Jul-8	21.19	34.23	13.04	27.71	0.00
Jul-9	22.10	35.76	13.66	28.93	0.00
Jul-10	20.22	32.64	12.42	26.43	0.00
Jul-11	15.43	27.91	12.49	21.67	0.00
Jul-12	14.67	22.88	8.21	18.78	0.00
Jul-13	14.44	27.71	13.27	21.07	0.00
Jul-14	12.26	30.06	17.80	21.16	0.00
Jul-15	13.61	32.03	18.43	22.82	0.00
Jul-16	16.86	32.23	15.38	24.55	0.00
Jul-17	20.93	32.70	11.76	26.82	0.00
Jul-18	21.22	35.97	14.75	28.60	0.00
Jul-19	22.78	35.94	13.16	29.36	0.00
Jul-20	23.07	36.51	13.44	29.79	0.00
Jul-21	25.03	36.67	11.64	30.85	0.00
Jul-22	19.03	31.20	12.16	25.11	0.00
Jul-23	14.43	29.12	14.69	21.78	0.00
Jul-24	14.58	31.00	16.42	22.79	0.00
Jul-25	18.73	35.17	16.44	26.95	0.00
Jul-26	20.07	34.21	14.14	27.14	0.00
Jul-27	22.11	34.69	12.58	28.40	73.56
Jul-28	17.52	31.58	14.06	24.55	12.31
Jul-29	18.87	31.68	12.81	25.28	0.00
Jul-30	16.86	33.27	16.41	25.06	0.00
Jul-31	19.69	33.76	14.06	26.72	0.00
Average	18.78	31.91	13.13	…	…
Total	…	…	…	…	85.87
30 yr AVG	20.00	33.17	13.77		100.58

Table A.5 Temperature and precipitation readings for August 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
Aug-1	19.18	35.12	15.94	27.15	0.00
Aug-2	18.46	32.00	13.55	25.23	0.00
Aug-3	19.85	34.61	14.76	27.23	0.00
Aug-4	22.22	35.22	13.00	28.72	0.00
Aug-5	20.67	35.02	14.35	27.85	0.00
Aug-6	20.21	33.86	13.64	27.03	0.00
Aug-7	20.23	31.69	11.46	25.96	0.00
Aug-8	16.59	32.04	15.45	24.31	0.00
Aug-9	12.43	28.95	16.52	20.69	0.00
Aug-10	16.71	31.54	14.83	24.13	0.00
Aug-11	19.37	33.06	13.69	26.22	7.00
Aug-12	18.58	30.25	11.66	24.42	35.13
Aug-13	14.27	23.95	9.67	19.11	0.00
Aug-14	12.96	29.11	16.15	21.04	0.00
Aug-15	15.50	30.79	15.28	23.15	0.00
Aug-16	19.18	31.95	12.77	25.56	9.12
Aug-17	17.89	25.51	7.62	21.70	0.00
Aug-18	17.64	31.77	14.13	24.71	6.12
Aug-19	18.18	27.26	9.08	22.72	0.00
Aug-20	19.42	33.07	13.65	26.25	4.25
Aug-21	21.08	31.69	10.61	26.38	10.04
Aug-22	22.81	33.63	10.82	28.22	0.00
Aug-23	19.73	32.53	12.80	26.13	0.00
Aug-24	18.85	28.60	10.02	23.59	0.00
Aug-25	16.70	31.04	14.35	23.87	0.00
Aug-26	17.00	31.39	14.39	24.20	0.00
Aug-27	18.00	31.54	13.54	24.77	0.00
Aug-28	16.96	31.03	14.08	24.00	0.00
Aug-29	17.27	30.72	13.45	24.00	0.00
Aug-30	17.13	31.33	14.20	24.23	0.00
Aug-31	16.49	31.23	14.74	23.86	0.00
Average	18.11	31.34	13.07	…	…
Total	…	…	…	…	71.67
30 yr AVG	19.06	32.44	13.38		80.77

