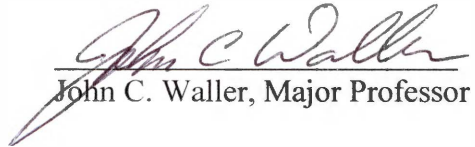
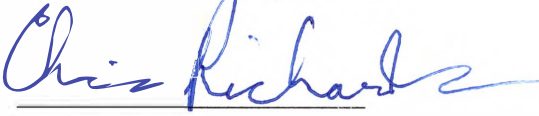


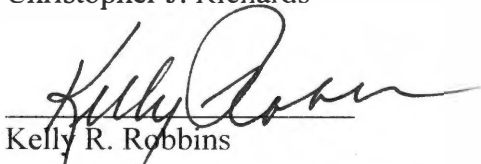
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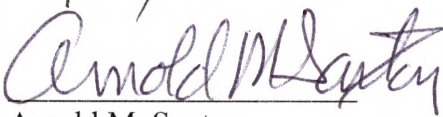
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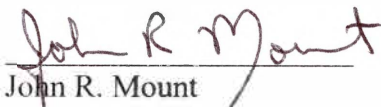

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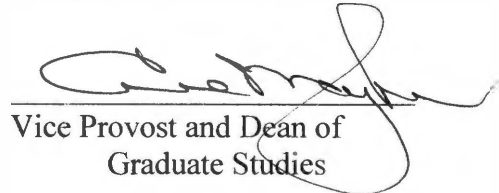

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**COMPARISON OF CHEMICAL FRACTIONS AND RUMINAL
DEGRADATION OF DRY MATTER *IN SITU* OF TALL FESCUE**

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Musa Yavuz
May 2004

AG-VET-MED.

Thesis
2004b
.438

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DEDICATION

This dissertation is dedicated to these verses “And the cattle, He has created them for you; in them there is warmth (warm clothing), and numerous benefits, and of them you eat. And wherein is beauty for you, when you bring them home in the evening, and as you lead them forth to pasture in the morning. And they carry your loads to a land that you could not reach except with great trouble to yourselves. Truly, your lord is full of Kindness, Most Merciful.” Surah 16, verse 5 to 7, and “And verily! In the cattle, there is a lesson for you. We give you to drink of that which is in their bellies, between excretions and blood, pure milk; palatable to the drinkers.” Surah 16, verse 66 of the Noble Qur’an.

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ABSTRACT

A series of laboratory experiments were conducted to test modifications of protocols used for evaluating nutritional value of feedstuffs for ruminants. The *in situ* ruminal degradation procedure was used to measure rumen disappearance of total feedstuff dry matter (DM) and a variety of feedstuff components as measured by the Cornell Net Carbohydrate Protein System (CNCPS). The *in situ* ruminal degradation procedure calls for the use of Dacron bags. We tested an alternate bag (F57) with and without post incubation washing with neutral detergent fiber (NDF) solution. Use of F57 bags permits sample analysis without removing feed residue from the bag as required when Dacron bags are used. Results of a series of analyses with seven common feedstuffs known to vary widely in both digestibility and composition (alfalfa, bermudagrass hay, tall fescue hay, corn, soybean meal, soybean hulls, and wheat straw) indicated that disappearance of total DM and a variety of CNCPS-measured components were similar for Dacron and F57 bags only after the F57 bags were washed with NDF solution following incubation. These same procedures were then employed to determine the nutritional value of hand clipped stockpiled forage. Three varieties of tall fescue were tested. It was determined that sample preparation (i.e. freeze-drying vs. oven-drying) significantly affected *in situ* rumen disappearance and values of CNCPS-measured feedstuff fractions. Regardless of the forage sample preparation method, differences in rate of dry matter disappearance were found among the tall fescue varieties tested, i.e. variety KY31E+ had lower DM disappearance rates than either Jessup E- or Jessup AR452. We applied these same methods to determine if there were any detectable differences in *in situ* rumen digestibility among masticate samples of forage from pastures composed of either pure

stands of KY31E+ or KY31E-, or a mixed stand of KY31E+ and clover. The samples from the KY31E+ pasture were found to have lower dry matter disappearance percentage than the other two forage samples, perhaps explaining, in part, the previously reported better performance of animals grazing clover containing pastures vs. KY31E+ pasture.

Key words: Tall fescue KY31, Jesup AR542, in situ, Cornell Net Carbohydrate Protein System

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PART 1
INTRODUCTION

Beef cattle producers often have to evaluate grazing practices of their herd due to economic and environmental pressure. Optimizing productivity requires balancing pasture productivity and sustainability with the animal's nutrient needs. It is important to know the nutrient value of a pasture when developing a strategy to meet animal requirements. Cattle are ruminants and digestion by ruminants needs to be evaluated differently than monogastric animals because ruminants can digest or utilize some feed sources (non-protein nitrogen and cellulose), which cannot be utilized by monogastrics. The rumen is the site of feed fermentation and resynthesis by the rumen microbes, which can decrease or increase value of feed for the host animal.

Chemical analysis can be used to evaluate forage samples. The Cornell Net Carbohydrate Protein Systems (CNCPS) are used to analyze feed protein and carbohydrate fractions. However, chemical analysis of the forage alone may not represent the true value of forage because digestibility of the forage is important. Digestibility can vary depending on whether the plants are grass or legume and the maturity of the plants. The *in situ* procedure has gained support as the standard method and is used to generate data for predicting ruminal digestion. *In situ* method has some limitations for use in laboratories because of maintaining fistulated animals, using standardized procedures, and correcting microbial attachment for protein analyses.

Tall fescue is a cool season perennial grass, which is widely adapted in the United States. Tall fescue occupies 3.5 million hectares of pasture in Tennessee (Fribourg et. al., 1991). When cattle consume endophyte (*Neotyphodium coenophialum*)

infected tall fescue, weight loss, reduced conception rates, fescue foot and bovine fat necrosis are general problems (Bacon, 1995). The problems can be costly for beef producers. Endophyte-infected tall fescue (E+) and clover mixtures have been used to reduce these problems. Tall fescue E+ and clover mixture increases production of pasture and beef cattle when compared to endophyte infected tall fescue alone. It is important to know the nutrient value of a pasture when developing a strategy to meet animal nutrient requirements. Forage production changes seasonally. Optimizing productivity requires balancing pasture productivity and sustainability with animal nutrient needs. When forage production is low, animal requirements need to be balanced with other feed sources. In those critical times, fast and accurate evaluation of the forages will improve their ability to develop a strategy to meet the animal requirements. Therefore our objectives were:

- 1) to adapt the CNCPS, *in situ* and purine procedure using different feedstuffs for the laboratories of the Department of Animal Science;
- 2) to estimate the carbohydrate and protein fractions of stockpiled tall fescue KY31 E+, Jesup E- and Jesup AR542 pastures, and to evaluate ruminal digestion; and
- 3) to estimate carbohydrate and protein fractions using masticate samples of tall fescue KY31 E+, KY31 E-, and KY31 E+ and clover mixture pastures for spring-summer for two years and to evaluate ruminal digestion.

PART 2

LITERATURE REVIEW

Nutritional Value of Feed

The sun, which provides light to the world, is the major energy source for life. Plants are capable of storing this energy as carbohydrates, proteins, lipids and vitamins (Lassiter and Edwards, 1982). Humans and animals are dependent on these nutrients. Food sources are limited in the growing population of the world. Most monogastric animals have to compete with humans for similar food sources, but ruminants can digest or utilize some feed sources (non-protein nitrogen and cellulose), which can't be utilized by monogastrics. The rumen is the site where feed is fermented and resynthesized by rumen microbes, which can decrease or increase the value of feed for the host animal. Carbohydrates and proteins are major components in ruminant rations that need to be balanced. In addition, these diets must contain enough lipids, vitamins and minerals. Nutrition value of ruminant feeds is usually determined by analyses for carbohydrate and proteins.

Carbohydrates. Carbohydrates are energy sources for most living organisms. Carbohydrates can be divided into five major fractions: hemicellulose, cellulose, pectin, starch and sugars (Van Soest, 1994). Hemicellulose and cellulose are the structural carbohydrates of plants and may be digested slowly and incompletely in the rumen. Pectin and starches are more readily digested and are the major carbon or energy sources for the microbial mass. Sugars are important carbon sources for some bacteria, but they are not a major component in ruminant feeds (Sniffen et al., 1983 and Van Soest, 1994). Carbohydrate fermentation differs in the rumen. Low quality forage can be a limiting factor for bacterial growth that decreases fermentation of forage and

incorporation of diet non protein nitrogen (NPN) to microbial protein. Balanced microbial growth requires a balanced ration of slowly and more rapidly digested carbohydrates and a balance between degradable protein and fermentable carbohydrates (Sniffen et al., 1983). Carbohydrate or energy requirement of animals can differ depending on animal, breed, age and production. Beef cattle requirements have been published in Nutrient Requirements of Beef Cattle (NRC, 1996).

Proteins. Proteins are essential for all living cells that synthesize proteins from amino acids through out the life cycle (Church and Pond, 1988). Protein requirements of animals differ depending on animal, breed, age and production. Beef cattle requirements have been published in Nutrient Requirements of Beef Cattle (NRC, 1996). The true protein value of feed can be found by measuring the amount of amino acids absorbed from the small intestine. In the ruminant, this may not be true because ruminal microbes can digest protein to non-protein nitrogen and can resynthesize non-protein nitrogen to protein (Lindberg, 1983). Ruminant protein requirements should be considered as either microbial or host animal. Understanding ruminal fermentation of feed sources can be beneficial for producers because microbial protein can supply 50% of protein requirements for beef cattle (Spicer et al., 1986).

The Cornell Net Carbohydrate Protein System (CNCPS)

The National Research Council (NRC) publishes estimates of the nutrient requirements of domestic animals. These publications are widely used by researchers and producers. Total digestible nutrient (TDN) values are used as the guidelines for these books. A TDN value does not account for solubility of nutrients in the rumen,

especially for protein fractions. These estimates have limitations when explaining ruminal mechanics and animal performance. The net carbohydrate and protein system has been included in NRC (1996) guidelines to reduce these limitations. The net carbohydrate and protein system assumes that a feedstuff contains five major components: carbohydrates, proteins, fats, ash and water (Figure 1¹). Carbohydrates and proteins are further subdivided based on chemical composition (Van Soest et al., 1991), physical characteristics, ruminal degradation, and postruminal digestibility characteristics (Figure 2)(Sniffen et al., 1992). Carbohydrates, proteins, fats, ash and water fractions can be derived from these standard laboratory chemical analyses (Official Method of Analysis, 1980): 1) dry matter (DM); 2) non-protein nitrogen (NPN, Procedure 1) (TCA-precipitable fraction, Winter et al., 1964; Shultz and Shultz, 1970; Van soest et. al.,¹ 1981; Krishnamoorthy et al., 1983; Sniffen et al., 1992); 3) crude protein (CP); 4) soluble protein (SolP, Procedure 2) (Pichard, 1977; Pichard and Van Soest, 1977); 5) Starch (Smith, 1969; Sniffen et al., 1992); 6) Neutral detergent fiber (NDF); 7) neutral detergent insoluble protein (NDIP, Procedure 3); 8) acid detergent fiber (ADF); 9) acid detergent insoluble protein (ADIP, Procedure 4); 10) acid detergent lignin (ADL); 11) ether extract (EE); and 12) ash (ASH).

Cornell protein fraction system. Ration formulation requires partitioning dietary protein into fractions of degraded intake protein (DIP) and undegraded intake protein (UIP) described by NRC (1985). The form of nitrogen has been evaluated by Pichard and Van Soest, (1977). Feed nitrogen was divided into three main fractions: NPN (A),

¹All figures and procedures are located in Appendix.

true protein (B), and unavailable insoluble protein (C) (Table 1). True protein can be fractionated into three subfractions: fraction B1 is a buffer soluble protein, fraction B2 is a buffer insoluble protein minus the protein insoluble in neutral detergent and fraction B3 is neutral detergent insoluble protein minus acid detergent soluble protein (Roe et al., 1990 and Sniffen et al., 1992). Roe et al. (1990) concluded that it is important to estimate the quantity and rate of digestion of each protein fraction, A, B1, B2, B3 and C. Commercial *Streptomyces griseus* protease has been used to estimate ruminal protein digestion for the CNCPS by Pichard and Van Soest, 1977; Roe et al., 1990; Roe et al., 1991. The natural availability of protein fraction versus time will be important to measure for ruminal degradation and passage rate, ruminal kinetics, and intake (Chalupa et al., 1991).

Based on the Cornell Net Carbohydrate Protein System chemical analyses, these equations were used to calculate protein fractions. (Nutrient Requirements of Beef Cattle. NRC,1996).

These equations were used to calculate the five protein fractions:

$$A (\% \text{ DM}) = \text{NPN} * 0.0001 * \text{SOLP} * \text{CP};$$

$$B1 (\% \text{ DM}) = \text{SOLP} * \text{CP} * 0.01 - A;$$

$$C (\% \text{ DM}) = \text{ADIP} * \text{CP} * 0.01;$$

$$B3 (\% \text{ DM}) = (\text{NDIP} - \text{ADIP}) * \text{CP} * 0.01;$$

$$B2 (\% \text{ DM}) = \text{CP} * 0.01; - A - B1 - B3 - C$$

Where:

CP (% DM) = Percentage of crude protein

NPN (% CP) = Percentage of non-protein nitrogen

SOLP (% CP) = Percentage of soluble protein in crude protein

NDIP (% DM) = Percentage of neutral detergent insoluble protein

ADIP (% CP) = Percentage of acid detergent insoluble protein in crude protein

A (% CP) = Percentage of non-protein nitrogen

B1 (% CP) = Percentage of rapidly degraded protein

B2 (% CP) = Percentage of intermediately degraded protein

B3 (% CP) = Percentage of slowly degraded protein

C (% CP) = Percentage of bound protein

Cornell carbohydrate fraction system. Total feed carbohydrates can be calculated when total CP, fat and ash are extracted from feed. The total carbohydrate can be divided into three main fractions A, B and C (Figure 1). Fraction B can be divided to two fractions B1 and B2. Sugars are fast soluble carbohydrates: fraction A. Starch and pectin are intermediate soluble carbohydrates, fraction B1. Cellulose and hemicellulose are slowly soluble and available cell wall, fraction B2. Lignin is unavailable cell wall, fraction C.

Based on the Cornel Net Carbohydrate Protein System chemical analyses, these

equations were used to calculate carbohydrate fractions. (Nutrient Requirements of Beef Cattle; NRC, 1996).

These equations are used to calculate the four carbohydrate fractions:

$$\text{CHO} = 100 - \text{CP (\% DM)} - \text{FAT (\% DM)} - \text{ASH (\% DM)}$$

$$\text{CC} = \text{NDF (\% DM)} * 0.01 * \text{LIGNIN (\% NDF)} * 2.4$$

$$\text{CB2} = \text{NDF (\% DM)} - \text{NDIP (\% CP)} * 0.01 * \text{CP (\% DM)}$$

$$\text{NFC} = \text{CHO} - \text{CB2} - \text{CC}$$

$$\text{CB1} = \text{STARCH (\% NFC)} * (\text{NFC})/100$$

$$\text{CA} = (\text{NFC} - \text{CBI})$$

Where:

CP (% DM) = Percentage of crude protein

CHO (% DM) = Percentage of carbohydrate

FAT (% DM) = Percentage of fat

ASH (% DM) = Percentage of ash

NDF (% DM) = Percentage of NDF

NDIP (% DM) = Percentage of neutral detergent insoluble protein

LIGNIN (% NDF) = Percentage of lignin in NDF

STARCH (% NFC) = Percentage of starch in the non-fiber carbohydrate

CA (% DM) = Percentage of sugar

CB1 (% DM) = Percentage of starch

CB2 (% DM) = Percentage of available fiber

CC (% DM) = Percentage of unavailable fiber

NFC (% DM) = Percentage of non-fiber carbohydrate

In Situ Method

Lindberg (1981a) studied the effects of sample size and sample structure on the disappearance of dry matter, nitrogen and cell wall from woven nylon bags measuring 170x75 mm (140x65 mm internal dimension) with a pore size of 10 microns. The rumen-cannulated cows were fed 4.0 to 6.0 kg DM of grass silage at 0800 and 1600. The bags were fastened by single stitches made with nylon and thread the seams were sealed with glue. All the samples were dried at 65° C overnight. In the first experiment, hay, barley and a concentrate mixture were milled to pass 1-mm screens. Nylon bags containing 0.9, 1.8, and 2.7 g samples were used to determine disappearances of DM, nitrogen and NDF at 2, 6, and 24 hours. DM, nitrogen and NDF disappearances were not significantly different for hay samples with nylon bag surface in relation to sample sizes (4.95, 9.89, and 14.85 mg dry sample/cm²). At 24 hours, DM and nitrogen disappearances were significantly decreased with increasing sample sizes for barley and concentrate mixture, 9.89 and 14.85 mg dry sample/cm², respectively. NDF disappearances were not significantly different between sample sizes for barley and concentrate mixture. In the second experiment, silage and concentrate mixtures were milled to pass 1 or 4.5-mm screens. Oats were milled to pass a 1-mm screen or crushed.

Nylon bags containing 1.8-g dry sample were used to determine disappearance of DM, nitrogen and NDF at 6, 24, and 48 hours. At 24 hour, DM and NDF disappearance were decreased with increased milling size from 1.0 mm to 4.5 mm for silage. Nitrogen disappearances were not significantly different for silage treatments. DM and nitrogen disappearances were significantly different for oat samples with milling size 1mm or crushed at 6, 24, and 48 hours. NDF disappearance levels were similar for the oat treatments. DM, nitrogen and NDF disappearance levels were similar for concentrate mixture with milling size 1.0 mm or 4.5 mm at 6, 24, and 48 hours.

Lindberg and Knutsson (1981) reported that all the samples were dried in a 65° C oven overnight and then milled to pass a 1 mm screen. All the experiments used 1 g of sample in nylon bags with the size of 120 mm X 60 mm outside and 100 mm X 50 mm inside (10 mg sample per cm² bag surface). In experiment one, grass hay number 1, grass hay number 110 and clover-grass hay number 17, and straw number 11 were used to determine loss of dry matter and water-soluble dry matter from the bags. Woven nylon cloths were used with apertures of 5, 6, 10, 20, and 36 µm. Each bag was placed into 800 mL plastic bottles with 500 mL water. All the bottles were shaken slowly 6 hours. Feed particles were removed from the water by centrifugation (10 min at 4500 rpm.). Loss of dry matter was not significant between nylon bags with 5, 6, and 10 µm pore; except, grass hay 110 had a significant difference between nylon bags with 6 and 10 µm pores. Loss of dry matter was highly significant between nylon bags with 10 and 20 µm pore. Loss of dry matter was slightly increased with increasing bag pore size 20 to 36 µm. In Experiment two, two cows with a 120-mm rumen cannula were fed 6.8 kg

DM grass silage, 0.9 kg DM straw and 0.9 kg DM beet pulp. Grass hay 1, clover grass hay number 17 and straw number 11 were used to determine the rate of digestion of ADF and lignin in the nylon bags with pore size 5 and 10 μm at 6, 12, 24, 48, and 72 hours. Differences in digestion of ADF were highly significant between nylon bags with 5 and 10 μm pore size during first 24 hours; however, straw ADF digestion was similar for pore size 5 and 10 μm . After 24 hours, the digestion of ADF deviated for hays and straw. The digestion of lignin was not significantly different for nylon bags with 5 and 10 μm pore size. In experiment three, the cows were fed 2.6 kg DM grass hay and 3.7 kg DM grass silage twice a day at 08.00 and 16.00. Grass hay and barley were used to determine digestion of ADF and lignin in nylon bags with 5, 6, 10, and 20 μm pore size at 6, 12, 48, and 72 hours. Digestion of ADF was significantly different between nylon bags with pore size 5 μm and 10 μm , and 10 μm and 20 μm for grass hay during 24 hours. At 48 and 72 hours, there were no significant differences between nylon bags with pore sizes 5 μm and 10 μm , and 10 μm and 20 μm for grass hay. Barley had similar ADF digestion levels for all the pore sizes and incubation times. Digestibility of lignin was similar between nylon bags with pore size 5 μm and 10 μm , and 10 μm and 20 μm . Digestibility of lignin was different between nylon bags with pore size 5 μm and 20 μm .