Table A.6 Temperature and precipitation readings for September 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
Sep-1	17.75	33.78	16.04	25.76	0.00
Sep-2	19.06	35.27	16.21	27.16	0.00
Sep-3	15.52	31.69	16.17	23.60	0.00
Sep-4	17.35	35.84	18.49	26.60	0.00
Sep-5	18.15	34.86	16.70	26.50	0.00
Sep-6	19.37	34.89	15.52	27.13	0.00
Sep-7	18.30	34.63	16.32	26.46	0.00
Sep-8	19.41	33.49	14.08	26.45	0.00
Sep-9	16.70	32.17	15.47	24.43	0.00
Sep-10	13.95	26.87	12.92	20.41	0.00
Sep-11	11.37	26.95	15.57	19.16	0.00
Sep-12	10.67	24.23	13.57	17.45	0.00
Sep-13	14.91	28.22	13.31	21.56	9.98
Sep-14	14.62	23.22	8.59	18.92	27.03
Sep-15	9.57	23.91	14.34	16.74	0.00
Sep-16	7.73	25.96	18.24	16.85	0.00
Sep-17	13.13	28.73	15.59	20.93	0.00
Sep-18	18.12	30.33	12.21	24.23	29.57
Sep-19	14.19	18.80	4.61	16.49	5.17
Sep-20	10.28	25.29	15.02	17.78	0.00
Sep-21	10.28	21.47	11.19	15.87	0.00
Sep-22	3.97	19.68	15.71	11.83	0.00
Sep-23	4.74	22.40	17.65	13.57	0.00
Sep-24	5.69	22.53	16.85	14.11	0.00
Sep-25	9.79	27.49	17.70	18.64	0.00
Sep-26	8.94	24.57	15.64	16.76	0.00
Sep-27	9.43	21.44	12.01	15.44	0.00
Sep-28	7.94	31.67	23.72	19.80	0.00
Sep-29	13.90	33.81	19.91	23.85	0.00
Sep-30	19.30	32.35	13.05	25.82	0.00
Average	13.14	28.22	15.08	…	…
Total	…	…	…	…	71.75
30 yr AVG	13.78	27.61	13.83		102.62

Table A.7 Temperature and precipitation readings for October 2002. (ºC)

Date	Minimum ºC	Maximum ºC	Range	Daily Avg.	Precip (mm)
Oct-1	18.25	31.94	13.69	25.10	25.11
Oct-2	10.89	21.71	10.82	16.30	23.71
Oct-3	10.83	20.91	10.08	15.87	18.80
Oct-4	3.83	17.86	14.03	10.84	0.00
Oct-5	2.72	21.67	18.95	12.19	0.00
Oct-6	5.13	19.83	14.70	12.48	0.00
Oct-7	0.77	18.86	18.09	9.82	0.00
Oct-8	6.43	21.26	14.83	13.85	0.00
Oct-9	9.14	23.22	14.08	16.18	0.00
Oct-10	6.47	21.66	15.19	14.06	0.00
Oct-11	10.05	22.32	12.26	16.18	0.00
Oct-12	2.71	16.68	13.97	9.69	0.00
Oct-13	-1.09	14.21	13.11	6.56	0.00
Oct-14	2.05	16.04	14.00	9.05	0.00
Oct-15	0.94	16.14	15.20	8.54	0.00
Average	5.94	20.29	14.20	…	…
Total	…	…	…	…	67.62
30-yr AVG	7.33	21.72	14.39		73.41

Appendix B - Analytical Procedures

Ash

References:

Ash of Animal Feed. (942.05). Official methods of Analysis. 1995. Association of Official Analytical Chemists, 16th Edition.

Undersander, D. D.R. Mertens, N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association. Omaha, Nebraska.

Procedure:

1. Weigh 2 g sample into a porcelain crucible and place in a temperature controlled furnace preheated to 600°.
2. Hold at this temperature for 2 hours and turn off oven.
3. When cooled to approximately 200°, transfer crucible directly to a desiccator, cool and weigh immediately.
4. Report % ash to the first decimal place.

Calculation: Percent Ash, DM Basis

$$\% \text{ Ash (DM Basis)} = \frac{(W3 - W1) \times 100}{(W2 - W1) \times \text{Lab DM} / 100}$$

Where: W1 = tare weight of crucible in grams

W2 = weight of crucible and sample in grams

W3 = weight of crucible and ash in grams

Comments:

If determining ash after fiber analysis, set furnace at 500°C and ash until carbon-free and grey ash color (3-5 hours). Lower ashing temperatures require longer ashing times.