Lindberg and Varvikko (1982) reported that samples of soybean meal, rape seed meal, barley, hay, silage and straw were dried at 65° C overnight and then milled to pass a 1-mm screen. All the experiments used 1 g samples and nylon bags with the internal

size of 100 mm X 50 mm. Fistulated cows were adapted for each diet in three week periods before the experiment started and they were fed twice a day at 0600 and 1400 h. In Experiment one, one cow was fed 6.0 kg (dry matter) grass hay. Woven nylon bags were incubated with pore sizes of 10, 20 and 36 μm for 2, 5, 12, 24, and 48 hours. The total number of bags incubated was 3 sizes X 6 samples X 5 times X 4 reps = 360. The pore size and times did not affect straw dry matter disappearance. In all the other feeds, DM disappearances were significantly different for bags with different pore size and times, except soybean meal after two hours. Nitrogen and NDF disappearances were similar for bags with different pore sizes. Nitrogen content of straw was increased with time. In Experiment two, one cow was fed 5.5 kg legume grass silage in period one and 6.0 kg DM grass hay in period two. Woven nylon bags with pore sizes of 10 and 36 μm for 5, 12, and 24 hours were used. In all samples, DM, nitrogen and NDF disappearance rates were generally higher in nylon bags with pore size 36 μm than 10 μm , however, straw samples were similar for cows on silage diets. In all samples, DM, nitrogen and NDF disappearance rates were generally higher in silage diets than basal hay diet. Nylon bags with pore size 10 μm had more differences between basal hay diet or silage diet. Lindberg and Varvikko (1982) concluded that sample DM, nitrogen and NDF disappearance rates can be affected by pore size and diet and each feed sample has different disappearance rate when compared to other feeds (Soybean meal, barley, silage straw etc.).

Lindberg, (1981b) studied the effect of basal diet on the ruminal degradation. Basal diets were: 1) 7.0 kg ammonia-treated barley; 2) 4.9 kg ammonia treated barley

straw and 2.1 kg crushed oats; 3) 2.1 kg ammonia treated barley straw and 4.9 kg crushed oats; 4) 7.0 kg grass hay; 5) 4.9 kg grass hay and 2.1 kg crushed oats, and 6) 2.1 kg grass hay and 4.9 kg crushed oats. Fistulated cows were fed twice a day at 08.00 and 16.00. Samples of barley, oats, soybean meal, peas, fishmeal, dried sugar beet pulp, hay number 105, hay number 110, hay number 1 and straw were dried at 65° C overnight and then milled to pass a 1-mm screen. All experiments used 1 g sample and nylon bags with the size of 120 mm X 60 mm from outside and pore size 10 microns. Each experiment was conducted after a three-day adaptation period. The bags were incubated for 2, 6, 12, 24, and 48 hours. Rumen samples were collected and pH was measured every two hours before and after feeding two days. The lowest rumen pH was 6.1 and 6.4 at 4-6 hours after feeding basal diets. The dry matter disappearance of hay number 105, hay number 110, hay number 1 and dried sugar-beet pulp were decreased with decreasing roughage levels in the basal diet. The nitrogen disappearance in hay number 105 and sugarbeet pulp showed significant differences in basal diets 1, 2 and 3. Hay number 110, hay I, fishmeal and soyameal showed significant differences between basal diets 4, 5, and 6. Others were similar for the nitrogen disappearances. The cell wall disappearance (NDF) of hay number 105, hay number 110, straw and dried sugarbeet pulp were decreased with increasing oats in the basal diets. Dry matter and nitrogen, DM and NDF, nitrogen and NDF had great correlation in most of the feeds except straw which had negative correlation between DM and nitrogen, and nitrogen and NDF, but a high positive correlation between dry matter and NDF degradation.

Vanzant et al. (1998) reviewed *in situ* methods to standardize the procedure. Standardized recommendations are as follows: 1) diet should consist of 70% forage; 2) animal should be fed at maintenance level in two feedings a day; 3) polyester bag should have pore size of 10 to 60 μm and sample size: surface area 10 mg/cm^2 4) sample should be ground through a 2 mm screen; 5) at least two animals should be used; and 6) simultaneous removal should be used if washing method is standardized (four rinses, with 45 L of cold water, a 1 min agitation, and a 2 min spin per rinse cycle with washing machine).

Wilkerson et al. (1995) studied *in situ* methods to evaluate the variation between laboratories and developed common procedures to standardize the method. The recommendations are: 1) samples are ground through a 2 mm screen in a Wiley mill; 2) weighed 5 g of sample into polyester bags (10 cm x 20 cm, average pore size 53 + 10 μm); 3) bags wrapped around a #8 rubber stopper and secured with two #18 rubber bands; 4) twenty four sample bags were placed in a mesh bag (36 cm x 42 cm); 5) Sample bags soaked in water (39° C) for 20 min; 6) mesh bags were placed into ventral sac for 16 hours just before feeding; 7) mesh bags were rinsed until the rinse water was clear; (8) polyester bags were rinsed before removing #8 rubber stopper in the lab, and (9) the rinsed bags were oven dried and stored until further analyses.

Estimation of Microbial Attachment in Nylon Bag Residues

Ruminal digested feed samples contain protein originating from three different sources, microbial protein synthesized in the rumen and feed protein and endogenous

protein (Siddons et al., 1982). Microbial attachment needs to be corrected for in situ method. If rumen microbial protein is not correctly estimated and corrected in the digested samples, microbial protein shows as an undigested protein in the feed. Estimation of protein digestion can change significantly. Purine and neutral detergent insoluble N methods were developed to correct microbial protein attachment. Microbial contribution to digested feed; not only nitrogen, but also DM, fat, carbohydrate and ash should be corrected.

Purine method. Purines are adenine, guanine, hypoxanthine and xanthine (Lehninger et al., 1993). Hypoxanthine and xanthine are important intermediates in synthesis and degradation of adenine and guanine. Adenine and guanine are major compounds for ribonucleic acids. Ribonucleic acid is present in bacteria, protozoa and feed. Marshak and Vogel (1951) reported a method that determines microbial purines and pyrimidines in biological materials in Procedure 6. The method combines hydrolysis of nucleotide, precipitation of free purines and pyrimidines, and measured spectrophotometrically. Microbial protein was estimated by the ratio of purines to nitrogen of isolated values, which is accepted as a constant for given conditions (Klopfenstein et al., 2001). They suggested that purines gave more accurate results for estimating microbial population. Over the years, purine procedure was improved by Zinn and Owens (1986), Obispo and Dehority (1999) and Makkar and Becker (1999). The method can be used to estimate net microbial protein synthesis in the rumen. The ratio of purines to nitrogen of isolated bacteria can be estimated to provide total protein

value of the bacterial protein. However, purine procedure requires several steps that increase variation.

Collection of rumen microbes and straining bacteria. A microbial sample is required for the purine method to estimate microbial population. Ruminal microbes need to be collected and separated from other particles. There are different methods used to collect and purify ruminal microbes. Obispo and Dehority (1999) procedure was: 1) collect rumen contents; 2) strain through four layers of cheesecloth to obtain 600 mL of ruminal fluid; 3) centrifuge at 200 x g for 10 min (to separate protozoa and particles); 4) strain supernatant with four layers of cheesecloth; 5) collected in a 1,000 mL flask; 6) gas with CO₂ for at least 15 min; 7) place in a 50 mL Teflon centrifuge tube; 8) centrifuge at 12,000 x g for 30 min; 9) remove supernatant with aspiration; 10) wash three times with 0.9% (wt/vol) saline; and 11) dry samples.

Makkar and Becker (1999) reported a similar procedure: 1) collect 1 L rumen contents 2 hours postfeeding ; 2) strain through two layer of muslin cloth; 3) keep in 1 L cylinder at 4° C for 30 min; 4) gas with CO₂; 5) place a 35 mL fluid into centrifuge tube; 6) centrifuge at 20,000 x g for 20 min at 4° C; 7) wash three times with distilled water; and 8) lyophilize and pool all the samples.

Coblentz et al. (1999) reported a different procedure: 1) collect rumen content 4 hour postfeeding; 2) mix 2 kg ruminal content with 2 L saline solution (9 g of NaCl/L); 3) strain through two layers of cheesecloth; 4) centrifuge at 500 x g for 20 min to

separate protozoa and particles; 5) centrifuge twice at 30,000 x g for 20 min; 6) wash with saline solution; and 7) freeze dry in Procedure 5.

In situ neutral detergent insoluble nitrogen method. Mass et al. (1999) studied the use of neutral detergent insoluble N for estimating microbial correction factor for the *in situ* method. Neutral detergent insoluble N was determined by omitting sodium sulfite from neutral detergent fiber solution described by Van Soest et al. (1991), because disulfide bridges can not be broken biologically (Licitra et al., 1996). The purine method was also used to estimate microbial attachment on the samples. Results showed that purine and neutral detergent insoluble N methods were not different for estimating microbial attachment. Mass et al. (1999) stated that neutral detergent insoluble N method is more accurate and less complex than the purine method. Klopfenstein et al. (2001) reported that eight forages were incubated 4 to 24 h in *in situ*. *In situ* digested samples CP, NDIP and purine were determined. Undegraded intake protein (UIP) was calculated from fractional rates of digestion and passage. Undegraded intake protein estimates were higher without microbial correction. *In situ* digested samples estimates of UIP were lower and similar for purine and NDF corrected samples.

In Vitro Enzymatic Methods

Different methods are currently used to estimate ruminal digestion of feedstuffs. *In situ* procedure was standardized and used to generate data for predicting ruminal digestion. It has some limitations for use commercially; such as the cost of maintaining fistulated animals, labor involved, concern over the use of surgically modified animals,

standardizing procedures, correcting microbial attachment for protein analyses, and variation between laboratories (Jones and Theodorou 2000; Roe and Sniffen, 1990). Due to these problems, researchers focused on replacing ruminal microbes with commercially available enzymes. Rumen microbes produce different enzymes to digest different chemical components of the feed sources. It is not easy to replace ruminal microbe enzyme activity with commercial enzymes for a variety of feed sources. Researchers have focused on cellulose or protein digestion to avoid complexity and increase accuracy of the results. There are several methods developed to estimate cellulose and protein digestion.

Enzyme techniques for estimating cellulose digestibility. Cellulase technique has been used to estimate dry matter digestion. Several procedures have been developed and evaluated. The procedure consists of two steps: 1) removal of cell contents with pretreatment; 2) followed by cell wall digestion with cellulase.

Jones and Hayward (1975) used acid pepsin pretreatment (AP). They compared four different cellulase activities with and without pepsin pretreatment. The *T. viride* cellulase was most active for solubilising 70% of herbage and cellulose in 24 h. AP pretreatment improved digestion of cellulose and correlation between *in vitro* digestibilities.

McLeod and Minsen (1978) studied the pepsin cellulase technique for estimating the dry matter digestibility of legumes and grass samples. Grinder screen size (0.4 mm or 1.0 mm), incubation temperature (39° C or 50° C) and sample size (0.2 g or 0.5 g) effects were examined. *Trichoderma viride* was used to extract Onozuka SS

(P-1500) cellulase (Maruzen chemicals, Co. Ltd., PO Box Higashi No.13, Osaka, Japan). They concluded 1mm screen size and 0.5 g sample gave better results, and increasing temperatures changed slightly but did not affect outcome significantly. This study resulted in improving cellulose technique.

Aufrere and Michalet-Doreau (1988) studied different methods for predicting digestibility of feeds. Two enzymatic methods were examined: pre treatment with pepsin (pepsin Merck no. 7190; 1:10000) in hydrochloric acid (0.2% pepsin in N HCl (Method A) or 0.1 N HCl (method B) in a water bath at 40° C for 24 h; starch hydrolyses in a water bath for 30 min at 80° C; and cellulase hydrolysis in a water bath at 40° C for 24 h followed by filtration and rinsing. Method A overestimates the digestibility of feed when rich in hemicellulose. Hemicellulose pentosans may affect digestion, because pentosans are considered poorly digestible in the rumen, but are hydrolyzed by hydrochloric acid. Method B gave predictions that are more accurate.

Dowman and Collins (1982) proposed using neutral detergent (ND) as a pretreatment for cellulose digestion. Procedure is as follows: 1) prepare 0.5 g of sample ground through 1 mm screen; 2) digest 1 h with 50 mL of neutral detergent solutions, 0.25 g of sodium sulfite plus 1 mL antifoam; 3) filter through a porosity 1 sintered glass crucible and wash with hot water; 4) transfer residue to polyethylene bottle and incubate with 30 mL of buffered cellulase (20 g liter⁻¹ buffer) for 24 h at 40° C; 5) filter through a porosity 2 sintered glass crucible and wash with hot water and acetone. *Aspergillus niger* and *Trichoderma viride* cellulases were compared. *Trichoderma viride* cellulase showed better results than *Aspergillus niger* cellulase. Both AP and ND pretreatment

have shown similar results. They concluded that ND pretreatment should be further investigated. McLeod and Minsen (1982) reported that ND pretreatment removed more DM than AP pretreatment (37% and 30%) because of removing more ash from the feed. ND cellulase removed more total DM than AP cellulase, 63% and 55%, respectively; however, the residual standard deviation was increased with ND cellulase.

Pace et al., (1984) compared known *in vivo* results with *in vitro*, AP cellulase and hot acid (HA) cellulase methods. There were significantly different results between the runs for *in vitro* method, but not with enzymatic methods. *In vitro* and AP cellulase method showed lower digestibility than *in vivo* results and HA cellulase method constantly showed higher digestibility than *in vivo* method.

Coelho et al., (1988) compared *in vivo* and enzymatic methods using alfalfa, mature ryegrass, common bermudagrass, Alicia bermudagrass, Pensacola bahiagrass, and 50:50 mixture of alfalfa and mature ryegrass hays. The enzymes used were: cellulase (*Aspergillus niger*) Type IV, No. C-7377, Sigma, St. Louis, MO; cellulase (*Trichoderma viride*) Type IV, No. C-4137, Sigma; cellulase Onozuka preparation (*Trichoderma viride*) Kanematsu-Gosho (U.S.A.), Ltd, CA; hemicellulase (*Aspergillus niger*) No. H-2125, Sigma; and pepsin (1:10000) from Nutritional Biochemical Corp., Cleveland, OH. AP cellulase and ND cellulase preparations were used with enzyme treatments: cellulase Onozuka preparation; cellulase *T. Viride* Type IV + hemicellulase *A. niger*; cellulase *A. niger* Type IV + Hemicellulase *A. niger*. The Onozuka cellulase preparation had greater cellulase activity. Cellulase *T. Viride* Type IV and hemicellulase *A. niger* with AP pretreatment had highest correlation coefficient ($r^2 = 0.88$). It was

close to the two-stage ruminal *in vitro* procedure. AP and ND pretreatment were not different. ADF was significantly correlated with DMD ($r^2 = 0.71$).

Givens et al. (1993) compared *in vivo*, *in vitro*, AP and ND cellulase techniques to estimate digestibility and energy values of grass silage conserved in big bales. ND cellulase method was highly correlated with *in vivo* method ($R^2 = 84\%$) and followed by AP cellulase and *in vitro* methods, $R^2 = 75.6\%$ and $R^2 = 74.5\%$, respectively.

Enzyme techniques for estimating protein digestibility. Pichard and Van Soest (1977) proposed using *in vitro* enzymatic (*Streptomyces griseus*) method to estimate ruminal protein digestion. In the experiment, rate of digestion was estimated for protein fractions for alfalfa, grass silage, corn silage and soybean meal. *In vitro* procedure was as follow: 1) air dry ground sample which contains 50 mg of available protein; 2) soak in rumen buffer for two hours; 3) add 1 mL of 1% sodium azide and 0.5 mL triton X-100 (1% solution); 4) incubate at 39.5° C; 5) after presoaking add 10 mL of enzyme solution (250 mg/100 mL); 6) samples swirled; 7) reaction stopped adding 2 mL 80% tertiary butyl alcohol; 8) samples filtered and washed (Whatman No. 54 paper); and 9) nitrogen determined in the digested samples for different fractions. Digestion kinetics were estimated.

Krisnamoorthy et al (1983) evaluated 12 grain mixtures for UIP using *in vitro* enzymatic, *in situ* and *in vivo* methods. *In vitro* enzymatic procedure was as follow: 1) determine protease activity unit (one activity unit of enzyme is able to hydrolyze casein to produce color equivalent to 1.0 μmol {181 μg } tyrosine/min at pH 7.5 and 37° C); 2)

an air dry ground (1mm) 0.5 g sample; 3) soak in borate-phosphate buffer { $\text{NaH}_2\text{PO}_4\text{H}_2\text{O}$ 7.6 g/l, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ 13.17 g/l}; 4) add 1 mL of 1% sodium azide; 5) incubate at 39.5° C for 0.5, 1, 2, 4, 6, 8, 10, 18, 24, 28, 32, and 48 h; 6) after presoaking add 33 units/mL; 10 mL of enzyme solution; 7) samples filtered (Whatman No. 54 paper) and washed with 400 mL distilled water; and 8) nitrogen determined in the undigested samples. The *in vitro* and *in vivo* results were compared for the 12-grain mixtures, ground maize and soybean meal. *In vitro* estimates and *in situ* methods were significantly correlated ($P < 0.01$; $r^2 = 0.61$) after 18h. Krisnamoorthy et al (1983) concluded that the *in vitro* enzyme method could be used to closely estimate to *in vivo* results.

Janicki and Stallings (1988) compared *in vitro* and *in situ* methods for estimating ruminal digestibility of alfalfa silage, alfalfa hays, corn silage and orchardgrass at 2, 12, 24, and 48 h. *In vitro* procedure was similar to Krisnamoorthy et al. (1983). Hay and silage digestion was different for different protein fractions due to different protein fraction concentrations. For silages, soluble CP correlation was $r^2 = 0.58$ between *in vitro* and *in situ* methods. For hays, NDIP correlation was $r^2 = -0.83$ between *in vitro* and *in situ* methods.

Roe et al. (1991) used the same *in vitro* procedure (Krisnamoorthy et al., 1983) with different enzymatic activity 0.33 units/mL; 10 mL. Four different soybeans and grains were used in the research. Correlation between *in vitro* and *in situ* methods were very high ($r^2 = -0.58$ to 96) when statistically analyzed for samples separately, but correlation between the two methods were lower for different incubation time when

data were pooled for all the samples; however, *in situ* 8 h incubation and *in vitro* 4 h incubation were highly correlated ($r^2 = 0.73$). Roe et al. (1991) reported that the difference between the sample digestions was due to different solubility of the protein because of the process or inhibitory effects of antitrypsin in soybean.

Feed carbohydrate was a concern using the *in vitro* procedure, because protein associated with carbohydrates might affect proteolysis. Abdulgadir et al. (1997) studied pretreatment with two carbohydrases on protease digestion. Two carbohydrases were cellulase (*Penicillium funiculosum*, C-0901, Sigma chemical, St. Louis, MO) or driselase (*Basidiomycetes*, D-9515, Sigma Chemical). Alfalfa hay and prairie hay were used in the experiment. Carbohydrase procedure was as follows: 1) determine carbohydrase and protease activities; 2) use 800 mg of carbohydrase per gram of hay; 3) dissolve enzyme in 20 mL of 20 mM sodium acetate buffer and, adjust to pH 4.8 using 1 M NaOH; 4) weight samples containing 15 mg of N; 5) add 20 mL carbohydrase solution; 6) add sodium azide (1mL; 1%, wt/vol) solution; 7) incubate 2 or 48 h at 39°C; and 8) at the end of the incubation, follow the *in vitro* procedure or chemical analyses. UIP results were very close to *in vivo* estimates. The 48 and 2 h preincubation time showed better results among the forages for cellulase and driselase, respectively. Two hour preincubation with cellulase caused filtration problems and increased UIP.