Crude Fat (Ether Extract) in Forages

References:

Undersander, D. D.R. Mertens, N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association. Omaha, Nebraska.

Laboratory Safety. (51.009, 51.011 and 51.054). Official Methods of Analysis. 1980. Association of Official Analytical Chemists. 13th Edition.

Equipment:

Goldfisch fat extraction apparatus, 6-flask unit, equipped with glass thimble holders and ether reclaiming tubes
Extraction thimbles, 22 x 80 mm, alundum (porous clay), coarse
Fat beakers, pyrex, with ground lip, engraved with a number, 50 x 85 mm
Drying oven, 102ºC gravity convection
Analytical balance, sensitive to 0.1 mg
Desiccator and tongs
Filter paper, Whatman #1, 11 cm or equivalent
Steambath in a hood (optional)
Gloves, white nylon, lintless

Reagents:

Anhydrous Diethyl Ether, purified for fat extraction Mallinkrodt #0844 or equivalent.
To prevent ether from absorbing water, purchase it in small containers and keep containers tightly closed.

Procedure:

Sample drying

1. Weigh 1.5 to 2 g of ground sample into a thimble recording the weight to nearest 0.1 mg (W1). Weigh a second subsample for dry matter determination.
2. Dry for 5 hours at 100ºC.
3. Dry beakers to be used for fat determination for at least 1 hour at 100ºC. Cool the appropriate number of fat beakers in a desiccator. Weigh and record the weight to the nearest 0.1 mg (W2).
4. When the drying period is over, remove the samples from the oven to a desiccator. (This is a convenient stopping point. The samples should remain in the desiccator if not immediately extracted.)

Extraction

5. Line the fat beakers up in front of the extractor and match the thimbles with their corresponding fat beakers.
6. Slip the thimble into a thimble holder and clip the holder into position on the extractor.
7. Add 40 ml of diethyl ether (one glass reclaiming tube full) to each fat beaker.
8. Wearing white gloves, slip the beaker into the ring clamp and tightly clamp the beaker onto the extractor. If the clamp is too loose, insert another gasket inside the ring.
9. Raise the heaters into position, leaving about a ¼ inch gap between the beaker and the heating element.
10. Turn on the heater switch, the main power switch and the condenser water.

11. After the ether has begun to boil, check for ether leakage. This can be detected by sniffing around the ring clamp. If there is leakage, check the tightness of the clamp, and if necessary, replace the gasket(s).
12. Extract for a minimum of 4 hours on a Hi setting (condensation rate of 5 to 6 drops per second), or for 16 hours on a Low setting (condensation rate of 2 to 3 drops per second).
13. After extraction, lower the heaters, shut off the power and water, and allow the ether to drain out of the thimbles (about 30 minutes). This is a good stopping point.

Ether Distillation and Weighing of Fat Residue

14. Remove the thimble from the holder, and rinse the holder with a small portion of diethyl ether from the washbottle. Clip an ether reclaiming tube in place and reattach the fat beaker.
15. Reposition the heaters and turn on the electricity and water. Proceed to distill the ether using a Hi setting. Watch Closely.
16. Distill until a thin layer of ether remains in the bottom of the beaker, and then lower the heater. Do not allow beakers to boil dry. Overheating will oxidize the fat. When the last beaker has finished, shut off the power and water.
17. Wipe the exterior of the beaker clean with a Kimwipe as it is being removed from the extractor.
18. Empty the reclaiming tubes into the "USED" diethyl ether container.
19. Place the tray of beakers in an operating hood to finish evaporating the ether. If there is no hurry, air moving through the hood will be sufficient without heat. A steam bath may be used to speed up the evaporation. Beakers should remain in the hood until all traces of ether are gone. Carefully sniff each

beaker to determine if any ether remains.

20. Place the beakers in a 102ºC gravity convection oven.
    Warning: If a beaker containing ether is placed in an oven an explosion may occur.
21. Dry for ½ hour. No longer. Excessive drying may oxidize the fat and give high results.
22. Cool in a desiccator and weigh and record weight to the nearest 0.1 mg (W2).
23. The fat beakers are best cleaned by warming on a steambath or on a hot plate on a low setting. Add some used ether to dissolve the fat. The use of a rubber policeman is helpful. After soaking the beakers in Alconox detergent, wash them using hot water and vigorous brushing. The thimbles are best cleaned by blowing out with air.