Coblentz et al. (1999) compared *in situ* and *in vitro* enzymatic methods for twenty forages. The *in vitro* enzymatic procedure was different from the Krisnamoorthy et al. (1983) procedure. *In vitro* enzymatic procedure was as follow: 1) determine protease activity unit (one activity unit of enzyme is able to hydrolyze casein to produce

color equivalent to 1.0 μmol {181 μg } tyrosine/min at pH 7.5 and 37° C); 2) an air dried ground (1mm) sample that contains 15 mg N; 3) soaked 1 h with 40 mL of borate-phosphate buffer { $\text{NaH}_2\text{PO}_4\text{H}_2\text{O}$ 7.6 g/l, $\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$ 13.17 g/l}; 4) add 1 mL of 1% sodium azide before soaking; 5) after presoaking add 6.6 or 0.066 units/mL; 10 mL of enzyme solution; 6) incubate at 39.5° C for 0.5, 1, 2, 4, 6, 8, 10, 18, 24, 28, 32, and 48 h; 7) swirl a couple of times in incubation flask; 8) prior to filtering, place all incubation flasks on ice to stop proteolytic activity; 9) samples filtered (Whatman No. 54 paper) and washed with 400 mL distilled water; and 10) nitrogen determined in the undigested samples. Enzyme activity of 6.6 units/mL samples were incubated for 1, 2, 3, 4, and 5 h. Enzyme activity of 0.066 units/mL samples were incubated for 24, 30, 36, 42, 48, and 54 h. Enzyme activity of 6.6 units/mL sample estimates were significantly ($P < 0.05$) correlated ($r^2 = 0.90$ to 0.93). Enzyme activity 0.066 units/mL sample estimates were significantly ($P < 0.05$) correlated ($r^2 = 0.81$ to 0.90). He concluded that *in vitro* enzyme method with enzyme activity 6.6 units/mL could be used effectively after two-hour incubation time.

Tall Fescue

Tall fescue (*Festuca arundinacea* Schreb.) is a perennial cool season grass that originated from Europe (Dibb and Darst, 1991). Tall fescue can be adapted to a wide range of climates. It can tolerate soil acidity, poor drainage, drought and is pest resistant (Bacon, 1995; Joost, 1995). It can be grazed September to December and March to July (Ball, 1996). Farmers rapidly accepted the release of the Kentucky-31 after the 1940s

(Fribourg et al., 1991). Tall fescue is grown on more than 35 million hectare in the United States (Dibb and Darst, 1991).

Tall fescue toxicosis. Fungal endophyte (*Neotyphodium coenophialum*) was found to have a symbiotic relationship with tall fescue in the 1970s (Bacon et al., 1986; Bacon, 1995). The endophyte can be found mostly in the roots, seed embryos or leaf blades (Fribourg et al., 1991). Ergovaline, erginine, ergot alkaloids, the pyrrolizidine alkaloids and unique pyrolopyrazine alkaloid peramine have been extracted from endophyte infected tall fescue (Porter, 1995).

Tall fescue toxicosis can be characterized by reduced feed intake, poor weight gain, elevated body temperature, reduced conception rates, long hair coats and nervousness (Bacon et al., 1986; Fribourg et al., 1991; Dibb and Darst, 1991). In wintertime, fescue foot can occur on animals when grazing endophyte infected tall fescue and is described as a tenderness of legs and loss of hoofs and tail (Fribourg et al., 1991; Dibb and Darst, 1991). Tall fescue pastures fertilized with poultry litter or high nitrogen can cause hard fat in adipose tissue and abdominal cavity, which is called bovine fat necrosis (Dibb and Darst, 1991; Fribourg et al., 1991). The most common problem is summer tall fescue toxicosis with estimated economic losses over \$600 million in the United States, and over \$85 million in Tennessee (Fribourg et al., 1991).

Effects of endophyte toxins on rumen microbes and host animals. Researchers have investigated the effects of endophyte toxins on ruminal microbes. *In vitro* studies by Bush et al. (1970) showed that perloline inhibited cellulose digestion regardless of the cellulose source (wood cellulose or tall fescue). Perloline decreased propionic acid

and increased butyric acid proportion by decreasing growth of cellulose degrading microbes *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and protozoa (Bush et al., 1972). Ergovaline (1.5 - 3.0 ppm) decreased intake and cellulose digestion, and increased rectal temperature with increasing environmental temperature (27-34° C) in sheep (Hannah et al., 1990).

Westendorf et al. (1991) studied the effect of alkaloids (pyrrolizidine alkaloid N-formyl and N-acetyl loline) in endophyte infected tall fescue on ruminal metabolism. In the first experiment, a combination of N-formyl and N-acetyl loline was incubated with ruminal fluid for 0, 24, or 48 hours. The N-formyl and N-acetyl loline were converted to loline. In the second experiment, endophyte infected feed was fed to abomasally cannulated sheep. Endophyte infected feed reduced dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein (CP) digestibility. Pyrrolizidine alkaloids metabolized to loline in the rumen and loline (100%) was absorbed. Ergot alkaloids were also absorbed and 5% of ergots were recovered from feces.

Jackson et al. (1984) reported that a tall fescue seed diet lowered feed and water intake and increased body temperature of steers when compared with an orchardgrass seed diet at room temperature 31° C.

McMurphy et al. (1990) studied steer performance on tall fescue pasture. Angus (AN), BrahmanXAngus (BA) and Simmental X BA (SBA) steers were grazed on three different pastures for three years. Pastures were: 1) high endophyte (76%) infected tall fescue pasture (HE); 2) high endophyte (74%) infected tall fescue pasture and clover

(HE + Clo); and 3) low endophyte (7%) infected tall fescue pasture (LE). All breeds had lower gain on HE pasture when compared with the other two pastures. Highest differences were observed between HE, HE + Clo and LE pastures during periods from March to May, low to high, respectively. The BA steers gained faster than AN and SBA steers in all the pastures, except that SBA steers gained more than BA steers on the HE pasture. The BA steer body temperature did not change with pasture treatment. The Angus and SBA steers' body temperatures were high when they were on HE pasture. Lusby et al. (1990) studied the effects of fescue endophyte on finishing performance of steers. Steers were removed from those three pastures (HE, LE and HE + Clo) and switched to bermudagrass-ryegrass pastures and then moved to holding facilities for 6 day, after which animals shipped 450 miles to a feedyard. Steers were fed a 90% concentrate-finishing ration in feedyard. Steers were slaughtered at 113 or 117 days. At the end of the study, HE + Clo pasture steers had heavier body weight than LE and HE steers, 596, 576, and 561 kg, respectively. The HE steers had lower quality grades, smaller ribeye areas and fewer choice carcasses than HE + Clo and LE. Steers. Body temperatures were still higher in the HE steers during 6-d periods than HE + Clo and LE (103.4, 103.1, and 102.8 F) and body temperatures were similar for all steers in feedyard. HE + Clo pasture could be used as a better management system.

Coffey et al. (1990) reported steer performance on different types of fescue pastures. Pastures were: 1) KY31 tall fescue infected with 65% endophyte (IF); 2) IF mixed with ladino clover (IFL); and 3) endophyte-free Missouri 96 tall fescue (MO). Steers were fed normal high concentrate diet after grazing period and then slaughtered.

Tall fescue with clover pasture steers had higher daily gain than IF or MO steers in 1987 (0.703 kg, 0.440 kg, and 0.485 kg, respectively); however, MO steers had higher pasture daily gain than IF or IFL steers in 1988 (0.613, 0.495, and 0.427 kg, respectively). This can be explained by the higher rainfall increasing clover production in 1987 and the dry season decreasing clover and increasing endophyte in 1988. Tall Fescue steers had lower feed-to-gain ratio than IFL or MO steers. IFL steers had better carcass quality than MO and IF steers.

Chestnut et al. (1991) studied steer performance on different tall fescue pastures. A factorial 2 by 2 by 2 arrangement of treatment was used with the following pasture treatments: 1) endophyte infected tall fescue (E+) or endophyte free tall fescue (E-); 2) N-fertilizer or clover; and 3) Bermuda grass with or without. Endophyte infected tall fescue pastured steers had lower average daily gain, forage dry matter intake, and higher rectal temperatures and hair coat scores than E- pasture steers. Bermudagrass and clover pastures both showed improved performances of steers on E+ and E- tall fescue pastures.

Forage Sample Collection

Vans Dyne and Torell reported (1964) that esophageal fistula has been used in different animals. Both esophageal fistula and rumen fistula have been used in ruminants to collect masticated samples. Rumen collection is made by: 1) completely emptying the animal rumen; 2) allowing the animal to graze; 3) taking a forage sample from the rumen; and 4) placing the original rumen contents back in the animal. The rumen evacuation technique is not easy for repeated sampling under range condition

because they are time consuming and difficult during cold weather. Samples can be taken one or two times a day. Sampling frequency varies widely according to the purpose of the investigation. Salivary contamination does not invalidate the samples as micro digestion samples and may increase nitrogen and ash content of rumen samples. Salivary contamination can be corrected by analyzing saliva and hand collecting samples; however, this method has been criticized because hand sampling may not represent the fistula sample composition of saliva secretions and may vary by time. Overall the fistula samples represent the best estimate available to evaluate the intake of the grazing animal (Vans Dyne and Torell, 1964).

Olsen reported (1991) that five Hereford steers were fistulated in the esophagus and rumen. Grazing and stall trials were used to evaluate three collection techniques: 1) rumen collection after evacuation (RC); 2) esophageal collection with the rumen evacuated (ECRE); and 3) esophageal collection with the rumen full (ECRF). In the grazing trial, five animals were allowed to stay on the same pasture. In the first two techniques, rumens were emptied of all of the solid and liquid material in early morning and the animals were allowed to graze for 30 to 40 minutes. They were returned to their pen and solid rumen samples were taken immediately without scraping the rumen wall or folds. All the rumen samples were placed in individual tubs and then sub sampled into plastic bags. All samples were chilled on ice. Original rumen contents were returned to the steers. The samples were frozen (-20° C) until processed. The samples were freeze-dried and were ground with a cyclone mill to pass a 1 mm screen. The samples were analyzed for nitrogen, NDF, ADF, ADL, hemicellulose, and cellulose.

Collection technique did not significantly affect any dependent variable except acid detergent lignin. The ECRF was lower than ECRE sample, 6.6% and 5.5% of dry matter, respectively. In the second experiment, animals were placed in to individual stalls and fed prairie hay in small bales. In this study, the hay was collected normally and stored at room temperature until further processing. The collection of samples and analysis were the same as the first trial. Dry matter was similar for RC and ECRF and they both were lower than the normal hay sample. Normal hay sample and ECRF organic matter nitrogen levels were similar. However, RC organic matter nitrogen level was 0.2-3% higher than the other samples. Acid detergent fiber level was significantly different ($P < 0.05$) for all the samples, normal hay sample (5%), ECRF (6%) and RC (7%). Hemicellulose level was similar for both ECRF (35%) and RC (35%), but normal hay had higher hemicellulose (36%).

Holechek and Vavra (1983) studied the botanical composition of diets selected from forest and grassland with esophageal fistulated cattle. Fistulated animals should be kept on the same pasture. This will help them to adapt to the vegetation and new environment. Grazing time should be 30 minutes for fistula sampling. Increased grazing time gives animals time to graze different areas in the pasture thereby affecting sample variety and amount. Animals may select a different grazing place in the same pasture depending on physical condition. Animals grazed open grassland in the morning and shaded places in the afternoon during the summer season. Consistency of sampling times should be important for evaluating differences between pastures.

Buritt et al. (1988) studied the effects of drying methods on the nutritive value of esophageal fistula forage samples collected from goats and sheep in wet and dry seasons. Air, oven (40° C) and freeze dryings were used in this study. Freeze-drying samples were frozen at -4° C and stored for 1 to 3 months prior to freeze-drying. Air and oven dryings samples were normally dried within 24 hours. Freeze-dried samples contained less cellulose and lignin than air or oven dried samples. This difference was higher in the wet season than in the dry season. *In vitro* organic matter digestibility (IVDMD) was lower in air and oven dried samples. Forage type may influence these differences. These results indicated that freeze-drying is the most reliable means of sample drying.

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Appendix

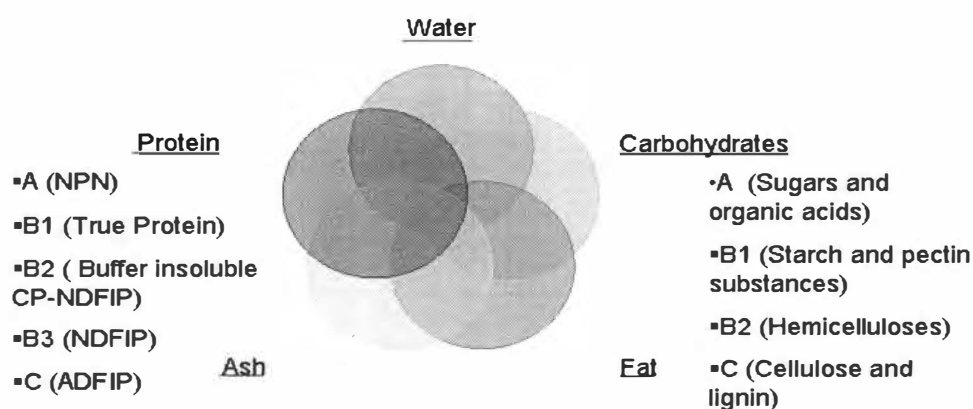


Figure 1. Major fractions and sub-fractions of feedstuff according to the Cornell Net Carbohydrate Protein System and interaction between the fractions (Sniffen et al., 1992).

Table 1. Carbohydrate and protein fractions and relative digestion rates.

Carbohydrate fractions		Protein fractions		Relative digestion
A	Sugars and organic acids	A	NPN	Fast
		B1	True protein	
B1	Starch, pectin substances	B2	Buffer insoluble CP- NDF Insoluble CP	Medium
B2	Hemi cellulose (NDF)	B3	NDF insoluble CP	Slow
C	Cellulose and lignin (ADF)	C	ADF insoluble CP	Indigestible

Source: Sniffen et al., 1992.

Determination of NPN using Trichloroacetic acid (TCA)

1.0 Reagents (use deionized H₂O)

1.0.1 Trichloroacetic acid 10% w/v in water. Keep refrigerated

1.1 Procedure

1.1.1 Weigh 0.5 g ground dry sample into a 125 mL Erlenmeyer flask.

1.1.2 Add 50 mL of distilled water. Allow to stand 30 min.

1.1.3 Add 10 mL 10% trichloroacetic acid. Let stand 20-30 min.

1.1.4 Filter on Whatman #54 or 541 paper by gravity.

1.1.5 Wash twice with trichloroacetic acid solution.

1.1.6 Use nitrogen analyzer to determine nitrogen contents or Kjeldahl procedure.

1.1.7 Calculate NPN by subtracting residual nitrogen from total nitrogen.

Value of NPN may be expressed as crude protein ($N \times 6.25$) or percent of total feed nitrogen.

(Modified from Licitra et al., 1996).

Soluble protein (buffer soluble nitrogen)

1.0 Reagents (use deionized H₂O)

1.0.1 Borate-phosphate buffer, pH 6.7-6.8 including

1.0.1.1 Monosodium phosphate (NaH₂PO₄·H₂O) 12.20g l⁻¹

1.0.1.2 Sodium tetraborate (Na₂B₄O₇·10H₂O) 8.91g l⁻¹

1.0.1.3 Tertiary butyl alcohol 100 mL l⁻¹

1.0.2 Sodium azide 10% solution freshly prepared.

1.1 Procedure

1.1.1 Weight 0.5 g ground dry sample into a 125 mL Erlenmeyer flask.

1.1.2 Add 50 mL borate-phosphate buffer.

1.1.3 Add 1 mL of sodium azide solution.

1.1.4 Let stand at room temperature for 3 h.

1.1.5 Filter through Whatman #54 or # 541 filter paper using mild vacuum.

250 mL cold distilled water.

1.1.6 Use nitrogen analyzer to determine buffer insoluble nitrogen contents or transfer samples into a Kjeldahl flask and determine nitrogen on residue according to standard Kjeldahl procedure. (This gives the insoluble protein)

1.2 Calculations

True soluble protein can be estimated with these formulas:

1.2.1 Soluble protein = Crude protein - Insoluble protein

1.2.2 True soluble protein = Soluble protein - TCA protein

(Modified from Licitra et al., 1996)

Neutral detergent insoluble nitrogen (NDIN)

1.0 Procedure

1.0.1 Follow the NDF procedure with Ankom fiber analyzer using 0.5 g sample. In the NDF procedure sodium sulfite is omitted. Pretreatment of urea-amylase should not be used in the procedure. Amylase can be added in the case of starchy foods.

1.0.2 Dry at 100° C for 8 h or overnight and weight if determining NDF.

1.0.3 Use nitrogen analyzer to determine NDF samples nitrogen contents or transfer samples into a Kjeldahl flask and determine nitrogen on residue according to standard Kjeldahl procedure.

1.0.4 Express NDIN as percent of total nitrogen or as $N \times 6.25$

(Modified from Licitra et al., 1996).

Acid detergent insoluble nitrogen (ADIN)

1.0 Procedure

1.0.1 Follow the ADF procedure with Ankom fiber analyzer using 0.5 g sample.

1.0.2 Dry at 100° C for 8 h or overnight and hot weight if determining ADF.

1.0.3 Use nitrogen analyzer to determine ADF samples nitrogen contents or transfer paper residue into a Kjeldahl flask and determine nitrogen on residue according to standard Kjeldahl procedure.

1.0.4 Express ADIN as percent of total nitrogen or as $N \times 6.25$

(Modified from Licitra et al., 1996).

Bacterial isolation from rumen contents

1.0 Reagents (use deionized H₂O)

1.0.1 Saline solution 9 g NaCl/L

1.1 Procedure:

- 1.1.1 Collect rumen content after 4 hour postfeeding
- 1.1.2 Thoroughly mix contents by hand and collect 2-4 kg in a bucket from all areas of the rumen
- 1.1.3 Mix 2 kg ruminal content with 2 L ice cold saline solution to kill bacteria
- 1.1.4 Blend 1 min at high speed in blender
- 1.1.5 Strain through two layers of cheesecloth
- 1.1.6 Place strained rumen fluid in 250 mL bottles
- 1.1.7 Centrifuge at 500 x g for 20 min (to separate protozoa and feed particle)
- 1.1.8 Carefully decant supernatant into 250 mL bottle
- 1.1.9 Centrifuge at 30,000 x g for 20 min
- 1.1.10 Carefully decant and discard supernatant fluid
- 1.1.11 Using rubber policeman and water, resuspend and pool bacterial pellets
- 1.1.12 Repeat centrifuge at 30,000 x g for 20 min and wash (2X)
- 1.1.13 Carefully decant and discard supernatant fluid
- 1.1.14 Freeze dry and grind for analysis

(Modified from Coblenz et al., 1999).

Purine contents of ruminal microbes and *in situ* residues

1.0 Reagents (use deionized H₂O)

1.1.1 0.2 M NH₄H₂PO₄

23 g NH₄H₂PO₄ to 1 liter in a volumetric flask with purified water

1.1.2 0.0285M NH₄H₂PO₄

143 mL of 2M NH₄H₂PO₄ to 1 liter in a volumetric flask with purified water

1.1.3 .4M AgNO₃

6.90 g AgNO₃ q.s. to 100 mL in a volumetric flask with distilled water

1.1.4 0.4M AgNO₃

6.90 g AgNO₃ q.s. to 100 mL in a volumetric flask with distilled water

Stirring with stir bar and heat help dissolve AgNO₃. Cover the storage container with aluminum foil or use dark container and keep in dark to prevent AgNO₃ precipitation.

1.1.5 0.5 N HCl

Fill a 1 liter volumetric flask 2/3 full with purified water

Add 41.85 mL HCl q.s. with purified water

1.1.6 2 M HClO₄ (Perchloric acid)

Fill a 2 liter volumetric flask 2/3 full with purified water

Slowly add 342 mL of 70% HClO₄ q.s. with purified water

1.1.7 12.5% HClO₄ in .0285 M NH₄H₂PO₄

Fill a 50 mL volumetric ½ full with .0285 M $\text{NH}_4\text{H}_2\text{PO}_4$

Slowly add 8.87 mL of 70% HClO_4 0285 M $\text{NH}_4\text{H}_2\text{PO}_4$

q.s. to volume

1.1.8 Precipitation solution

In a 100 mL volumetric combine:

5.0 mL 12.5% M HClO_4 in 0285 M $\text{NH}_4\text{H}_2\text{PO}_4$

5.0 mL of .4M AgNO_3

90.0 mL of .2M $\text{NH}_4\text{H}_2\text{PO}_4$

1.1 Procedure:

1.1.1 Standard preparation and hydrolyzing Procedure:

Weigh 0, .100, .200 and .300 g of pure Yeast RNA (Sigma) into 25-mL screw cap culture tubes and proceed through with steps 1.1.2-1.1.10 of the Hydrolyzing procedure.

The supernatant should contain the following concentrations of RNA.

<u>Stock</u>	<u>Pure RNA (g)</u>	<u>mg RNA/mL filtrate</u>
A	0.0	0.00
B	0.10	5.00
C	0.20	10.00
D	0.30	15.00

1.1.2 Weigh .4 g *in situ* residue (analyze only *in situ* samples that were incubated- i.e. do not analyze 0 h or original forage samples) or 0.2 g bacteria into 25-mL screw cap culture tube.

1.1.3 Perform this and the next step in a perchloric acid-rated hood. Add 2.5 mL 2.0 M HClO_4 , tightly cap tube (use caps with good

seals) and incubate in 90 to 95°C water bath for 1 h (note: make sure water bath level is above solution level in test tubes). After 15 min of incubation, vortex tubes and return to water bath.

- 1.1.4 Remove from water bath, cool, add 17.5 mL dilute buffer (.0285M $\text{NH}_4\text{H}_2\text{PO}_4$) and break up charred mass by vortexing. To increase precision, reinsert tubes into water bath for 30 min after vortexing.
- 1.1.5 Remove from water bath, cool and filter using Whatman 2 (or glass filter i.e. Whatman GF/D). Transfer .5 mL filtrate into 15 mL centrifuge tubes (polypropylene), add .5 mL .4M AgNO_3 and 9 mL .2M $\text{NH}_4\text{H}_2\text{PO}_4$. Vortex tubes gently or parafilm and shake. Allow tubes to stand for 30 min in a refrigerator (5°C) or in the dark. Increased precision may be obtained by refrigerating overnight. A set of working standards should be included in each run.
- 1.1.6 Centrifuge at 25,000 x g for 20 min. Carefully aspirate supernatant fluid and discard. Wash pellet with 10 mL precipitation solution, use stirring rod to scrape precipitate from side of tube, vortex, and centrifuge as before. Aspirate supernatant fluid and discard. Be careful not to disturb the pellet while performing this last step.
- 1.1.7 Add 10 mL of .5N HCl and incubate in 90 to 95°C water bath for 5 min. Stopper tubes with rubber stoppers and vortex at fast speed until thoroughly mixed.
- 1.1.8 Cover tube with marble and incubate in 90 to 95°C water bath for 45 min.
- 1.1.9 Remove tubes from water bath and stopper (use different stoppers from step 6 or match tubes with stoppers from step 6 to prevent cross contamination). Vortex at fast speed and cool. Centrifuge as before. A pinkish-purple precipitate may form.

- 1.1.10 Filter supernatant through hardened filtr paper (i.e. Whatman 541) and immediately read supernatant at 260 and 280 nm. Check 260/280 ratio for consistency between sample types and similarity to standard (Torula yeast RNA) 260/280 ratio. Standard 260/280 ratio generally is 1.8.
- 1.1.11 Use these supernatants to create working standards using the table below.

Working Standards	mL A	mL B	mL C	mL D	mL H ₂ O	RNA mg/mL	RNA mg/0.5mL
1	1.0	0.0	0.0	0.0	19.0	0.0000	0.0000
2	0.0	0.5	0.0	0.0	19.5	0.1250	0.0625
3	0.0	1.0	0.0	0.0	19.0	0.2500	0.1250
4	0.0	0.0	1.0	0.0	19.0	0.5000	0.2500
5	0.0	0.0	0.0	1.0	19.0	0.7500	0.3750
6	0.0	0.0	0.0	1.5	18.5	1.1250	0.5625

1.2 Calculations

- 1.2.1 Absorbance at 260 nm convert to mg RNA/.5mL filtrate from standard curve.
- 1.2.2 Total mg RNA (purines) = mg RNA/.5 mL x 40
- 1.2.3 mg purines/g DM = Total mg RNA/gram dry sample assayed

(Modified from Ushida et al., 1985, Zinn and Owens, 1986, Obispo and Dehority, 1999, and Makkar and Becker, 1999).

PART 3

COMPARISON OF F57 AND DACRON BAGS ON

RUMINAL DEGRADATION OF DRY MATTER *IN SITU*

Abstract

The Cornell Net Carbohydrate Protein System (CNCPS) is a widely used system to predict animal performance based on feedstuff chemical composition, physical characteristics, ruminal degradation, and postruminal digestibility characteristics. The *In situ* ruminal digestion procedure is becoming the most accepted procedure for estimating ruminal digestion. This procedure traditionally uses Dacron bags, designed for ruminal incubations. After incubation, digested samples must be removed from the Dacron bag for further analyses. Filter bags (F57, 4 cm x 5 cm, 30- μ m pore size, ANKOM Technology, Fairport, NY), do not contain ash or nitrogen (ANKOM Technology, Fairport, NY). These bags can be used for *in situ* procedures and residue can be further analyzed for CP, NDF, ADF, and ash procedures without changing bags. *In situ* procedure requires correction for microbial contaminations, and the purine method has been used to correct microbial attachment; however, it is a complex and time-consuming procedure. The neutral detergent method has been proposed as an alternative to the purine method. F57 bags could be used with NDF procedure to correct microbial attachment on *in situ* digested samples. Objectives of this experiment were to evaluate the F57 bag as a replacement for the Dacron bag and to compare purine and NDF method for microbial correction. Chemical analyses were performed using the CNCPS on alfalfa hay, bermudagrass hay, tall fescue hay, corn, soybean hulls, soybean meal and wheat straw. Based on chemical analyses, carbohydrate and protein fractions were calculated for each feedstuff. In Experiment 1, alfalfa hay, bermudagrass hay, tall

fescue hay, corn, soybean hulls, and soybean meal were inserted into the rumen of a cannulated cow for 6, 12, 24, 72, and 96 h to determine *in situ* digestion. Residues of *in situ* digested tall fescue samples were selected to do microbial correction with purine and NDF methods. In Experiment 2, alfalfa hay, tall fescue hay, soybean hulls, and wheat straw were incubated in four ruminally cannulated steers at 12, 24, 48, and 72 h. *In situ* digested samples were analyzed to determine CP, NDF and NDIP. Chemical compositions and carbohydrate and protein fractions were similar to those published in Nutritional Requirements of Beef Cattle (NRC, 1996). In Experiment 1, DM disappearances were similar for Dacron and F57 bags that were washed with NDF solution. Without NDF wash, F57 bag results were different from Dacron bag and the NDF washed F57 bags. The average microbial purine to nitrogen ratio was determined to be 11.43. Purine and NDF corrections for microbial protein of *in situ* digested tall fescues were similar within all hours, except 96 h. In Experiment 2, *in situ* digested samples CP and NDIP values were different for NDF-F57 and Dacron bags. Based on this study, F57 bags washed with NDF solution post *in situ* digestion can be used to replace the more expensive Dacron bags.

Key words: Net carbohydrate protein system, F57, *in situ* digestion, purine, neutral detergent.

Introduction

The National Research Council (NRC) publishes estimates of the nutrient requirements of domestic animals. These publications are widely used by researchers and producers. The Cornell Net Carbohydrate Protein System (CNCPS) has been

included in the NRC (1996) guidelines to predict animal performance on given feedstuffs. The CNCPS assumes that the feedstuff contains five major components: carbohydrates, proteins, fats, ash and water. Carbohydrates and proteins are further subdivided based on chemical composition (Van Soest et al., 1991), physical characteristics, ruminal degradation, and postruminal digestibility characteristics (Sniffen et al., 1992).

Chemical composition of nitrogen compounds has been evaluated by Pichard and Van Soest, 1977. Feed nitrogen was divided into three main fractions: NPN (A), true protein (B), and unavailable insoluble protein (C). True protein can be fractioned into three sub-fractions: fraction B1, a buffer soluble protein; fraction B2, a buffer insoluble protein minus the protein insoluble in neutral detergent; and fraction B3, neutral detergent insoluble protein minus acid detergent soluble protein (Roe et al., 1990 and Sniffen et al., 1992). Total feed carbohydrates can be calculated when total CP, fat and ash are extracted from feed. The total carbohydrate can be divided into three main fractions A, B and C. Fraction B can be divided into two fractions, B1 and B2. Sugars are fast soluble carbohydrates; fraction A. Starch and pectin are intermediate soluble carbohydrates, fraction B1. Cellulose and hemicellulose are slowly soluble and have an available cell wall, fraction B2. Lignin is unavailable cell wall, fraction C. Carbohydrate and protein fractions can be determined using common laboratory procedures and formulas that were developed for the CNPCS.

The *in situ* rumen procedure is a technique that generates data used to predict ruminal digestion (Qrskov, 1982; Vanzant et al., 1998). There are several sources of

variation associated with the *in situ* procedure (Madsen and Hvelplund, 1994).

Technician error can increase variation (Madsen and Hvelplund, 1994). Common steps in this procedure are weighing, filtering, and transferring samples into new containers. These are repeated steps and they can increase the risk of error with loss of sample due to retention in the filter or container. Technician error can be reduced by making simple changes in the lab. Dacron bags have been used widely for the *in situ* procedure due to their porosity size, and feed sample size: surface area ratios are standardized (Vanzant et al., 1998). The *in situ* procedure often requires further chemical analyses to identify the chemical properties of feed disappearance in the rumen. Dacron bags were designed primarily for use in ruminal incubations. After the incubation, digested samples must be removed from the Dacron bag for further analyses, resulting in sample loss and variation Spackman et al. (2001) reported using F57 bags for *in situ* procedure. Filter bags F57 (porosity 30 μm , ANKOM Technology, Fairport, NY) are designed for *in vitro* procedures and fiber and nitrogen analysis, and do not contain ash or nitrogen and can withstand up to 72% sulfuric acid (ANKOM Technology, Fairport, NY). These bags can be utilized for *in situ* procedures and carried over to CP, NDF, ADF, and ash procedures without changing bags. The F57 bags can reduce error resulting from sample transfer, speed up the procedure, and reduce the cost.

It has been reported that microbial attachment to the feed substrate can cause a lower estimate of protein digestion. Microbial attachment needs to be corrected for the *in situ* method. If rumen microbial protein is not correctly estimated and corrected for the digested samples, microbial protein appears as an undigested protein in the feed.

Estimation of protein digestion can change significantly. The purine and neutral detergent insoluble N methods were developed to correct microbial protein attachment in *in situ* digested samples. The purine method has been used to correct microbial attachment. The purine procedure requires both skilled technicians and laboratory equipment such as the spectrometer. The neutral detergent fiber method is a common procedure that is used in most labs, but it is a new method for correcting microbial attachment (Mass et al., 1999) and needs to be evaluated for *in situ* digested samples. Since it is recommended that F57 bags be washed with NDF for the *in vitro* method after 48 h (DAISYII200/220, ANKOM Technology, Fairport, NY), NDF method should be tested for F57 bags for *in situ* method also. The primary objectives of these experiments were to determine the efficacy of F57 bag as a replacement for the Dacron bag traditionally used for *in situ* procedures and to evaluate neutral detergent fiber method with purine procedure to estimate microbial attachment for *in situ* digested samples.

Materials and Methods

Sample preparation. Air-dried samples of alfalfa hay, bermudagrass hay, tall fescue hay, corn, soybean hulls, soybean meal and wheat straw were used in Experiments 1 and 2. A total of 200 g sample was collected and ground to pass through a 2 mm screen and a subsample weighing 50 g ground to pass through 1 mm in a Wiley mill.

Chemical analysis for the Cornell Net Carbohydrate Protein System. Sample DM was determined by drying samples at 100° C for 12 h (AOAC, 1998). Ash content

was determined in a temperature-controlled furnace at 600° C for overnight (AOAC, 1998). Sample ether extract was determined per AOAC (1998) standard procedure. Samples were analyzed for NDF, ADF and ADL (sulfuric acid method) using the ANKOM200 Fiber Analyzer (ANKOM Technology, Fairport, NY).

Feedstuffs were analyzed for total N, neutral detergent fiber insoluble protein (NDIP), and acid detergent fiber insoluble protein (ADIP) using a nitrogen analyzer (LECO Corporation, St. Joseph, MI). Total soluble protein (SolP) was determined by incubating the sample with a bicarbonate-phosphate buffer (Pichard and Van Soest, 1977) and insoluble N was determined on filtered samples using Kjeldahl analyses (AOAC, 1998). Non-protein nitrogen (NPN) or trichloroacetic acid (TCA) precipitation fraction (Winter et al., 1964; Shultz and Shultz, 1970; Krishnamoorthy et al., 1983; Sniffen et al., 1992) was determined using the Kjeldahl method (AOAC, 1998). NPN was determined as the difference between total forage N and TCA filtrate residue N. Based on these chemical analyses, Nutrient Requirements of Beef Cattle (1996) equations were used to calculate carbohydrate and protein fractions.

Animal. In Experiment 1, a Jersey cow fitted with a ruminal cannulae was used. The cow was managed according to Knoxville Experiment Station dairy farm standard procedures and fed individually. The cow was used under a protocol approved by The University of Tennessee Animal Care and Use Committee. Alfalfa cubes (80% of diet DM) and corn-based supplement (20% of diet DM) were offered twice daily (0800 and 1600). The animal was fed a maintenance energy level diet during the study from February 8 to March 1, 2002. The animal was adapted to the diet for ten days. Chemical

analyses were conducted on the individual feed ingredients and total mixed diet and the results are reported in Table 1. Samples were inserted into the rumen at 11 to 14 days. The animal remained on the diet for 3 days after the removal of the samples and then a second run was conducted.

In Experiment 2, four Angus steers fitted with ruminal cannulae were used. Steers were housed at Johnson Animal Research Teaching Unit and fed individually. Steers were used under a protocol approved by The University of Tennessee Animal Care and Use Committee. Steers were fed a diet of alfalfa cubes (70% of diet DM) and corn-based supplement (30% of diet DM) at a maintenance energy level with adequate degradable intake protein in two equal feedings (0830 and 1630) during the study. Chemical analyses of feed ingredients and total diet are reported in Table 2.

In situ method. The *In situ* method was used to evaluate the disappearance of DM using F57 filter bags (4 cm x 5 cm, 30- μ m pore size, ANKOM Technology, Fairport, NY). The bags were labeled with a permanent marker and processed according to the *in vitro* techniques recommended by the manufacture. Bags were pre-rinsed with acetone for three minutes and dried. Ground 2 mm samples were placed in F57 filter bags in duplicate. Filter bags (F57) were sealed with a heat sealer. Sample name, replication, incubation time, F57 filter bag identification number and mesh bag numbers were recorded.

Disappearance of samples was also evaluated using #R1020 Dacron bags (10 cm x 20 cm, 50- μ m to 70- μ m pore size, ANKOM Technology, Fairport, NY). A permanent

marker was used to label these bags. Dry Dacron bags were weighed and recorded. Duplicate ground samples (5 g; 2-mm screen) were placed into identified Dacron bags. Dacron bags were sealed with a heat sealer and placed in mesh bags. Mesh bags (used to prevent loss of *in situ* bags in the rumen) contained a maximum of 12 Dacron bags and 12 F57 bags. Mesh bags were soaked in 39° C water for 15 min in an insulated thermos container after that they were placed in the rumen directly under the particulate mat. Samples were inserted sequentially (96, 72, 24, 12, 6, and 0 h prior to removal) and removed at the same time. Upon removal, samples were pre washed with cold water and then the *in situ* bags were rinsed in a washing machine (low water level, 45 L capacity; 10 times; each rinse cycle consisted of one min agitation and two min spin) until bags were clear as described by Vanzant et al. (1998). Bags were dried at 100° C in a forced draft oven for 12 h. Dry weights were recorded for all samples. Results using F57 bags for *in situ* DM disappearance are referred to as F57 in tables and figures. When F57 bags are used for *in vitro* DM disappearance, a post incubation wash with NDF solution is recommended (DAISYII200/220, ANKOM Technology, Fairport, NY). Washing with NDF solution is to remove microbial contamination present in the F57 bags after incubation in rumen fluid during an *in vitro* study or after incubation in the rumen of the animal during an *in situ* study. After the F57 bags were subjected to washing in NDF solution, they were dried and weighed. These weights are used to calculate the residue in the bag remaining after washing in NDF solution and are referred to as NDF-F57 in tables and figures. Zero hour samples were not inserted into the rumen. They were soaked in 39° C water for 20 min. After the soaking, the zero hour samples received the

same treatment as the ruminal samples except F57 bags were not washed with NDF solution, since the zero hour samples were not exposed to rumen microbes.

In Experiment 2, the same procedures were used except samples were inserted sequentially at 72, 48, 24, 12, and 0 h prior to removal. After washing, the bags were dried at 55° C in a forced draft oven for a 2-day period and dry weights measured each Dacron bags. *In situ* digested samples were pooled based on feed type and incubation times and stored in a glass jar until further analyses. DM was determined on the pooled samples and *in situ* digested bags were corrected for DM content. Crude protein, NDF, and NDIP were determined for *in situ* digested samples. F57 bags were processed in the same way as Dacron bags, but samples were not pooled for F57 bags. The F57 bags were washed with NDF solution and the results are presented as NDF-F57. NDIP was determined on these samples, also. *In situ* digested samples CP and NDIP values are not comparable with undigested samples CP or NDIP values; because *In situ* digested samples dry matter loss has to be compensated for in the calculation. *In situ* digested samples CP and NDIP values were corrected based on *in situ* dry matter loss.

Ruminal fluid collection. In Experiment 1, two kg of ruminal contents was collected four h post feeding on days 11 and 17. In Experiment 2, a total of six kg ruminal contents were collected. One kg from each of six steers and the contents were mixed in a mechanical mixer. The ruminal fluid was stored in a pre-warmed insulated thermos container until transported to the Animal Science Laboratories. Two kg ruminal fluid was weighed and mixed with ice-cold saline solution (9 g of NaCl/L). It was blended in a high-speed blender and strained through two layers of cheesecloth into 250

mL bottles. The rumen fluid was then centrifuged at 500 x g for 20 min (to separate protozoa and feed particles). Supernatant fluid was collected into 250 mL bottles and centrifuged at 30,000 x g for 20 min. The supernatant fluid was discarded and water was added. Bacterial pellets were pooled and re-suspended. These steps were repeated three times. Separated bacteria were lyophilized, ground and stored in a glass jar until further analyses.

Microbial correction procedures and chemical analyses. To correct for microbial protein attachment to feed substrate the purine method described by Zinn and Owens, (1986); Obispo and Dehority (1999); Makkar and Dehority (1999) was used. Additional analyses included CP, NDF, and NDIP.

Statistical methods. Data were analyzed using Mixed Model Procedures in SAS (2001). Analyses of variance were done using a complete randomized design. PDMIX800 and MMAOV macros were used for this SAS procedure (Saxton, personal communication, 2001). In Experiment 1, *in situ* digestion was run two times. Treatments were arranged in a factorial combination of two *in situ* bags with five different incubation times. In Experiment 2, *in situ* digestion was done on four animals at same time. Each number represents the mean value of four animals. Treatments were arranged in a factorial combination of two *in situ* bags with four different incubation times. *In situ* DM disappearances three fold higher or lower than standard error were not used in the data summary. Standard deviations were calculated for replicate chemical analyses. If the standard deviation was higher than those reported by the National Forage Testing Association web site, analyses were repeated. *In situ* digested

samples DM disappearances, CP and NDIP were analyzed separately for each individual feedstuff. DM disappearance rates were calculated by SAS model reported by Mertens (1988).

Results and Discussion

The Cornell Net Carbohydrate Protein System chemical values and fraction calculations. The Cornell Net Carbohydrate Protein System required chemical analyses of selected feedstuffs (Table 3). Nutrient Requirements of Beef Cattle (NRC, 1996) reported nutrient values were compared to our findings. There are some differences between our findings and the reported values due to plant differences such as maturity, production environment, drying and storage.

Soybean meal had the highest CP value (53.86%) among the selected feedstuffs. Bermudagrass and corn had the lowest CP values among the feedstuffs, (8.14 and 8.61%, respectively). Alfalfa had the highest CP value (19.8%) among the forages. Non-protein nitrogen was determined using the nitrogen analyzer (LECO Corporation, St. Joseph, MI) the first time and readings were incorrect due to the TCA solutions, which reacted with the filter. Non-protein nitrogen procedure was repeated using the Kjeldahl method. Non-protein nitrogen values (67% and 80%) were higher than reported values in the literature for bermudagrass and tall fescue respectively. Others had similar values. Soluble protein, determined using the Kjeldahl, were higher than NRC reported values. NDIP and ADIP were higher than values reported in the literature. Madsen and Hvelplund (1994) also reported that larger variations were seen on nitrogen analyses than on other analyses between laboratories.