Calculations:

$$\text{\% Crude Fat (DM Basis)} = \frac{(W3 - W2) \times 100}{W1 \times \text{Lab DM} / 100}$$

Where : W1 = initial sample weight in grams
W2 = tare weight of beaker in grams
W3 = weight of beaker and fat residue in grams

Comments:

Monitor unattended operations with equipment that will automatically shut down process if unsafe condition develops. Perform operations behind safety barrier with hot water, steam, or electric mantle heating. Use effective fume removal device to remove flammable and toxic vapors as produced. Set up apparatus on firm supports and secure all connections. Leave ample headroom in flask and add boiling chips before heating is begun. All controls, unless vapor sealed, should be located outside vapor area. Dispose of waste and flammable solvents by evaporation as above unless other provisions for safe disposal are available. Avoid contact with skin. Take necessary precautions to protect eyes, skin and clothing. Diethyl Ether is extremely flammable and should be stored

protected from light. Unstable peroxides can form upon long standing or exposure to sunlight in bottles. Can react explosively when in contact with Cl, O_3, $LiAlH_4$, or strong oxidizing agents. Use effective fume removal device. Avoid static electricity.

Neutral Detergent Insoluble Nitrogen (NDIN)

References:

Licitra, G., T.M. Hernandez, P.J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science Technology, 57: 347- 358.

Equipment:

LECO Nitrogen Analyzer

Reagents:

NDF Solution
Acetone
0.01 N Hydrochloric Acid (HCl)

Procedure:

1. Follow the SOP for Neutral Detergent Fiber (NDF). Weigh paper if NDF value is desired.
2. Transfer sample residue into a vial. Determine N on residue according to SOP 'Determination of N by LECO'.
3. Titrate distillate with 0.01 N HCl.
4. Express NDIN as percent of total N or N x 6.25.

Comments:

Sodium sulfite should not be used in the NDF procedure because it reduces the protein content. Similarly, the use of urea-amylase to remove resistant starch can not be used because urea will dissolve proteins in the B_3 Fraction.

Laboratory Dry Matter, 105ºC

References:

Undersander, D. D.R. Mertens, N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association. Omaha, Nebraska.

Procedure:

1. Dry 57 mm aluminum weigh pans at 105ºC for at least 2 hours.
2. Place pans in a desiccator and allow cooling at room temperature (about 20 minutes).
3. Remove pans from desiccator one at a time and record weight to the nearest 0.1 mg. Keep the desiccator lid in place between removals.
4. Tare the pan and add 1-2 g of ground sample. Record the weight to the nearest 0.1 mg.
5. Dry samples in oven at 105ºC for 16 hours (overnight).
6. Move samples to a desiccator and allow to cool at room temperature for 30 minutes.
7. Again, remove pans from the desiccator one at a time and weigh to the nearest 0.1 mg. Record weight.

Calculations:

$$\%\ DM = \frac{(\text{Dry sample} + \text{Pan Weight}) - \text{Pan Weight}}{\text{Initial Sample Weight}} \times 100$$

Comments:

Always use tongs or gloves when handling containers, for oils and moisture from hands can cause weighing errors.

Aluminum pans can be ashed, but temperatures should not exceed 450ºC.

Acid Detergent Insoluble Nitrogen (ADIN)

References:

Licitra, G., T.M. Hernandez, P.J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science Technology, 57: 347- 358.

Equipment:

LECO Nitrogen Analyzer

Reagents:

ADF Solution

0.01 N Hydrochloric Acid (HCl)

Acetone

Procedure:

1. Follow the SOP for Acid Detergent Fiber (ADF). Weigh paper if ADF value is desired.
2. Transfer sample residue into a vial. Determine N on residue according to SOP 'Determination of N by LECO'.
3. Titrate distillate with 0.01 N HCl.
4. Express ADIN as percent of total N or N x 6.25.

Comments:

ADIN represents the C Protein Fraction.

Acid Detergent Fiber (ADF)

References:

Undersander, D. D.R. Mertens, N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association. Omaha, Nebraska.

Equipment:

Digestion apparatus – Ankom Fiber Analyzer

Filtration device - Ankom F57 filter bags

Impulse bag sealer – requires high enough temperatures to melt and seal polymer filter bags.

Desiccator- Ankom *MoistureStop* weigh pouch F39

Reagents:

Acid Detergent Solution:

To Prepare Mix:

1 liter 1.00N Sulfuric acid, plus or minus 0.005N. Normality must be verified by titration with a primary base standard before adding CTAB. A solution approximately 1.0 N sulfuric acid can be made by adding 51.04 g (27.7 ml) of concentrated reagent grade sulfuric acid (95-98% purity) to 972.3 ml water (AOAC 935.70). Titrate and add water (if normality is too high) or sulfuric acid (if normality is too low) to adjust normality to 1.00N, plus or minus 0.005N.

20g Cetyl trimethylammonium bromide (CTAB), technical grade

Acetone, reagent grade

Procedure:

1. Weigh filter bag (W1), record weight and tare balance.
2. Weigh 0.5 g of air-dried sample (W2), ground to pass through a

1 mm screen, directly into the filter bag. Weigh one blank bag and include in digestion to determine blank bag correction (C1).

3. Seal the bags closed within 0.5 cm from the open edge using the heat sealer.
4. Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.
5. A maximum of 24 bags may be place in the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. The weight is placed on top of the empty 9th tray to keep the bag suspender submerged.
6. When processing 24 sample bags, add 1900-2000ml of ambient ADF solution into ANKOM Fiber Analyzer vessel. If processing less than 20 bags, add 100 ml/bag of detergent solution (minimum of 1500 ml (ensure Bag Suspender is covered)).
7. Place bag suspender with samples into the samples into the solution in vessel. Turn *Agitate* and *Heat* ON and confirm that Bag Suspender is agitating properly. Set timer for 75 minutes and push *Start*. Close and seal lid of vessel.
8. After 75 minutes (timer will beep) turn *Agitate* and *Heat* OFF, open the drain valve and exhaust hot solution before opening lid. **WARNING: The solution in vessel is under pressure. The valve should be opened first to remove pressure before lid can be opened. Ensure exhaust hose is securely positioned for safe disposal of effluent.**
9. After the solution has been exhausted, close valve and open the lid. Add approximately 2000ml of hot (90-100ºC) water. Lower lid but do not tighten. Turn Agitate On and leave Heat OFF. Each rinse should last 3-5 minutes. Exhaust water and repeat rinse two more times (total of three times).

10. Remove filter bags from bag suspender and gently press out excess water. Place in beaker and soak in acetone. Allow bags to soak 3 minutes then remove and lightly press out excess acetone.
11. Spread bags out and allow acetone to evaporate. Complete drying in oven at 105ºC for at least 2 hours. **WARNING: Do not place bags in the oven until acetone has completely evaporated.** Longer drying period may be required depending on oven and frequency of sample introduction into the oven. Remove bags from oven, place directly into *MoistureStop* weigh pouch and flatten to remove air. Cool to ambient temperature and weigh bags (W3).

Calculations:

$$\text{ADF (as-is basis)} = \frac{(W3 - (W1 \times C1)) \times 100}{W2}$$

$$\text{ADF (DM basis)} = \frac{(W3 - (W1 \times C1)) \times 100}{W2 \times DM}$$

$$\text{ADF(OM) (DM basis)} = \frac{(W4 - (W1 \times C2)) \times 100}{W2 \times DM}$$

Where: W1 = bag tare weight

W2 = sample weight

W3 = weight after extraction

W4 = weight of Organic Matter (OM) (loss of weight on ignition of bag and fiber residue.)

C1 = blank bag correction (final oven-dried weight/original blank bag weight)

C2 = ash corrected blank bag (loss of weight on ignigtion of blank bag/original blank bag weight)

Comments:

Always add sulfuric acid to water.

Wear face shield and heavy rubber gloves. If acid is splashed on skin, wash immediately with copius amounts of water.

CTAB powder will irritate mucous membranes, eyes and skin. Wear gloves and dust mask while handling.