NDF values were similar to those reported in the literature. Forages and soybean hulls had NDF values ranging from 40% to 76%. Higher NDF values were associated with higher standard deviations as reported by the National Forage Testing Association (NFTA, 1984). In the first run, the corn NDF value was higher than reported values, because corn contains large amounts of starch and it is partially soluble with NDF solution. The NDF procedure requires inclusion of alpha amylase to digest high starch feedstuffs. Alpha amylase was used to correct NDF values in the second run. Corn and soybean meal had lower NDF levels, 10.11 and 13.76%, respectively. Lignin values were close to reported values, except corn and soybean meal had higher lignin levels than reported values, 7.04 and 6.71%, respectively. Alfalfa had the highest lignin value 14.93%. Starch values were lower than reported values. Fat content was similar to the reported values for corn (4.18%) and soybean meal (1.68%). Other feedstuff fat values were slightly less than the reported values. Ash content was lower for bermudagrass (5.52%), tall fescue (5.72%), and corn (0.98%). Others ash values were similar to the reported values.

Selected feedstuff chemical values were obtained by chemical analyses according to The Cornell Net Carbohydrate Protein System, and are reported in Table 3. These values were used in equations described by Nutrient Requirements of Beef Cattle (NRC, 1996). Calculated carbohydrate and protein fractions of feedstuffs are presented in Tables 4 and 5, respectively. Since NRC data were used to evaluate our data, carbohydrate and protein fractions were compared with our results. Carbohydrate and protein fractions were calculated from NRC data and are reported in Tables 6 and 7.

Calculated carbohydrate fractions for our data and reported data are presented in Tables 4 and 6. Fraction A (sugars and organic acids) was similar, however, soybean meal and soybean hull percentages seemed to be higher than reported values. Lower starch values may have affected the results. Fraction B1 (starch and pectin substances) was lower for all the forages because forages usually do not store glucose as a starch. Corn had the higher value of B1 fractions (67%). Soybean meal and hulls had a lower level of B1 fractions than reported values. All of the feedstuffs had similar values for fraction B2. Fraction C was similar to the reported values, except corn and soybean meal were higher.

Calculated protein fractions for our data and reported data are presented in Tables 3 and 5. Fraction A was slightly higher than reported values, except soybean hulls that had lower (0.2%) than reported values (1.58%). Alfalfa and soybean meal had a higher B1 fraction than reported values. Tall fescue had smaller fraction B1 values (0.8%) than reported values (2.5%). Fraction B2 was slightly smaller for all the feedstuffs from reported values. Fraction B3 had higher value than reported values. Fraction C had similar values for all the feedstuffs.

In situ dry matter disappearances and in situ digested samples CP and NDIP values. Alfalfa dry matter disappearance percent is shown in Figure 1¹. Alfalfa dry matter disappearances were 28 to 34% at zero h for F57 and Dacron bags, respectively. The zero h value represents a sample that was soaked in warm water and washed following the standard washing procedure used for all samples. This process resulted in

¹All figures are located in the Appendix.

the loss of the most soluble fractions. Alfalfa had higher CP (19%) than other forages and the zero h loss was higher than in other forages. Most of the DM disappearance was at 24 h. Extended dry matter disappearance was 73% at 96 h for F57 bags. Dacron and NDF-F57 bags had similar DM disappearances at all time points except 0 h. Samples digested in F57 bags were different from samples digested in Dacron bags for all incubation times.

Bermudagrass dry matter disappearance percentages are shown in Figure 2. Bermudagrass had the highest NDF values (76%) among the feedstuffs used. Bermudagrass dry matter disappearances were 13 to 16% at zero hr with NDF-F57 or Dacron bags, respectively. Dry matter disappearances were 56 and 55% at 96 h for NDF-F57 bags and Dacron bags, respectively. This trend in digestion was similar for bermudagrass and tall fescue; however, 96 h disappearance of bermudagrass was lower than tall fescue. Results with the Dacron bags were similar to those of NDF-F57 bags at 24, 72, and 96 h. F57 bags were different from Dacron bags for all incubation times.

Tall fescue dry matter disappearance at different times of incubation is shown in Figure 3. Tall fescue dry matter disappearances were 14 to 18% at zero h for NDF-F57 and Dacron bags, respectively. Tall fescue DM disappeared slowly and after 72 h, it did not change much due to a higher NDF value (73%). Dry matter disappearances were 70% at 96 h for both the NDF-F57 bags and Dacron bags, respectively. Results with the Dacron bags were similar to results with NDF-F57 bags for all the incubation times. Samples inserted in F57 bags dry matter disappearances were different from samples inserted in Dacron bag.

Corn dry matter disappearances were 27 to 31% at zero h for NDF-F57 and Dacron bags, respectively. Since corn is high in starch, DM rapidly disappeared. Extended dry matter disappearances were 96 and 94% at 96 h for NDF-F57 and Dacron bags, respectively (Figure 4). Results with Dacron bags were similar to results with NDF-F57 bags for all the incubation times. F57 bags were different from Dacron bags for all the incubation times.

Soybean meal dry matter disappearance percent is shown in Figure 5. Soybean meal dry matter disappearances were 35 to 42% at zero h for NDF-F57 and Dacron bags, respectively. Soybean meal had the highest CP value at 53%. At 24 h, soybean meal had the highest DM disappearance rate (96 and 99% for NDF-F57 and Dacron bags, respectively). Extent of dry matter disappearances was 95 and 93% at 96 h for samples inserted in NDF-F57 and Dacron bags, respectively. Samples inserted in Dacron bags were similar to samples inserted in NDF-F57 bags at 6, 24, 72, and 96 h.

Soybean hulls dry matter disappearance percent is shown in Figure 6. Soybean hull dry matter disappearances were 16 to 17% at zero h for NDF-F57 and Dacron bags, respectively. It seemed that DM disappearances were high in the first 6 h, and then slowed the next six hours then increased again, and could have been affected by NDF. Extended dry matter disappearances were 91 and 92% at 96 h for NDF-F57 and Dacron bags, respectively.

Calculated DM disappearances rates were similar for NDF-F57 and Dacron bags (Table 8). There were differences among feedstuffs. Alfalfa and soybean meal had similar DM disappearance rates between 20 and 26% per h. Bermuda grass, tall fescue

and soybean hulls had similar DM disappearance rate between 6.0 to 8.1% per h. Corn DM disappearances rate was 15-16% per h and was different from all the others. These results indicate that either the Dacron or NDF-F57 bag can be used to determine the rate of *in situ* DM disappearance of several feedstuffs. These findings are the first to show that laboratories have an alternative to the Dacron bags when determining rates of *in situ* DM disappearances. The use of the F57 bags fits better into the laboratories that are using the ANKOM technology system for fiber analyses and *in vitro* DM disappearance.

In Experiment 2, samples inserted in F57 bags were washed with NDF and results were presented as NDF-F57. *In situ* digested CP and NDIP values for samples in Dacron and F57 bags were determined and values compared in this experiment.

Undigested alfalfa samples had 22.14% CP however, when alfalfa digested in Dacron bags the CP was 2.83 and 1.37% at 12 and 72 h incubation, respectively (Table 10). For *in situ* digested alfalfa samples in Dacron bags, CP and NDIP values were different except 72 h. Overall Dacron and NDF-F57 bags NDIP values were different except 12h.

In situ digested tall fescue CP and NDIP values are shown in Table 13. Tall fescue samples had 12.96% CP. *In situ* digested tall fescue samples in Dacron bags had 11.85 and 3.44% CP at 12 and 72 h incubation, respectively (Table 11). For samples in Dacron bags, CP and for samples in NDF-F57 bags, NDIP was similar for 48 and 72 h. For samples in NDF-F57 and Dacron bags, NDIP values were only different at 48 h.

Predigested wheat straw samples had the lowest (3.45% CP) value among the feedstuffs. CP values of *in situ* digested samples in Dacron bags were 20% and 5% CP for 12 and 72 h incubations, respectively (Table 12). These were higher than the undigested samples CP values. *In situ* digested samples in Dacron bags had different CP and NDIP values. *In situ* digested samples in Dacron and NDF-F57 bags values were similar at 12 and 24 h.

Predigested soybean hulls samples had 13.78% CP value. *In situ* digested samples in Dacron bags had 3.97 and 1.10% CP for 12 and 72 h incubations, respectively (Table 14). Crude protein and NDIP values were similar for *in situ* digested samples in Dacron bags were similar at 24, 48, and 72 h for *in situ* digested samples in Dacron and NDF-F57 bags, NDIP values were different for all the time.

The F57 bag manufacturer suggested that weighing 0.25 g of samples gives better results for the *in vitro* method, (DAISYII200/220, ANKOM Technology, Fairport, NY). This resulted in a sample size: surface area ratio of 6.25 mg/cm², but the recommended sample size: surface area ratio is 10 mg/cm² (Madsen and Hvelplund, 1994; Vanzant et. al., 1998). Varvikko and Vanhatalo (1990) reported that sample size: surface area is more important than the pore size for the disappearance of feed components. A smaller sample size surface area should lower the risk of using smaller porosity bags (30 µm) instead of using recommended porosity of 40 to 60 µm (Vanzant et. al., 1998). Apparent trends were observed at zero h. F57 bag sample disappearance percent was lower than Dacron bags at zero hours. Smaller porosity prevented small particles from passing out of the bag (Qrskov, 1982; Madsen and Hvelplund, 1994). Our

results indicated that samples in F57 bags and Dacron bags were not similar for *in situ* digestion without NDF washing of F57 bags. Dry matter, CP, and NDF disappearance can be affected by pore size and each feed sample has different disappearance rates (Lindberg and Knutsson, 1981; Lindberg and Varvikko, 1982). Use of neutral detergent fiber procedure has been suggested as a method to correct for microbial attachment on *in situ* samples (Mass et al., 1999 and Klopfenstein et al., 2001). Microbial attachment can contribute extra DM and CP to samples, which can generate low estimates of DM and CP digestion. NDF analysis with the F57 bags was not a problem after the *in situ* procedure. The NDF procedure automatically corrects for microbial attachment, but there are concerns about using the NDF procedure when NDF soluble particles have not fully disappeared. The time of NDF soluble particle disappearance should be determined, since it could give an over estimate of DM disappearance. For samples in Dacron bags, CP and for samples in NDF-F57 bags, NDIP was similar after 24 h for all the feedstuff except wheat straw. After 24 h, NDIP values were similar for samples in Dacron and NDF-F57 bags and it may be scientifically acceptable.

In situ digested sample microbial protein correction estimates with purine procedure. In Experiment 1, *in situ* digested alfalfa samples were first used to determine microbial purine concentration. Because of inexperience and equipment problems associated with conducting purine analysis, the digested alfalfa samples were exhausted. As a result, *in situ* digested tall fescue samples were used to compare microbial correction with purine and NDF methods. *In situ* digested tall fescue microbial protein corrected protein values are presented in Table 9. Microbial purine (111.83 mg

purines/g DM) and nitrogen (9.78 % DM) ratio were determined to be 11.43. *In situ* digested tall fescue sample microbial purine concentrations were determined and N contamination corrected based on the microbial purine:protein ratio. *In situ* digested tall fescue samples were also washed with NDF and nitrogen was determined on the same samples before and after NDF wash. *In situ* digested tall fescue CP values were compared after microbial corrections. Data from purine and NDF methods were similar within the same time, except 96 h purine corrected sample protein, values were different than 96 h NDF corrected sample protein values. Purine and NDF values were significantly different ($P \leq 0.05$) at 96 h. If there were more data available, the two methods could be better compared.

In Experiment 2, purine procedure was used to determine microbial protein on *in situ* digested samples; however, purine procedure results were not consistent. Therefore, microbial protein correction could not be calculated for this experiment using the purine method.

Purine method has been widely used to determine and correct for microbial attachments to the feed substrate. However, it was a new procedure for our laboratories, and is a time consuming multiple step procedure. The neutral detergent fiber method was proposed to replace purine method. It is simple and can be done easily. Both methods use small amounts of sample; however, in Experiment 1, after reruns there was not enough sample left to run NDIP or purine except tall fescue. *In situ* digested tall fescue results were similar for microbial correction with purine and NDF as reported by Mass et al., (1999) and Klopfenstein et al., (2001).

In Experiment 2, purine procedure results were not consistent across samples, even though the standards were repeatable between runs. There appeared to be some interference when reading the samples because of the many fine particles in solution. A similar experience was reported by Ushida et al. (1985). In Experiment 2, *in situ* digested samples standard errors were not acceptable for purine procedure. *In situ* digested samples NDIP value were determined, however we could not conduct the comparison test between NDF and purine method.

Implications

The F57 bags require NDF wash after *in situ* digestion for the results to be comparable to those obtained with Dacron bags. *In situ* digested samples CP values in Dacron bags and NDIP values in NDF-F57 bags were similar after 24 h., therefore NDF-F57 bags can be used for determining extent of digestion. Comparison of purine and NDF methods was not possible because of difficulties encountered in developing reliable purine procedures for some of these feedstuffs. More research on procedures for diverse feedstuffs is needed.

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Appendix

Table 1. Chemical analyses of feed ingredients and diet in Experiment 1.

Chemical Analyses	Alfalfa Cubes (80% of Diet DM)	Corn-base supplement ^a (20% of Diet DM)	Total Diet
DM, %	91.79	88.50	-
CP ^b , %	13.68	8.61	12.66
NDF, %	57.00	10.11	47.62
ADF, %	43.26	2.45	35.09
ASH, %	10.00	0.98	8.19

^aFree access of mineral salt block containing 0.4% Zn, 0.2% Fe, 0.2% Mn, 0.03% Cu, 0.007% I, 0.005% Co, in 94% minimum NaCl.

^bNutrients on a DM Basis.

Table 2. Chemical analyses of feed ingredients and diet in Experiment 2.

Chemical Analyses	Alfalfa Cubes (70% of Diet DM)	Corn-based Supplement ^a (30% of Diet DM)	Total Diet
DM, %	91.79	90.73	-
CP ^b , %	13.68	10.02	12.58
NDF, %	57.00	25.63	47.58
ADF, %	43.26	3.30	31.27
ASH, %	10.00	2.8	7.84

^aSupplement was 98.16% corn, 1% molasses, and 0.83% mineral mix (27 % Ca, 0.4.5% P, 19.2% NaCl, 0.3% Mg, 0.1% K, 24 ppm Co, 36 ppm I, 20 ppm Se, 1000 ppm Cu, 3000ppm Mn, 0.002 Se, 3500ppm Zn, 240,000 IU/lb Vitamin A, 45,000 IU/lb Vitamin D-3 and Vitamin E 140 IU/lb).

^bNutrients on a DM Basis.

Table 3. Carbohydrate and protein fractions, fat and ash in selected feedstuffs^a in Experiments 1 and 2.

Common name	DM, % AF	CP, % DM	NDF, % DM	Lignin, % NDF	SolP, % CP	NPN, % SolP	NDIP, % CP	ADIP, % CP	Starch, % NSC	Fat, % DM	Ash, % DM
Alfalfa	90.13	19.8	43.22	14.93	41.42	72.16	15.78	6.64	0.10	1.29	9.26
Bermudagrass	92.05	8.14	76.74	9.67	40.00	67.72	50.61	11.71	0.00	0.65	5.52
Tall fescue	90.49	11.56	73.91	7.85	36.21	80.89	50.40	7.12	0.00	1.82	5.72
Corn	88.50	8.61	10.11	7.04	20.57	55.86	32.90	5.27	90.00 ^b	4.18	0.98
Soybean meal	89.05	53.86	13.76	6.71	22.64	52.22	6.49	3.91	0.00	1.68	6.54
Soybean hulls	91.86	14.27	62.20	3.53	29.70	49.41	54.08	17.73	0.97	1.40	4.51
Wheat straw	92.91	3.45	84.98	10.08	32.73	94.86	15.78	6.64	0.00	0.31	5.82

^aSolP = soluble protein; NPN = trichloroacetic acid (TCA) soluble nitrogen; NDIP = neutral detergent insoluble protein; and ADIP = acid detergent insoluble protein.

^bCorn starch value was calculated from Nutrient Requirements of Beef Cattle, (NRC, 1996).

Table 4. Calculated carbohydrate fractions based on chemical values of selected feedstuffs^a in Experiments 1 and 2.

Common name	Carbohydrate Fractions ^b , % DM			
	A	B1	B2	C
Alfalfa	12.22	0.01	41.90	15.49
Bermudagrass	-7.93	0	75.79	17.82
Tall fescue	-6.16	0.04	73.09	13.93
Corn	7.48	67.36	9.66	1.71
Soybean meal	24.02	0	11.66	2.21
Soybean hulls	13.58	0.13	60.81	5.27
Wheat straw	-14.19	-0.14	84.16	20.57

^aBased on chemical analyses, carbohydrate fraction equations were used to calculate from Nutrient Requirements of Beef Cattle (1996).

^bFraction A = sugars; fraction B1 = starch and pectin; fraction B2 = hemicellulose; and fraction C = cellulose and lignin.

Table 5. Calculated protein fractions based on chemical values of selected feedstuffs^a in Experiments 1 and 2.

Common name	Protein Fractions ^b , % DM				
	A	B1	B2	B3	C
Alfalfa	5.90	2.28	8.47	1.80	1.31
Bermudagrass	2.20	1.05	0.76	3.16	0.95
Tall Fescue	3.38	0.80	1.54	5.00	0.82
Corn	0.98	0.78	4.01	2.37	0.45
Soybean Meal	6.37	5.82	38.16	1.39	2.10
Soybean Hulls	0.20	3.83	5.64	3.20	1.39
Wheat straw	1.074	0.05	1.00	0.50	0.81

^aBased on chemical analyses, protein fraction equations were used to calculate from Nutrient Requirements of Beef Cattle (1996).

^bFraction A = non-protein N; fraction B1 = Soluble CP in borate phosphate buffer; fraction B2 = insoluble in borate phosphate buffer but soluble in neutral detergent; fraction B3 = insoluble in neutral detergent but soluble in acid detergent; and fraction C = acid detergent insoluble CP.

Table 6. Calculated carbohydrate fractions based on reported chemical values^a.

Common name	Carbohydrate Fractions ^b , % DM			
	A	B1	B2	C
Alfalfa hay, early bloom	10.03	1.11	39.81	17.03
Bermudagrass hay, late vegetative	-9.55	-0.60	75.90	15.75
Fescue Ky31 hay, full bloom	-3.80	-0.24	65.85	11.99
Fescue Ky31 hay, mature	-6.72	-0.58	69.8	16.80
Corn grain, cracked	7.37	66.41	10.31	0.57
Soybean meal-44	2.66	23.96	13.90	0.76
Soybean meal-49	3.10	27.92	6.71	0.46
Soybean hulls	1.14	10.28	64.59	4.75

^aCarbohydrate fraction equations were used to calculate from Nutrient Requirements of Beef Cattle (1996).

^bFraction A = sugars; fraction B1 = starch and pectin; fraction B2 = hemicellulose; and fraction C = cellulose and lignin.

Table 7. Calculated protein fractions based on reported chemical values^a.

Common name	Protein Fractions ^b , % DM				
	A	B1	B2	B3	C
Alfalfa hay, early bloom	5.36	0.40	10.54	1.39	2.18
Bermudagrass hay, late vegetative	0.51	1.50	3.11	1.97	0.69
Fescue K31 hay, full bloom	0.84	2.49	5.14	3.26	1.14
Fescue K31 hay, mature	0.08	3.91	3.82	0.67	0.19
Corn grain, cracked	0.78	0.29	7.25	0.98	0.49
Soybean meal-44	5.48	4.49	37.42	1.49	0.99
Soybean meal-49	5.94	4.86	40.50	1.62	1.08
Soybean hulls	1.58	0.61	7.56	0.73	1.70

^aProtein fraction equations were used to calculate from Nutrient Requirements of Beef Cattle (1996).

^bFraction A = non-protein N ($N \times 6.25$); fraction B1 = Soluble CP in borate phosphate buffer; fraction B2 = insoluble in borate phosphate buffer but soluble in neutral detergent; fraction B3 = insoluble in neutral detergent but soluble in acid detergent; and fraction C = acid detergent insoluble CP.

Table 8. *In situ* dry matter disappearance rates, %/h for Dacron and NDF-F57 bags in Experiment 1.

Samples	Bag type	k, %h
Alfalfa	Dacron	20.0 ± 1.8 ^a
	NDF-F57	23.0 ± 1.3 ^a
Bermuda grass	Dacron	6.2 ± 1.8 ^c
	NDF-F57	6.1 ± 1.3 ^c
Tall Fescue E+	Dacron	6.0 ± 1.3 ^c
	NDF-F57	6.6 ± 1.3 ^c
Corn	Dacron	15.0 ± 1.3 ^b
	NDF-F57	16.0 ± 1.3 ^b
Soybean meal	Dacron	26.0 ± 1.8 ^a
	NDF-F57	25.0 ± 1.8 ^a
Soybean Hulls	Dacron	7.9 ± 1.8 ^c
	NDF-F57	8.1 ± 1.3 ^c

^{a,b,c} Least square means within a row with different letters differ ($P < 0.05$).