Acetone is highly flammable. Do not let vapors accumulate in work area. Use effective fume removal device. Also avoid inhaling or contact with skin. Make sure all traces of acetone have evaporated from the bags containing fiber residue before placing them in the oven.

If black discoloration occurs during drying, repeat the analysis.

Neutral Detergent Fiber (NDF)

References:

Undersander, D. D.R. Mertens, N. Thiex. 1993. Forage Analysis Procedures. National Forage Testing Association. Omaha, Nebraska.

Equipment:

Digestion apparatus – Ankom Fiber Analyzer

Filtration device - Ankom F57 filter bags

Impulse bag sealer – requires high enough temperatures to melt and seal polymer filter bags.

Desiccator- Ankom *MoistureStop* weigh pouch F39

Reagents:

Neutral Detergent Solution:

To make approximately 18 liters:

17.82 L	Distilled Water
540 g	Sodium lauryl sulfate, USP
335 g	Ethylenediaminetetraacetic acid (EDTA), disodium salt (may substitute 72 g sodium hydroxide (NaOH) and 263 g free acid EDTA as a less expensive alternative).
122.6 g	Sodium borate, decahydrate ($Na_2B_4O_7$ $10H_2O$), reagent grade
82.1 g	Sodium phosphate, dibasic (Na_2HPO_4), anhydrous, reagent grade

180 ml Triethylene glycol, reagent grade

Acetone, reagent grade

When making the NDF Solution, mix in the following manner:

1. Mix EDTA and $Na_2B_4O_7.10H_2O$ in a 4 L flask. Add deionized water (2-3 L), heat and stir until dissolved.
2. Add above solution to solution containing sodium lauryl sulfate and 2-ethoxy-ethanol (ethylene glycol triethyl ether).
3. Put Na_2HPO_4 in 4 L flask. Add some deionized water, heat and stir until dissolved, then add to solution containing other ingredients.

Be sure to keep an accurate recording of amount of water added above. Add balance of water and mix. Check pH (range 6.9-7.1).

Procedure:

1. Weigh filter bag (W1), record weight and tare balance.
2. Weigh 0.5 g of air-dried sample (W2), ground to pass through a 1 mm screen, directly into the filter bag. Weigh one blank bag and include in digestion to determine blank bag correction (C1).
3. Seal the bags closed within 0.5 cm from the open edge using the heat sealer.
4. Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.
5. A maximum of 24 bags may be place in the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. The weight is placed on top of the empty 9th tray to keep the bag suspender submerged.
6. When processing 24 sample bags, add 1900-2000ml of ambient ADF solution into ANKOM Fiber Analyzer vessel. If processing less than 20 bags, add 100 ml/bag of detergent solution

(minimum of 1500 ml (ensure Bag Suspender is covered)).

7. Place bag suspender with samples into the samples into the solution in vessel. Turn *Agitate* and *Heat* ON and confirm that Bag Suspender is agitating properly. Set timer for 75 minutes and push *Start*. Close and seal lid of vessel.
8. After 75 minutes (timer will beep) turn *Agitate* and *Heat* OFF, open the drain valve and exhaust hot solution before opening lid. **WARNING: The solution in vessel is under pressure. The valve should be opened first to remove pressure before lid can be opened. Ensure exhaust hose is securely positioned for safe disposal of effluent.**
9. After the solution has been exhausted, close valve and open the lid. Add approximately 2000ml of hot (90-100ºC) water. Lower lid but do not tighten. Turn Agitate On and leave Heat OFF. Each rinse should last 3-5 minutes. Exhaust water and repeat rinse two more times (total of three times).
10. Remove filter bags from bag suspender and gently press out excess water. Place in beaker and soak in acetone. Allow bags to soak 3 minutes then remove and lightly press out excess acetone.
11. Spread bags out and allow acetone to evaporate. Complete drying in oven at105ºC for at least 2 hours. **WARNING: Do not place bags in the oven until acetone has completely evaporated.** Longer drying period may be required depending on oven and frequency of 1 sample introduction into the oven.
    Remove bags from oven, place directly into *MoistureStop* weigh pouch and flatten to remove air. Cool to ambient temperature and weigh bags (W3).