Table 9. *In situ* digested tall fescue samples CP values after microbial protein correction with purine and neutral detergent fiber methods in Experiment 1.

Time H	Purine	NDF
	Corrected CP, % (DM)	
6	7.55 ± 0.33 ^{a,b,c}	7.43 ± 0.33 ^{b,c,d}
12	8.43 ± 0.23 ^a	8.14 ± 0.23 ^{a,b}
24	8.19 ± 0.33 ^{a,b}	7.66 ± 0.23 ^{a,b,c}
72	6.48 ± 0.23 ^{d,e}	5.88 ± 0.23 ^e
96	6.86 ± 0.23 ^{c,d}	5.97 ± 0.23 ^e

^{a,b,c,d,e} Least square means with unlike letters differ ($P < 0.05$).

Table 10. *In situ* Alfalfa CP and NDIP values for Dacron and NDF-F57 bags for different incubation times in Experiment 2.

Time, h	Residual as % of Initial Dry Sample Weight		
	Dacron	Dacron	NDF-F57
	CP	NDIP	NDIP
12	2.83 ± 0.16 ^b	1.38 ± 0.16 ^{c,d}	1.30 ± 0.11 ^d
24	1.76 ± 0.16 ^c	1.01 ± 0.16 ^d	3.38 ± 0.16 ^a
48	2.52 ± 0.16 ^b	1.34 ± 0.16 ^{c,d}	2.56 ± 0.11 ^b
72	1.37 ± 0.16 ^{c,d}	0.90 ± 0.16 ^d	1.66 ± 0.11 ^c

^{a,b,c,d}Least square means with different letters differ ($P < 0.05$).

Table 11. *In situ* tall fescue CP and NDIP values for Dacron and NDF-F57 bags for different incubation times in Experiment 2.

Time, h	Residual as % of Initial Dry Sample Weight		
	Dacron	Dacron	NDF-F57
	CP	NDIP	NDIP
12	11.85 ± 0.71 ^a	3.77 ± 0.71 ^c	2.21 ± 0.58 ^{c,d,e}
24	6.99 ± 0.71 ^b	2.09 ± 0.71 ^{c,d,e}	3.08 ± 0.58 ^c
48	2.74 ± 0.71 ^{c,d,e}	0.81 ± 0.71 ^e	3.29 ± 0.58 ^c
72	3.44 ± 0.71 ^c	1.04 ± 0.71 ^{d,e}	2.77 ± 0.50 ^{c,d}

^{a,b,c,d,e}Least square means with different letters differ ($P < 0.05$).

Table 12. *In situ* wheat straws CP and NDIP values for Dacron and NDF-F57 bags for different incubation times in Experiment 2.

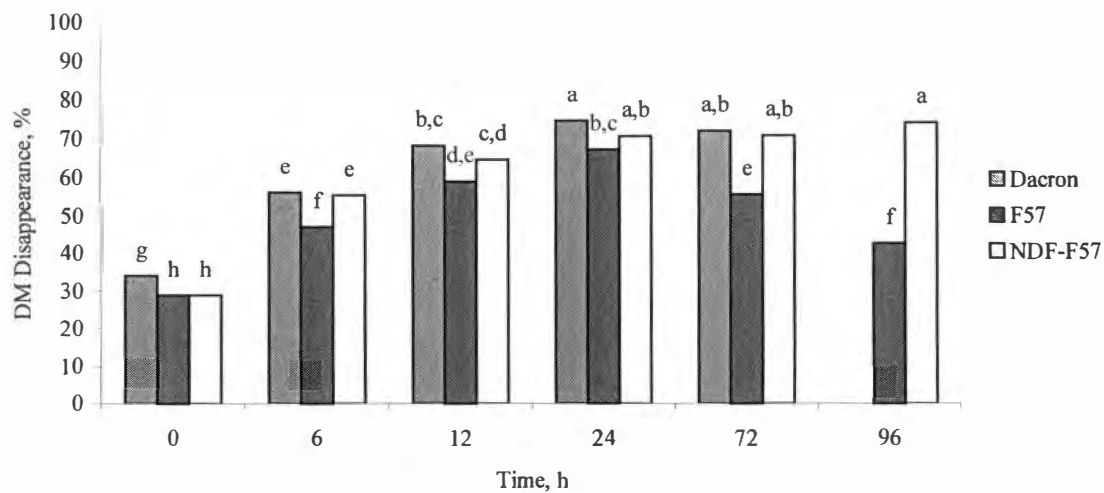
Time, h	Residual as % of Initial Dry Sample Weight		
	Dacron	Dacron	NDF-F57
	CP	NDIP	NDIP
12	20.23 ± 0.28 ^a	1.35 ± 0.20 ^{e,f}	1.11 ± 0.16 ^{e,f,g}
24	17.99 ± 0.20 ^b	1.54 ± 0.50 ^e	1.46 ± 0.14 ^e
48	12.49 ± 0.28 ^c	1.54 ± 0.20 ^e	0.81 ± 0.16 ^{f,g}
72	5.03 ± 0.20 ^d	0.69 ± 0.20 ^g	1.57 ± 0.14 ^e

^{a,b,c,d,e,f,g} Least square means with different letters differ ($P < 0.05$).

Table 13. *In situ* Soybean hulls CP and NDIP values for Dacron and NDF-F57 bags for different incubation times in Experiment 2.

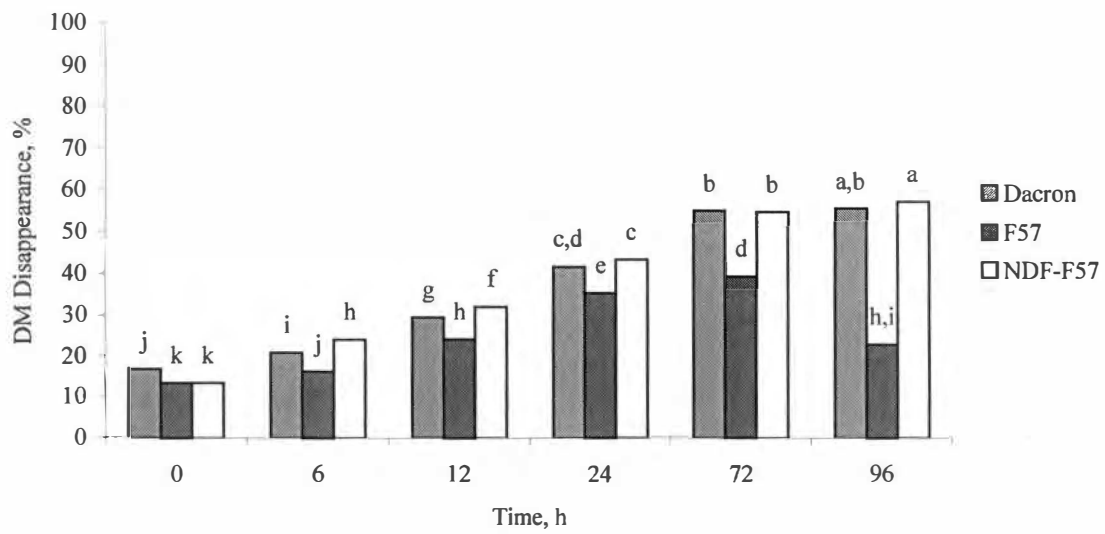
Time, h	Residual as % of Initial Dry Sample Weight		
	Dacron	Dacron	NDF-F57
	CP	NDIP	NDIP
12	3.97 ± 0.20 ^a	2.47 ± 0.20 ^c	1.58 ± 0.16 ^{d,e}
24	2.02 ± 0.20 ^{c,d}	1.52 ± 0.20 ^{d,e}	3.38 ± 0.14 ^b
48	1.09 ± 0.20 ^{e,f}	0.88 ± 0.28 ^{e,f}	2.44 ± 0.14 ^c
72	1.10 ± 0.20 ^{e,f}	0.69 ± 0.28 ^f	1.91 ± 0.14 ^d

^{a,b,c,d,e,f} Least square means with different letters differ ($P < 0.05$).



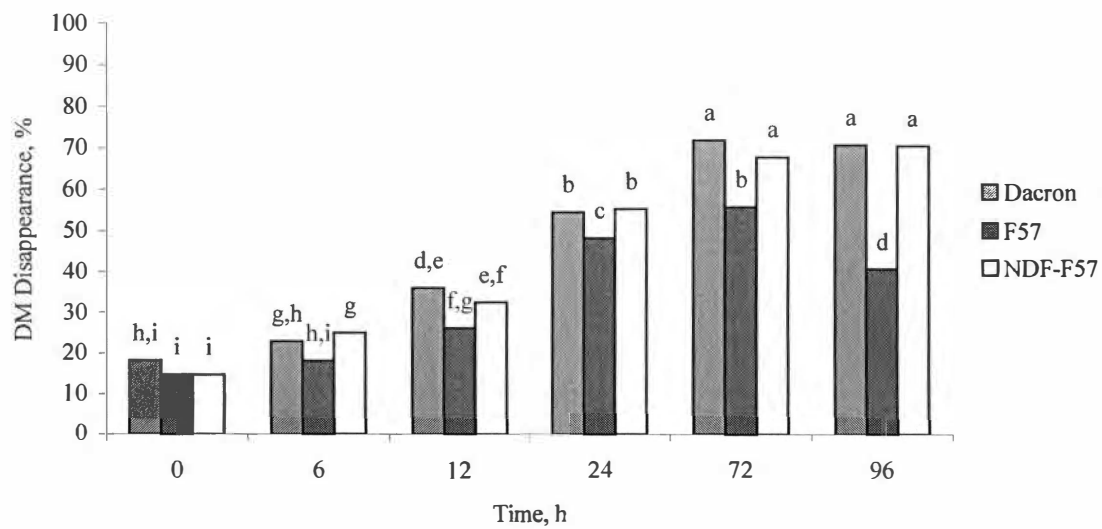
a,b,c,d,e,f,g,h Least square means having different letters differ ($P < 0.05$).

Figure 1. *In situ* alfalfa dry matter disappearance in Dacron, F57, or NDF washed F57 in Experiment 1.



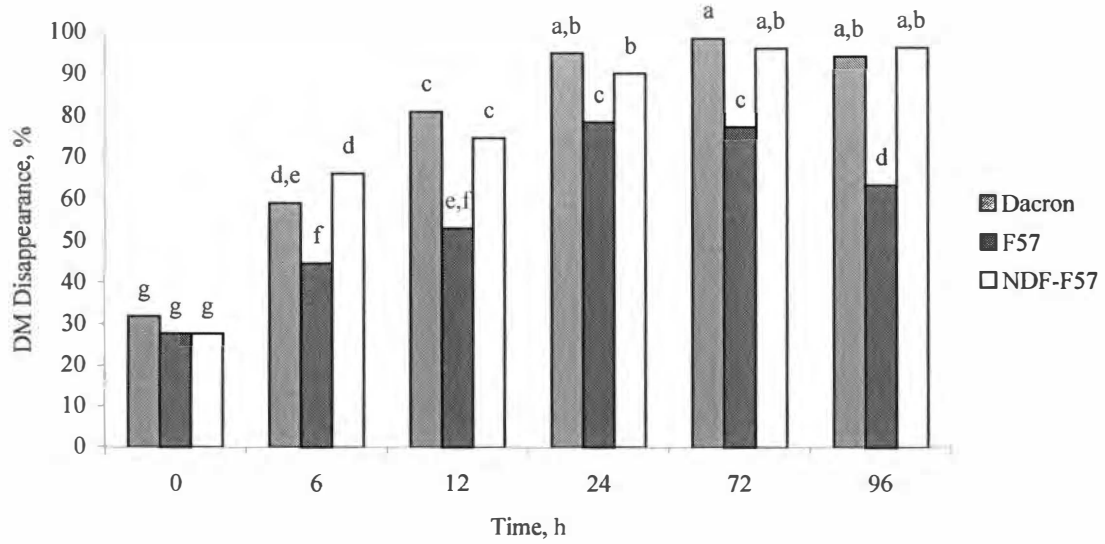
a,b,c,d,e,f,g,h,i,j Least square means having different letters differ ($P < 0.05$).

Figure 2. *In situ* bermudagrass dry matter disappearance in Dacron, F57, or NDF washed F57 in Experiment 1.



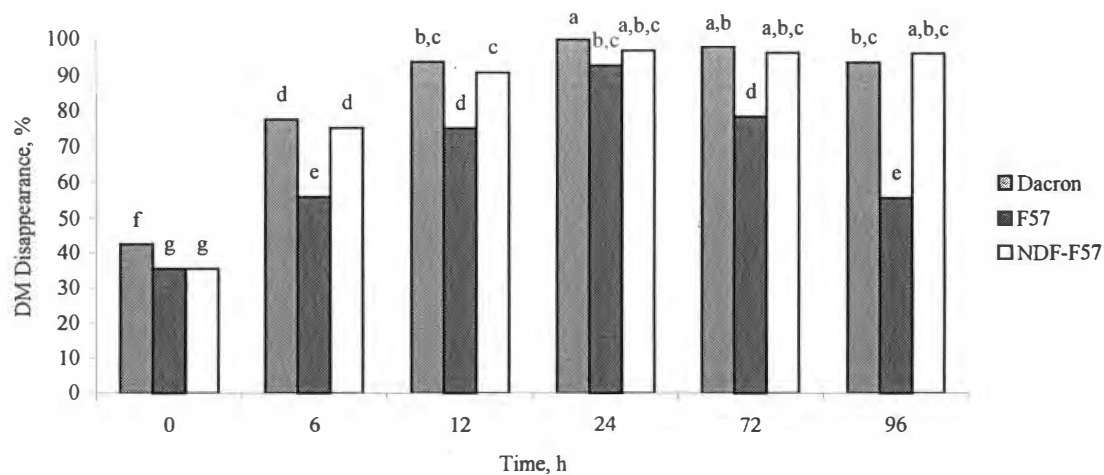
a,b,c,d,e,f,g,h,i Least square means having different letters differ ($P < 0.05$).

Figure 3. *In situ* tall fescue dry matter disappearance in Dacron, F57, or NDF washed F57, in Experiment 1.



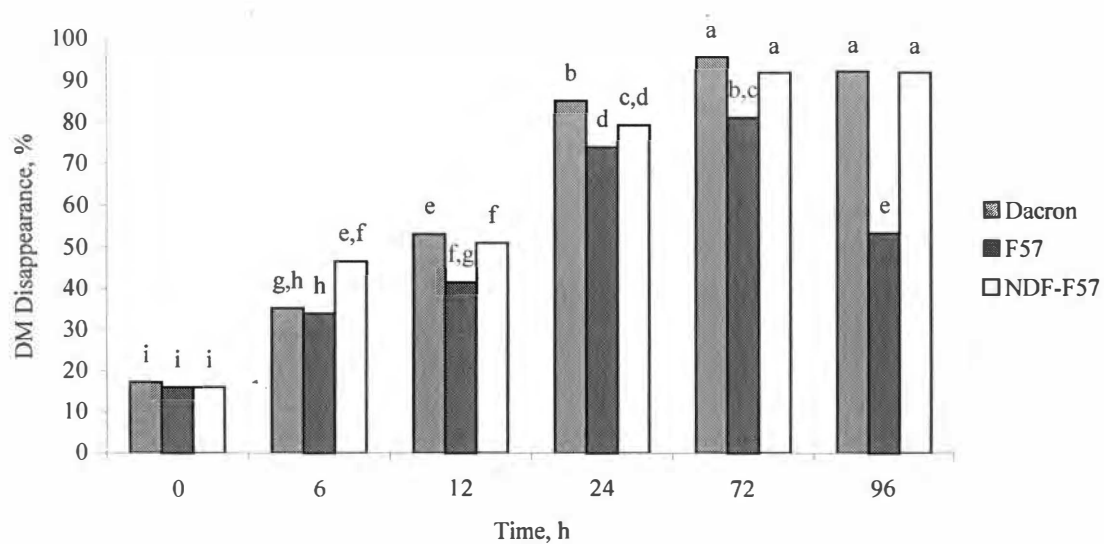
a,b,c,d,e,f, Least square means having different letters differ ($P < 0.05$).

Figure 4. *In situ* corn dry matter disappearance in Dacron, F57, or NDF washed F57 in Experiment 1.



a,b,c,d,e,f,g Least square means having different letters differ ($P < 0.05$).

Figure 5. *In situ* soybean meal dry matter disappearance in Dacron, F57, or NDF washed F57 in Experiment 1.



a,b,c,d,e,f,g,h,i LS Means having different letters differ ($P < 0.05$).

Figure 6. *In situ* soybean hulls dry matter disappearance in Dacron, F57, or NDF washed F57 in Experiment 1.

PART 4

**COMPARISON OF CHEMICAL FRACTIONS AND *IN SITU*
RUMINAL DRY MATTER DEGRADATION OF STOCKPILED KY31 E+,
JESUP AR542, AND JESUP E- TALL FESCUES**

Abstract

Tall fescue (*Festuca arundinacea schreb.*) is a perennial cool season grass. Fungal endophyte (*Neotyphodium coenophialum*) was found to have a symbiotic relation with tall fescue. Endophytes cause toxicity and lower animal performance. Tall fescue, free of endophytes (E-), solves the toxicity problem; however, the E- fescue has poor persistence in drought conditions and has low pest resistance. Non-toxic endophyte strains have been found in the Mediterranean region. One strain, AR542, was introduced in Jesup tall fescue to create a toxin-free endophyte-infected cultivar. Our objective was to compare chemical and ruminal digestion characteristics of tall fescue KY31 E+, Jesup E- and Jesup AR542. Stockpiled KY31 E+, Jesup E-, and Jesup AR542 tall fescues were hand clipped. Fresh forages were chopped and freeze-dried. Some fresh chopped samples were air-dried. Air-dried samples were ground to pass 2 mm screen. Fresh chopped, air-dried chopped, and air dried 2 mm screen size samples were put into Dacron bags. Air-dried and freeze dried samples were analyzed for chemical properties to determine protein and carbohydrate fractions. Samples were ruminally digested *in situ* at 96, 72, 36, 24, 12, 9, 6, and 3 h prior to removal. *In situ* digested sample fractions were determined for different times of incubation. CP and NDIP were determined for *in situ* digested samples. Chemical values were significantly affected by processing method. Freeze dried samples had higher protein fractions A, B1, B2. Air-dried samples had higher carbohydrate B2 and C fractions, and protein B2, B3 and C fractions.

Dry matter disappearances were different ($P < 0.001$) between Jesup E-, Jesup AR542, and KY31 E+ samples. KY31 had a lower DM disappearance percentage than Jesup E- or Jesup AR542 all time points. KY31 had 3.5 to 7 %/h lower DM disappearance rate than Jesup AR542. CP and NDIP values for *in situ* digested samples were similar among the three forages. Processing method significantly ($P < 0.001$) affected the percentage of DM disappearance. Results of chemical analyses and ruminal digestion characteristics in this study suggests that Jesup AR542 tall fescue can replace KY31 E+ while reducing the adverse affects of the endophyte toxins on grazing animals.

Key words: Tall fescue AR542, *in situ*, NDF

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is a perennial cool season grass that originated in Europe (Dibb and Darst, 1991). Tall fescue can be adapted to a wide range of climates. It can tolerate soil acidity, poor drainage, and is drought and pest resistant (Bacon, 1995; Joost, 1995). Tall fescue is grown on more than 15 million hectares in the United States (Dibb and Darst, 1991) and about 1.5 million hectares are currently growing in Tennessee (Fribourg et al., 1991).

Fungal endophyte (*Neotyphodium coenophialum*) was found to have a symbiotic relationship with tall fescue in the 1970s (Bacon et al., 1986; Bacon, 1995). Ergovaline, erginine, ergot alkaloids, the pyrrolizidine alkaloids and unique pyrolopyrazine alkaloid peramine have been extracted from endophyte-infected tall

fescue (Porter, 1995; Westendorf, 1991). Tall fescue toxicosis can be characterized by reduced feed intake, poor weight gain, elevated body temperature, reduced conception rates, long hair coats and nervousness (Bacon et al., 1986; Fribourg et al., 1991; Dibb and Darst, 1991). The most common problem is “summer slump” or “tall fescue toxicosis” with estimated economic losses of over \$600 million in the United States, and over \$85 million in Tennessee (Fribourg et al., 1991).

Researchers have removed endophytes from tall fescue and produced endophyte-free tall fescue (E-). Tall fescue E- had the same growth characteristics as endophyte-infected tall fescue, but did not exhibit toxic effects on animals. However, E- tall fescue did not tolerate drought or maintain pest resistances. Endophyte-free pastures have not had the longevity normally associated with E+ tall fescue. The University of Georgia and the Agricultural Research Center of New Zealand produced a new variety of tall fescue (Jesup, AR542) (Bouton et al., 2000). Non-toxic endophyte strains were introduced to tall fescue (Latch and Christensen, 1985; Christensen and Latch 1991). It was reported that this new variety does not cause toxic effects on cattle and sheep (Parish et al., 2003), and was resistant to drought conditions and insects (Pennington Seed, 2002).

Stockpiling tall fescue is a common practice for the reduction the amount of hay feeding during the winter season in which forage is limited for grazing (Ball et al., 1996). Stockpiling is accomplished by fertilizing tall fescue in late August or early September, allowing the forage growth in the fall to accumulate before grazing in early December. Tall fescue E+ and E- have been studied for chemical composition

and ruminal degradation. Since Jesup AR542 is a new variety available to farmers, chemical composition and ruminal digestion should be evaluated. Air-dried or freeze-dried processed samples have been used to measure ruminal digestion of feedstuffs. It has been reported that freeze dried samples induce better chemical and ruminal digestion (Abdalla et al., 1988 and Burritt et al., 1988). Mincing fresh samples could be a better sample preparation than freeze drying and grinding or chopping 6-mm length samples (Burke et al., 2001). The objectives of this research were to determine any chemical and ruminal digestion differences among stockpiled KY31 E+, Jesup E-, and JesupAR542 tall fescues, and to determine any chemical and ruminal digestion differences between fresh and air-dried forage samples.

Materials and Methods

Animals. Six Angus steers fitted with ruminal cannulas were utilized in this experiment. Steers were housed at Johnson Animal Research Teaching Unit and fed individually. Steers were used under a protocol approved by The University of Tennessee Animal Care and Use Committee. They were fed a diet of alfalfa cubes (70% of diet DM) and corn-based supplement (30% of diet DM) at a maintenance energy level with adequate degradable intake protein in two equal feeding (0830 and 1630) during the study. Diet chemical analyses are presented in Table 1¹. The steers were adapted to the diet for 15 days.

Sample preparation. Samples (six kg) of fresh stockpiled Jesup E-, Jesup AR542, and KY31 E+ were collected randomly by hand clipping with shears five to

seven cm above the ground at the Pasture Research Unit on Blount farm of the Knoxville Experiment Station in November 2002. Samples were placed in dark garbage bags. They were immediately brought to the laboratory and gassed with CO₂ to reduce oxidation. Samples were then chopped with a meat chopper (Fatosa Cutters 20 liter), with cutters at 3000 rpm. Two kg of fresh forage samples was chopped an average of eight minutes until the chopper produced a sample particle size similar to that previously reported by Burke et al., 2001. Chopped samples were taken to the Animal Science Laboratories, gassed with CO₂, and stored at 4° C until the next morning. Forty grams of fresh sample was calculated to be equal to four g of dry material. Therefore, forty grams of fresh sample was placed into numbered 10 cm x 20 cm Dacron bags. Burke et al. (2001) used 30 g of fresh sample for 10 cm x 10 cm Dacron bag. Dacron bags were stored in plastic Ziploc bags based on scheduled *in situ* insertion times. Ziploc bags were gassed with CO₂, sealed and stored in a freezer until *in situ* digestion took place. Another two kg of fresh chopped forage was divided into two subsamples. One kg of forage was air dried at 55° C for 48 h. The other kg of forage was placed into a freezer and later freeze-dried. Chopped air-dried forage samples were then divided into two parts. One-half of each air-dried forage sample was placed in Dacron bags based on scheduled *in situ* insertion time. A second half of each forage sample was ground to pass through a 2 mm screen in a Wiley mill. The 2 mm ground forage sample was weighed out and placed into Dacron bags based on scheduled *in situ* insertion times. The remaining samples were stored in glass jars prior to chemical analyses.

¹All tables are located in Appendix.

Freeze dried fresh samples were ground to pass through a 2 mm screen in a Wiley mill and stored in a glass jars prior to chemical analyses.

Chemical analyses. The Cornell Net Carbohydrate Protein Systems standard laboratory procedures and chemical analyses were used to determine forage fractions for samples. Non-protein nitrogen was determined with Kjeldahl method. CP, NDIP, ADIP, and SOLP were determined with nitrogen analyzer (LECO Corporation, St. Joseph, MI). NDF, ADF, and ADL were determined with the ANKOM200 Fiber Analyzer (ANKOM Technology, Fairport, NY). Based on these chemical analyses, Nutrient Requirements of Beef Cattle (NRC, 1996) equations were used to calculate carbohydrate and protein fractions.

In situ method. The *In situ* method was used to evaluate the disappearance of DM using #R1020 Dacron bags (10 cm x 20 cm, 50- μ m to 70- μ m pore size, ANKOM Technology, Fairport, NY). A permanent marker was used to label the bags. The dry Dacron bags were weighed and recorded. Duplicate or triplicate ground samples (5 g: 2-mm screen) were placed into identified Dacron bags. The Dacron bags were sealed with a heat sealer and Dacron bag identification numbers and mesh bag numbers were recorded. Mesh bags (used to prevent the loss of *in situ* bags with in the rumen) contained a maximum of 24 Dacron bags. The mesh bags were soaked in 39° C water for 15 min in a water thermos before being placed into the rumen under the particulate mat. The samples were inserted sequentially 96, 72, 36, 24, 12, 9, 6, and 3 h and removed at same time. Upon removal, samples were rinsed with cold water. The rinsed *in situ* bags were washed in a washing machine (low water level 45 L capacity; 10 times

with each rinse cycle consisting of 1 min agitation and 2 min spinning) until the bags were clean as described by Vanzant et al. (1998). The bags were dried at 55° C in a forced draft oven for 48 h. Dry weights were recorded for all samples. Duplicate samples were combined for each incubation time and stored in a glass jar until further analyses. DM was determined for combined samples, the final weight was corrected based on this DM. Crude protein and NDIP was determined on *in situ* digested samples. The CP and NDIP values of the *in situ* digested samples are not comparable with the CP or NDIP values of undigested samples since the dry matter loss of *in situ* digested samples has to be compensated in the calculation. The CP and NDIP values of digested samples were corrected based on *in situ* dry matter disappearance.

Statistical methods. Data were analyzed using Mixed Model Procedures in SAS (2001). A complete randomized design was used for analyses of variance. PDMIX800 and MMAOV macros were used for this SAS procedure (Saxton, personal communication, 2001). *In situ* digestion was done using six animals at the same time. Therefore, each number represents the mean value of six animals. Animals were used as a block in randomized block design; however, animal effects were not significant. Data were analyzed again as a complete randomized design. Treatments were arranged in a factorial combination of three stockpiled forage cultivars, with three types of processing: 1) chopped fresh, 2) chopped air-dried, and 3) 2-mm air-dried; and nine incubation times. If the DM disappearances values of *in situ* digested samples were threefold higher or lower than the standard error, they were removed from the data set. Standard deviations were calculated for each replicate of chemical analyses. If the

standard deviation was higher than those reported by the National Forage Testing Association web site, analyses were repeated. Dry matter disappearance rates were calculated by a SAS model reported by Mertens (1988).

Results and Discussion

Freeze-drying and air-drying were used to compare chemical differences between fresh and air-dried samples presented in Tables 2 and 3. Air-dried Jesup AR542 had the highest CP (24.6%). The percent CP of Jesup E- and KY31 E+ were 21.25 % and 23.24%, respectively. Air-dried Jesup AR542 and KY31 E+ samples had slightly higher CP than freeze-dried samples. Freeze-dried Jesup E- and AR542 samples had higher soluble protein, and NPN; however, KY31 E+ samples had higher nitrogen values for air-dried samples than freeze-dried samples. Air-dried samples had higher NDF, ADF and ash for all the samples. Percent NDIP and ADIP were also higher in air-dried samples. Ether extract (fat) was twice as high in freeze-dried samples compared to air-dried samples. Based on the chemical values shown in Table 2, the carbohydrate and protein fractions of each sample were calculated. Jesup E- and Jesup AR542 had higher carbohydrate fractions A and B1 than KY31E+. However, KY31 E+ had a higher B2 fraction than the other tall fescue cultivars. Air-dried samples had higher carbohydrate B2 fractions. Air-dried Jesup E- had lower carbohydrate C fraction (3.55%) than the other forages. Protein fractions were calculated as a percent of total protein. Fraction A (32.11%) was higher in air-dried Jesup AR542. KY31E+ had the higher B1 fractions. Air-dried samples had lower B2 fractions than freeze-dried samples. KY31E+ had higher B2 fractions: 7.69 and

32.63% for air- and freeze-dried samples, respectively. Air-dried B3 and C fractions were higher than freeze-dried values since the NDIP and ADIP values were higher. The overall drying process affected the chemical property of the stock piled tall fescue samples. Abdalla et al. (1988) reported that freeze-dried samples had 12% lower CP values and 47% lower NDIP values than air-dried samples. Our results did not indicate a large difference between air-dried and freeze-dried samples. The difference between our results and those reported by Abdalla et al. (1988) may be related to their use of liquid nitrogen was used to freeze the samples immediately after collection. Buritt et al. (1988) reported that hemicellulose and lignin concentrations were increased with air-drying compared to freeze-drying. Their findings were similar to our results.

Dry matter disappearances were different ($P < 0.001$) between Jesup E-, Jesup AR542, and KY31 E+ samples. Steer effects were not significant over all treatments. There were sample time interactions between the samples (Figure 1¹). Percent DM disappearances were lower in KY31 E+ than Jesup E- or Jesup AR542 at all time points. Percent DM disappearances were higher for Jesup AR542 at all times, except 0, 3, and 96 h incubation times in which case they were similar to Jesup E-. The percent DM disappearance of Jesup E- and KY31 E+ were similar at 72 and 96 h of incubations. Estimated rates of *in situ* dry matter disappearance are reported in Table 8. Fresh chopped Jesup AR542 had the highest DM disappearance rate (22.97 %/h). Fresh chopped samples had higher rates of digestion than chopped air-dried or 2-mm air-dried for each cultivar. Jesup E- digestion rates were similar for chopped air-dried and 2-mm

¹All figures are located in Appendix.

air-dried Jesup AR542, and fresh chopped KY31 E+ samples. KY31 E+ had lowest *in situ* digestion rate (8.99 %/h) for chopped air-dried processing. The percent CP of Jesup AR542 samples were similar to the percent CP of Jesup E- samples (Table 5). The percent CP of *in situ* digested KY31 E+ samples were higher than other forages. *In situ* digested KY31 E+ samples were similar for percent CP to others after 48 h. The percent NDIP of *in situ* digested samples were almost half of the percent CP of *in situ* digested samples. Forage availability from Jesup AR542 pastures is similar to toxic tall fescue (Bouton, 2002). Bondurant et al. (2001) reported that animals did not exhibit any adverse symptoms from grazing on Jesup AR542 pastures. Watson et al. (2001) reported that body weight, body condition scores and average daily gain were better in animals grazing on Jesup AR542 pastures compared to toxic fescue pastures. Based on chemical composition and ruminal digestion of Jesup AR542 compared to KY31 E+, livestock producers can use Jesup AR542 as an alternative to KY31 E+ and reduce incidence of tall fescue toxicosis. Kallenbach et al. (2003) reported that the nutritive value of stockpiled tall fescue was similar when the wild type of endophyte in KY31 was compared to another nontoxic endophyte (HiMag). They concluded that stockpiling tall fescue was a management practice producers could use to avoid toxicosis problems in fall and winter.

Processing affected ($P < 0.001$) percent of DM disappearance. There were processing and time interactions as shown in Figure 2. Fresh chopped samples had a higher percent of DM disappearance than chopped air-dried samples at 0, 3, 6, and 12 h incubations. After the 24 h incubation of samples, percent DM disappearances were

similar for both air-dried and fresh chopped samples. These results can be explained by changing in carbohydrate and protein fractions of freeze-dried and air-dried samples (Tables 3 and 4). Fast and medium soluble or digestible carbohydrate and protein fractions were higher in freeze-dried samples. After 24 h, slow digestible fractions could remain and be present at levels similar fresh chopped or chopped air-dried samples. The percent DM disappearances of 2-mm air-dried samples were higher at 0, 24, 48, 72, and 96 h than chopped air-dried samples, because the loss of smaller particles was greater from the 50- μ m to 70- μ m pore size Dacron bag. However, fresh samples had higher percent disappearances than 2-mm air-dried samples between 0 to 24 h incubations. *In situ* dry matter disappearance rates are shown in Table 8. Dry matter disappearance rates were higher in fresh chopped samples than air-dried samples. Fresh chopped Jesup AR542 had highest DM disappearances rate 22.97 %/h. Chopped air-dried KY31 E+ had the lowest rate of digestion. Overall DM disappearances, rates were similar for chopped air-dried and 2-mm air-dried samples. Processing method significantly ($P < 0.001$) affected *in situ* digested CP values. There were processing and time interactions as shown in Table 6. *In situ* digested fresh chopped samples had lower CP values than air-dried samples until 48 h incubation. After 48 h, effects of processing disappeared. Sample processing accounted for the differences in the NDIP values of the *in situ* digested samples. *In situ* digested fresh chopped NDIP values were lower than *in situ* digested air-dried NDIP values at 3, 6 and 12 h. After 48 h incubation, NDIP values were similar for all the samples. Abdalla et al. (1988) reported that freeze-drying increased solubility of

protein compared to air-dried samples, thus probably affecting rate of digestion during the first 24 h. In our study, fresh samples were compared to air-dried samples. Air-dried sample did not accurately represent fresh forage digestion.

Implications

Percent DM disappearances and rate of digestion was better in Jesup AR542 than in KY31 E+ stockpiled tall fescue samples. Fresh and air-dried samples were different for all variables until 24 h incubations. Based on the results of this study, care must be used in making predictions of ruminal digestion fresh forage from data obtained on air-dried samples.

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Appendix

Table 1. Chemical analyses of feed ingredients and diet.

Chemical Analyses	Alfalfa Cubes (70% of Diet DM)	Corn-based Supplement ^a (30% of Diet DM)	Total Diet
DM, %	91.79	90.73	-
CP ^b , %	13.68	10.02	12.58
NDF, %	57.00	25.63	47.58
ADF, %	43.26	3.30	31.27
ASH, %	10.00	2.80	7.84

^aSupplement: 98.16% corn; 1% molasses; and 0.83% mineral mix (27 % Ca, 0.4.5% P, 19.2% NaCl, 0.3% Mg, 0.1% K, 24 ppm Co, 36 ppm I, 20 ppm Se, 1000 ppm Cu, 3000ppm Mn, 0.002 Se, 3500ppm Zn, 240,000 IU/lb Vitamin A, 45,000 IU/lb Vitamin D-3 and Vitamin E 140 IU/lb).

^bNutrients on a DM basis.

Table 2. Chemical analyses of stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue^a.

Tall Fescue	Drying	DM, % AF	CP, % DM	NDF, % DM	Lignin, % NDF	SolP, % CP	NPN, % SolP	NDIP, % CP	ADIP, % CP	Fat, % DM	Ash, % DM
Jesup E-	Air	92.27	21.25	53.13	2.79	40.81	68.98	27.88	2.88	1.21	10.33
Jesup E-	Freeze	91.85	21.52	47.77	3.68	42.33	73.94	15.18	2.44	2.35	9.75
Jesup AR542	Air	92.90	24.17	54.11	4.47	43.41	73.98	29.24	3.17	1.65	11.21
Jesup AR542	Freeze	92.79	23.04	50.06	2.77	42.75	68.15	17.33	2.38	2.21	10.64
KY31 E+	Air	91.80	23.24	60.75	4.62	38.25	48.42	32.72	3.94	0.40	11.75
KY31 E+	Freeze	93.11	22.00	59.98	3.27	42.96	65.94	14.59	2.98	1.76	10.87

^aSolP = soluble protein; NPN = trichloroacetic acid (TCA) soluble nitrogen; NDIP = neutral detergent insoluble protein; and ADIP = acid detergent insoluble protein.

Table 3. Calculated carbohydrate fractions based on chemical analyses of stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue^a.

Tall Fescue	Drying	Carbohydrate Fractions ^b , % DM			
		A	B1	B2	C
Jesup E-	Air	11.03	0.11	52.51	3.56
Jesup E-	Freeze	14.76	0.15	47.24	4.22
Jesup AR542	Air	3.79	0.04	53.35	5.80
Jesup AR542	Freeze	11.15	0.11	49.51	3.33
KY31 E+	Air	-1.94	-0.02	59.84	6.73
KY31 E+	Freeze	1.32	0.01	59.33	4.71

^aBased on chemical analyses, carbohydrate fraction equations were used for calculations (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = sugars; fraction B1 = starch and pectin; fraction B2 = hemicellulose, and fraction C = cellulose and lignin.

^cStarch value were accepted as zero because these forages contain little starch. In the calculation starch = 1 was used to eliminate mathematical error while multiplying numbers to calculate fraction B1 instead number zero.

Table 4. Calculated percent protein fractions based on total crude protein of stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue^a.

Common Name	Drying	Protein Fractions ^b , % DM				
		A	B1	B2	B3	C
Jesup E-	Air	5.98	2.69	6.65	5.31	0.61
Jesup E-	Freeze	6.74	2.37	9.15	2.74	0.53
Jesup AR542	Air	7.76	2.73	6.61	6.30	0.77
Jesup AR542	Freeze	6.71	3.14	9.20	3.45	0.55
KY31 E+	Air	4.30	4.59	6.75	6.69	0.92
KY31 E+	Freeze	6.23	3.22	9.34	2.55	0.65

^aBased on chemical analyses, protein fraction equations were used for calculations (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = non-protein N; fraction B1 = Soluble CP in borate phosphate buffer; fraction B2 = insoluble in borate phosphate buffer but soluble in neutral detergent; fraction B3 = insoluble in neutral detergent but soluble in acid detergent; and fraction C = acid detergent insoluble CP.

Table 5. Crude protein of *in situ* digested stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue after different times of incubation.

Residual CP as % of Initial Dry Sample Weight			
Time, h	Jesup E-	Jesup AR542	KY31 E+
3	9.49 ± 0.2 ^b	10.20 ± 0.2 ^a	10.29 ± 0.2 ^a
6	7.84 ± 0.2 ^d	7.67 ± 0.2 ^d	8.82 ± 0.2 ^c
12	4.48 ± 0.2 ^f	3.89 ± 0.2 ^f	5.92 ± 0.2 ^e
24	2.05 ± 0.2 ^h	1.82 ± 0.2 ^{h,i}	2.80 ± 0.2 ^g
48	1.22 ± 0.2 ^{ij}	1.07 ± 0.2 ^j	1.55 ± 0.2 ^{h,ij}
72	1.18 ± 0.2 ^j	1.05 ± 0.2 ^j	1.46 ± 0.2 ^{h,ij}
96	1.18 ± 0.2 ^j	1.15 ± 0.2 ^j	1.53 ± 0.2 ^{h,ij}

^{a,b,c,d,e,f,g,h,i,j,k,l} Least square means having different letters differ ($P < 0.05$).

Table 6. NDIP of *in situ* digested stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue after different times of incubation.

Residual NDIP as % of Initial Dry Sample Weight			
Time, h	Jesup E-	Jesup AR542	KY31 E+
3	4.85 ± 0.2 ^{b,c}	5.11 ± 0.2 ^{a,b}	5.41 ± 0.2 ^a
6	3.86 ± 0.2 ^d	3.86 ± 0.2 ^d	4.53 ± 0.2 ^c
12	1.93 ± 0.2 ^f	1.87 ± 0.2 ^f	2.97 ± 0.2 ^e
24	0.91 ± 0.2 ^{h,i}	0.83 ± 0.2 ^{h,i}	1.39 ± 0.2 ^g
48	0.62 ± 0.2 ⁱ	0.60 ± 0.2 ⁱ	0.89 ± 0.2 ^{h,i}
72	0.71 ± 0.2 ^{h,i}	0.78 ± 0.2 ^{h,i}	1.15 ± 0.2 ^{g,h}
96	0.75 ± 0.2 ^{h,i}	0.80 ± 0.2 ^{h,i}	1.10 ± 0.2 ^{g,h}

^{a,b,c,d,e,f,g,h,i,j,k,l} Least square means having different letters differ ($P < 0.05$).