Calculations:

$$NDF\ (\text{as-is basis}) = \frac{(W3 - (W1 \times C1)) \times 100}{W2}$$

$$NDF\ (\text{DM basis}) = \frac{(W3 - (W1 \times C1)) \times 100}{W2 \times DM}$$

$$NDF(OM)\ (\text{DM basis}) = \frac{(W4 - (W1 \times C2)) \times 100}{W2 \times DM}$$

Where: W1 = bag tare weight

W2 = sample weight

W3 = weight after extraction

W4 = weight of Organic Matter (OM) (loss of weight on ignition of bag and fiber residue.)

C1 = blank bag correction (final oven-dried weight/original blank bag weight)

C2 = ash corrected blank bag (loss of weight on ignition of blank bag/original blank bag weight)

Comments:

Sodium sulfite and amylase are not being used as reagents in this procedure because sodium sulfite reduces the protein content of the forage, and amylase dissolves proteins in the B3 fraction.

CTAB powder will irritate mucous membranes, eyes and skin. Wear gloves and dust mask while handling.

Acetone is highly flammable. Do not let vapors accumulate in work area. Use effective fume removal device. Also avoid inhaling or contact with skin. Make sure all traces of acetone have evaporated from the bags containing fiber residue before placing them in the oven.

LECO N Analyzer

Instrument: LECO FP – 2000 Serial # 3273

Calibration Standard: NIST SRM 1515 Apple Leaves, SRM 1547 Peach Leaves, or other suitable standard

Accessories: Gel Caps

Instrument Settings

Flow Profile: High-High-High/ 10-30-END

Furnace Temperature: 850ºC

Atm. Blank: 0.04

Crucible Change Interval: 50

Aliquot loop size: 10cc

Sample Weight: 0.10 g

Analysis Time: Approximately 2.5 minutes.

Procedure:

1. Set up system as outlined in the operator's instruction manual following the leak check, blank and calibrating procedures.

2. To Analyze Samples:

   a. Weigh approximately 0.10 g sample into a gel cap and

   b. Analyze according to analysis procedure as enter weight.

   outlined in the operator's instruction manual.

Acid Detergent Lignin (ADL)

References:

Official Methods of Analysis of the Association of Official Analytical Chemists, 1980. 13th ed, Ed. Horowitz, W., Washington, D.C. p. 135

Van Soest, P.J. 1963. Use of Detergents in the Analysis of Fibrous Feeds. II. A Rapid Method for the Determination of Fiber and Lignin. Journal of the AOAC. 46: 829-835.

Van Soest, P.J. 1973. Collaborative Study of Acid-Detergent Fiber and Lignin. Journal of the AOAC. 56: 781-784.

Equipment:

Digestion apparatus – Ankom Fiber Analyzer

Filtration device - Ankom F57 filter bags

Impulse bag sealer – requires high enough temperatures to melt and seal polymer filter bags.

Desiccator- Ankom *MoistureStop* weigh pouch F39

Fritted glass crucibles

Reagents:

Sulfuric Acid (72% by wt.) Add 1200g of H_2SO_4 to 440 ml of H_2O in 1 L MCA vol. flask with cooling.

Procedure:

1. Follow the SOP for Acid Detergent Fiber.
2. Weigh paper if ADF is desired.
3. Submerge ANKOM bagged samples into glass beaker of 72% H_2SO_4.
4. Use a second smaller beaker to agitate samples, soaking bags.
5. Allow samples to soak in H_2SO_4 for 3 hours, agitating every 30 minutes.

6. Remove samples from acid and rinse thoroughly.
7. Dry in forced air oven overnight.
8. Remove samples from oven and allow cooling in a desiccator and weigh.
9. Place sample bags in a previously weighed glass crucible and ash in muffle oven at 500° C for 8 hours.
10. Remove from muffle oven and allow cooling in a desiccator and weigh.

Calculations:

$$\%\text{Acid Insoluble Lignin} = \frac{(W1-W2-W3)}{S}$$

Where: W1 = Initial drying in forced air oven.

W2 = Weight after ashing

W3 = Blank

$$S = \frac{\text{g sample x g oven dried matter}}{\text{g air dried or wet matter}}$$

Comments:

Always add H2SO4 to H2O. Wear face shield and heavy rubber gloves to protect against splashes.

References:

Abdelgadir, I.E.O., R.C. Cochran, E.C. Titgemeyer, and E.S. Vanzant. 1997. In vitro determination of ruminal protein degradability of alfalfa and prairie hay via a commercial protease in the presence or absence of cellulose or driselase. Journal of Animal Science. 75:2215-2222.

Coblentz, W.K., I.E.O. Abdelgadir, R.C. Cochran, J.O. Fritz, and K.C. Olsen. 1998. Degradability of forage proteins by in situ and enzymatic methods. Journal of Animal Science 76 (Suppl.1):347.

Krishnamoorthy, U., C.J. Sniffen, M.D. Stern, and P.J. VanSoest. 1983. Evaluation of a mathematical model of rumen digestion and in vitro simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. Br. J. Nutr. 50:555.

Mathis, C.P., R.C. Cochran, E.S. Vanzant, I.E.O. Abdelgadir, J.S. Heldt, K.C. Olson, J. Caton, D. Faulkner, T. Klopfenstein, K. Moore, S. Paisley, and C. Sheaffer. 1998. Evaluation of in situ and in vitro techniques for estimating degradable intake protein content in forages. J. Anim. Sci. 76(Suppl. 2):77.

Roe, M.B. C.J. Sniffen, and L.E. Chase. 1990. Techniques for measuring protein fractions in feedstuffs. In: Proc. Cornell Nutr. Conf. Feed Manuf., Syracuse, NY. P.81. Cornell Univ., Ithaca, NY.

Roe, M.B., L.E. Chase, and C.J. Sniffen. 1991. Comparison of in vitro techniques to the in situ technique for estimation of ruminal degradability of protein. J. Dairy Sci. 74:1632.

Reagents:

Borate-Phosphate (BP) Buffer:

pH 7.8-8.0:

$NaH_2PO_4.H_2O$ 7.6 g/L

$Na_2B_4O_7.10H_2O$ 13.17 g/L

Filter through Whatman #541 filter paper

Protease Solution:

Protease type XIV Streptomyces griseus, (SGP) Sigma Chemical Co. (P-5147). Always use "fresh" solution (i.e. prepared on the same day of use, preferably just before use) when conducting these assays. If the solution needs to be stored temporarily before use, place in refrigerator).

Concentration of protease solution: 0.33 units/mL

Example: To make 1000ml of the 0.33 units/ml protease solution requires use of 330 units of enzyme. The units of activity/mg powder are noted on the receptacle in which the enzyme is delivered. If the activity is 4.6 units per mg solid, then one would need to add 71.7 mg (330/4.6=71.7) or 0.0717 g to 1000 ml of BP buffer. Filter solution through Whatman #541 filter paper after enzyme addition.

Procedure:

1. Determine total N content of forage samples.
2. Weigh out the equivalent of 15 mg feed N (0.015 / sample N) into a 125 ml Erlenmeyer flask. Record exact amount. Prepare samples in duplicate.
3. Add 20 ml of BP buffer to Erlenmeyer flask and let stand 5 minutes.
4. Add 20 ml more of BP buffer to Erlenmeyer flask for a total of 40 ml of BP buffer solution in each flask.
5. Incubate at 39° C for 1 hour.

6. Add 10ml of “fresh” protease solution, swirl to mix the protease solution with the sample.
7. Incubate forage samples exposed to the 0.33 units/ml solution for 48 hours.
8. At the end of the incubation time filter the sample through Whatman #541 filter paper (use cone shaped funnel rather that Buchner funnel).
9. Wash residue with 400 ml distilled water.
10. Measure N in the residue and record.

Calculations:

DIP (%) = 100 – [(g residual N / g Total N) *100]

Calculating % DIP in situations where the final sample is split between two filter papers:

1. Calculate the g N in the original sample:

   (as-is original sample wt) x (%N in original sample, as-is basis) = g N (original)

2. Calculate the g N in the residual sample:

   Assume the original as-is sample wt. was arbitrarily split into two parts (A and B)

   [(% residual N for part A) * (A/A+B)] + [(%residual N for part B) * (B/A+B)]= %N in the residue (weighted average; as-is basis)

   (as-is original sample wt.) x (% N in residue, as-is; weighted avg.) = g N (residue)