Table 7. Effect of processing on crude protein of *in situ* digested stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue after different times of incubation.

Residual CP as % of Initial Dry Sample Weight			
Time, h	Air dried 2mm	Fresh Chopped	Air dried Chopped
3	10.30 ± 0.4 ^{a,b}	8.93 ± 0.4 ^c	10.75 ± 0.4 ^a
6	8.51 ± 0.4 ^c	6.11 ± 0.4 ^d	9.72 ± 0.4 ^b
12	4.94 ± 0.4 ^e	3.63 ± 0.4 ^f	5.72 ± 0.4 ^d
24	2.02 ± 0.4 ^h	1.90 ± 0.4 ^{h,i}	2.74 ± 0.4 ^g
48	1.12 ± 0.4 ^j	1.29 ± 0.4 ^j	1.44 ± 0.4 ^{h,i,j}
72	1.04 ± 0.4 ^j	1.28 ± 0.4 ^j	1.38 ± 0.4 ^{i,j}
96	1.01 ± 0.4 ^j	1.35 ± 0.4 ^{i,j}	1.50 ± 0.4 ^{h,i,j}

^{a,b,c,d,e,f,g,h,i,j} Least squares means having different letters differ ($P < 0.05$).

Table 8. Effect of processing on percent NDIP of *in situ* digested stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue after different times of incubation.

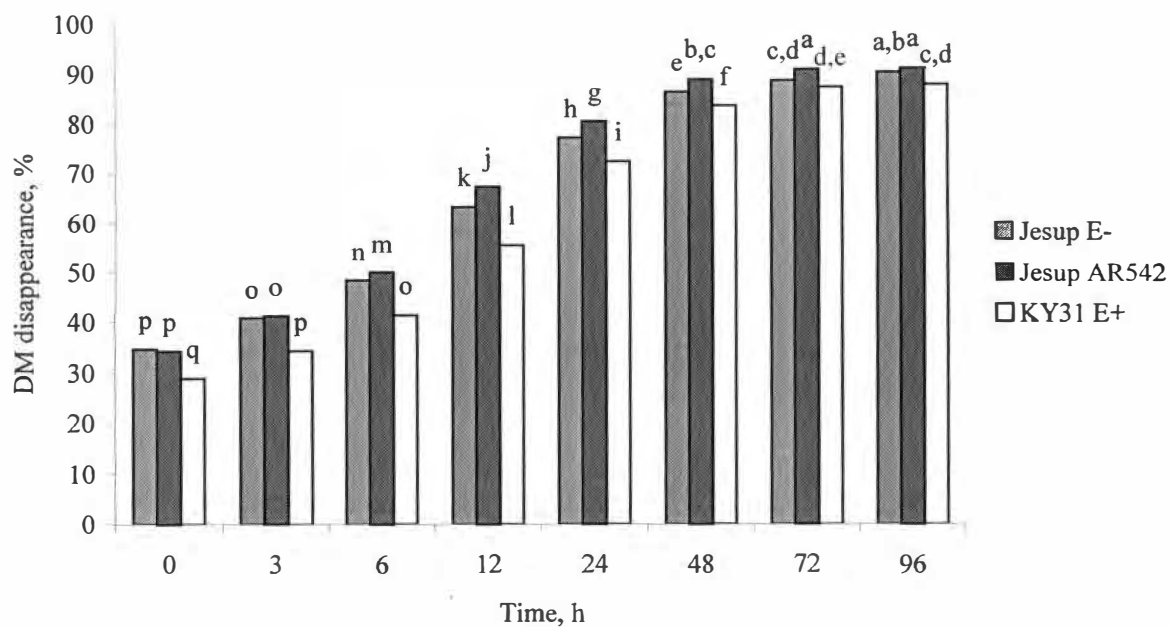
Residual NDIP as % of Initial Dry Sample Weight			
Time, h	Air dried 2mm	Fresh Chopped	Air dried Chopped
3	5.45 ± 0.2 ^{a,b}	4.11 ± 0.2 ^c	5.81 ± 0.2 ^a
6	4.41 ± 0.2 ^c	2.75 ± 0.2 ^d	5.10 ± 0.2 ^b
12	2.41 ± 0.2 ^d	1.52 ± 0.2 ^e	2.83 ± 0.2 ^d
24	0.97 ± 0.2 ^{f,g,h}	0.81 ± 0.2 ^{g,h}	1.36 ± 0.2 ^{e,f}
48	0.60 ± 0.2 ^h	0.75 ± 0.2 ^{g,h}	0.77 ± 0.2 ^{g,h}
72	0.77 ± 0.2 ^{g,h}	0.78 ± 0.2 ^{g,h}	1.08 ± 0.2 ^{e,f,g}
96	0.66 ± 0.2 ^{g,h}	0.95 ± 0.2 ^{f,g,h}	1.04 ± 0.2 ^{f,g,h}

^{a,b,c,d,e,f,g,h,i,j,k,l} Least square means having different letters differ ($P < 0.05$).

Table 9. *In situ* dry matter disappearance rates, %/h for stockpiled Jesup E-, Jesup AR542, and KY31 E+ tall fescue subjected to three different processing methods.

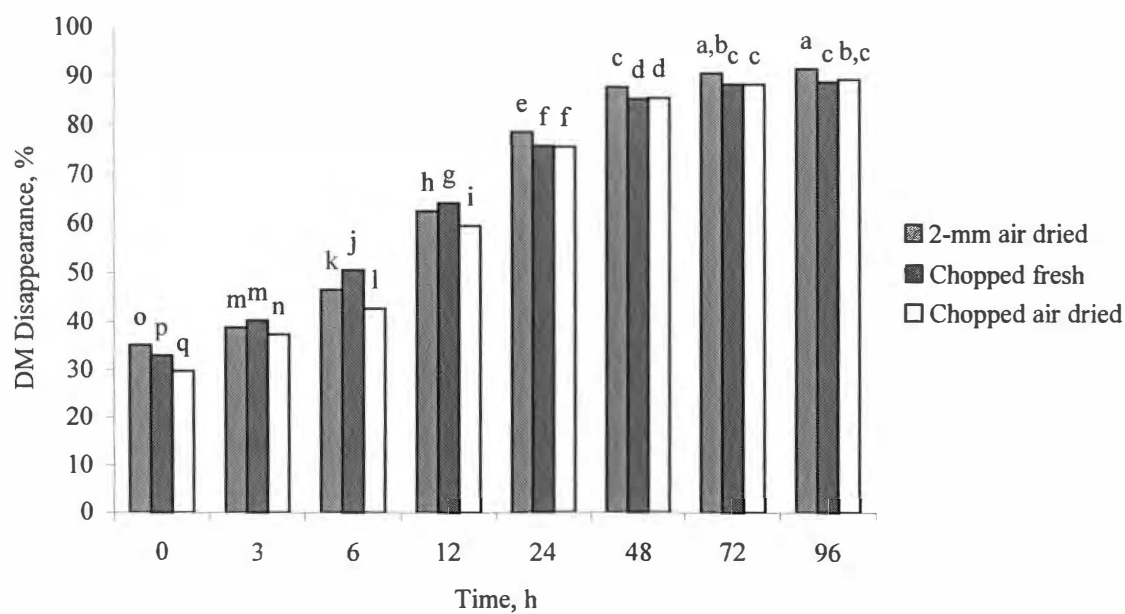
Samples	Processing	k, %h
Jesup E-	Air dried 2 mm	14.08 ± 1.3 ^{b,c}
	Fresh chopped	16.64 ± 1.3 ^b
	Air dried chopped	14.23 ± 1.3 ^{b,c}
Jesup AR542	Air dried 2 mm	13.79 ± 1.3 ^{b,c}
	Fresh chopped	22.97 ± 1.4 ^a
	Air dried chopped	13.00 ± 1.3 ^{b,c}
KY31 E+	Air dried 2 mm	10.87 ± 1.3 ^{c,d}
	Fresh chopped	13.14 ± 1.3 ^{b,c}
	Air dried chopped	8.99 ± 1.32 ^d

^{a,b,c,d}Least squares means having different letters differ ($P < 0.05$).



a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q Least square means having different letters differ ($P < 0.05$).

Figure 1. *In situ* Jesup E-, Jesup AR542, and KY31 E+ dry matter disappearance after different times of incubation.



a,b,c,d,e,f,g,i,j,k,m,n,o,p,q Least square means having different letters differ ($P < 0.05$).

Figure 2. *In situ* dry matter disappearance of fresh chopped, chopped air-dried and 2-mm ground samples after different times of incubation.

PART 5

CARBOHYDRATE AND PROTEIN FRACTIONS OF TALL FESCUE

WITH AND WITHOUT CLOVER

Abstract

Tall fescue (*Festuca arundinacea schreb.*) is a perennial cool season grass. A fungal endophyte (*Neotyphodium coenophialum*) was found to have a symbiotic relationship with tall fescue that causes reductions in animal performance. Several pasture management strategies have been tested to dilute or reduce the endophyte effects on animal performance. Rumen fistulated beef steers were used to collect masticate samples from tall fescue pastures with KY31 E+, KY31 E-, and KY31 E+ and clover in May, June, July, and August for two years. The objective of this study was to determine nutritional values of KY31 E+, KY31 E-, and E+ and clover mixture pastures. The chemical fractions were determined for all samples. Crude protein and NDIP were determined after 24 and 72 h of *in situ* digestion. KY31 E+ and KY31 E- masticate samples were similar in chemical composition and calculated fractions. Crude protein was high in KY31 E+ and clover samples. Neutral detergent fiber and ADF were higher in KY31 E+ and KY31 E- masticate samples than in KY31 E+ and clover mixture. KY31 E+ and clover had higher ($P < 0.001$) percent dry matter disappearances than KY31 E+ or KY31 E-. *In situ* digested KY31 E+ and clover had higher ($P < 0.001$) CP and NDIP than other forages. KY31 E+ and KY31 E- masticate samples were statistically similar for carbohydrate and protein fractions and ruminal digestion. Improved performance of animals grazing KY31 E+ by adding clover can be explained in part by the enhanced nutrient composition and changes in ruminal digestion found in this study.

Key words: Tall fescue, clover, *in situ*, masticate

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is a perennial cool season grass that originated in Europe (Dibb and Darst, 1991). Tall fescue can be adapted to a wide range of climates due to its ability to tolerate soil acidity, poor drainage, and drought. It is also pest resistant (Bacon, 1995; Joost, 1995). In the United States, tall fescue covers more than 35 million hectares in the United States (Dibb and Darst, 1991).

Fungal endophyte (*Neotyphodium coenophialum*) was found to have a symbiotic relationship with tall fescue (Bacon et al., 1986; Bacon, 1995). Ergovaline, erginine, ergot alkaloids, pyrrolizidine alkaloids and unique pyrolopyrazine alkaloid peramine have been extracted from endophyte infected tall fescue (Porter, 1995; Westendorf, 1991). Tall fescue toxicosis can be characterized by reduced feed intake, poor weight gain, elevated body temperature, reduced conception rates, long hair coats and nervousness (Bacon et al. 1986; Fribourg et al., 1991; Dibb and Darst, 1991). The economic due to tall fescue toxicosis is estimated at to be over \$600 million in the United States, and over \$85 million in Tennessee (Fribourg et al., 1991). Currently, there are several management practices established to reduce endophyte toxicities in animals. The endophyte can be removed from tall fescue resulting in a new endophyte-free (E-) tall fescue variety. Unfortunately, the endophyte free has a reduced persistence during drought conditions. McMurphy et al. (1990), Coffey et al. (1990) and Chestnut et al. (1991) have reported mixed pastures with tall fescue E+ and clover or E- tall fescue resulted in improved animal performance and reduced toxic effects. Therefore,

the objectives of the current experiment were to determine the nutritional value of KY31E+, KY31E-, and KY31E+ and clover mixture pastures using masticate samples collected by rumen fistulated animals.

Materials and Methods

Animal. Three Angus steers fitted with ruminal cannulas were used in this experiment. Steers were housed at Johnson Animal Research Teaching Unit and fed individually. Steers were used within a protocol approved by The University of Tennessee Animal Care and Use Committee. Steers were fed a diet of alfalfa cubes (70% of diet DM) and corn-based supplement (30% of diet DM) at a maintenance energy level with adequate degradable intake protein in two equal rations (0830 and 1630) throughout the study. Dietary chemical analyses were performed and results are shown in Table 1¹.

Sample preparation. Forage treatments were as follows: endophyte-infected tall fescue KY31 (E+): endophyte-free tall fescue KY31 (E-) and endophyte-infected tall fescue and white clover (KY31E+ and clover). Three different pastures were sampled using ruminally cannulated steers using procedure described by Holechek and Vavra (1983), and Olsen (1991) at Pasture Research Unit on Blount farm of the Knoxville Experiment Station, in June, July, and August 1997 and May, June, July, and August, 1998. Samples were placed in aluminum containers, put into plastic bags and chilled on ice, transported to the Animal Science Laboratories and stored in a freezer

¹All tables are located in the Appendix.

prior to processing. They were then lyophilized (Buritt et al., 1988). A total of 50 g sample was collected and ground to pass through a 2-mm screen and a 20 g subsample was ground to pass 1-mm screen in a Wiley mill.

Chemical analysis. Selected standard laboratory procedures and chemical analyses were used to determine forage composition. They included methods for DM, CP, NPN, SOLP, NDF, NDIP, ADF, ADIP, ADL, EE, and ASH. Non-protein nitrogen was determined with the Kjeldah method. CP, NDIP, ADIP, and SOLP were determined with a nitrogen analyzer (LECO Corporation, St. Joseph, MI). NDF, ADF, and ADL were determined with the ANKOM200 Fiber Analyzer (ANKOM Technology, Fairport, NY). Based on these chemical analyses, Nutrient Requirements of Beef Cattle (NRC, 1996) equations were used to calculate carbohydrate and protein fractions.

In situ method. The *in situ* method was used to determine the DM disappearance of samples using Dacron bags (5 cm x 10 cm, 50- μ m to 70- μ m pore size, ANKOM Technology, Fairport, NY). Permanent markers were used to label the bags. Dry Dacron bags were weighed and the weights were recorded. Ground samples (5 g) were placed into identified Dacron bags. At the end of the *in situ* procedure, samples were combined based on the incubation time for each run to produce a composite sample. Further analyses were conducted on composite samples. Dacron bags were sealed with a heat sealer and Dacron bag identification numbers and mesh bag numbers were recorded. Mesh bags (used to prevent loss of *in situ* bags in rumen) contained a maximum of 24 Dacron bags. Mesh bags were soaked for 15 min in 39 °C water before

being placed in the rumen under the particulate mat. Samples were inserted sequentially at 72 and 24 h prior to removal, and removed at same time. Upon removal, the *in situ* bags were washed with cold water and then rinsed in a washing machine (low water level 45 L capacity; 10 rinsing; each rinse cycle being one min agitation and two min spin per rinse) until the bags were clear as described by Vanzant et al. (1998). The bags were dried at 55° C in a forced draft oven for 96 h. Dry weights were recorded for all samples. Duplicate samples were combined for each time and stored in a glass jar until further analyses. Neutral detergent insoluble protein of *in situ* digested samples was determined. The CP and NDIP values of *in situ* digested samples are not comparable with those of undigested samples since *in situ* digested samples dry matter loss has to be compensated in the calculation. The CP and NDIP values of *in situ* digested samples were corrected based on *in situ* dry matter loss.

Statistical methods. Dry matter disappearance data were analyzed using Mixed Model Procedures in SAS (2001) as a completely randomized block design with multiple sampling. Samples were collected in June, July and August of 1997 and May, June, July and August of 1998. Year and month were used as blocks in the design. Treatments were arranged in a factorial combination of three forages with two incubation times. PDMIX800 and MMAOV macros were used for this SAS procedure (Saxton, personal communication, 2001). The *in situ* DM disappearances values of digested samples were not used if they were three times higher or lower than the standard error. Standard deviations were calculated for each replicate of chemical analyses. If the standard deviation was higher than those reported by the National

Forage Testing Association web site, analyses were repeated.

Results and Discussion

Chemical analyses of KY31 E+, E-, and KY31 E+ and clover masticate samples are shown in Tables 2, 3, and 4. Carbohydrate and protein fractions were calculated based on these results.

Carbohydrate fractions were calculated for KY31 E+, KY31E-, and KY31 E+ and clover masticate samples (Tables 5, 6, and 7). Year and month effects were not significant for chemical values or carbohydrate fraction values as reported earlier by Elizalde et al. (1999). Carbohydrate fraction A represents the non-fiber carbohydrates minus the starch. KY31 E+ and clover masticate samples had 14.56 % fraction A and were different ($P < 0.001$) from the other forages. Carbohydrate fraction A was -1.50 and -0.86% for KY31 E+ and KY31E- masticate samples, respectively. Carbohydrate B1 was expected to be higher in KY31 E+ and clover masticate samples because cool season forages generally contain little starch. Carbohydrate fraction B1 was -0.015 and -0.008% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover masticate samples had 0.14 % carbohydrate fraction B1. Neutral detergent fiber was 61.80 and 62.82% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover masticate samples had 44.19 % NDF and were different ($P < 0.001$) from the other pastures. Carbohydrate fraction B2 results were similar to NDF values. Carbohydrate fraction C, which represents undigestible carbohydrates, was 10.94% and 10.46% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover masticate samples had lower (7.47 %) carbohydrate fraction C. Ether

extracts and ash contents were not different among the samples. Elizalde et al. (1999) reported that carbohydrate fractions were similar in springtime for KY31 E+ and KY31 E- pastures.

Protein fractions were calculated for KY31 E+, KY31E-, and KY31 E+ and clover masticate samples (Tables 5, 6, and 7). Protein fraction A was 2.45% and 2.67% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover masticate samples had 4.16 % protein fraction A and were different ($P < 0.05$). Non-protein nitrogen values were 2.44, 2.68, and 4.16% for KY31 E+, KY31E-, and KY31 E+ and clover masticate samples, respectively. KY31 E+ and clover masticate samples had higher non-protein nitrogen. Protein fraction B1 values were 1.16 and 1.41% for KY31 E+ and KY31 E- masticate samples, respectively. KY31 E+ and clover masticate samples had 1.85 % protein fraction B1 value, and were similar to the B1 fraction of KY31 E- masticate samples. Fraction B1 and soluble protein values were different from B1 fractions. Soluble protein was 3.6 and 3.7% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover masticate samples had almost double (6.01%) the soluble protein of the others. Protein fraction B2 was 1.94, 1.62, and 3.33% for KY31 E+, KY31E-, and KY31 E+ and clover-masticate samples, respectively. Protein fraction B3 represents the differences between NDIP and ADIP. The NDIP of KY31 E+ and KY31E- masticate samples were 11.67 and 11.69%, respectively. KY31 E+ and clover masticate samples had 15.26% NDIP. ADIP was 3.70 and 8.82% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover-masticate samples had 5.01% NDIP and were different ($P < 0.04$). Protein fraction B3 was 8.12

and 7.68% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover-masticate samples had 10.24% protein fraction B3, and were different ($P < 0.009$) from the others. Protein fraction C which represents the undigestible protein (mostly ADIP) and was 3.65 and 3.95% for KY31 E+ and KY31E- masticate samples, respectively. KY31 E+ and clover masticate samples had 5.01% protein fraction B1 and were similar to KY31 E- samples C fraction. Protein values were reported to be similar for KY31 E+ and KY31E- pastures by Elizalde et al. (1999). Fraction B3 was reported to be lower in KY31 E- than in KY31 E+ pasture samples. Emile et al (2000) reported no difference in chemical composition and *in vitro* dry matter disappearance in E+ and E- hays. Other fractions were similar in KY31 E+ and E- pasture samples. Martz et al. (1999) reported that animal preferences play a crucial role in pasture grazing and the characteristics of masticate samples. In our research, the same two rumen cannulated animals were used to collect all the masticate samples. Additionally, these samples were combined for analyses to eliminate animal variation. Therefore, animal preference was minimized in the collection of masticate samples for this study. Animal performance has been shown to be increase by interseeding clover with tall fescue pastures. Martz et al. (1999) reported that clover in cool season pastures increased CP values in late August due to more available clover than spring season forages. Our results indicate that KY31 E+ and clover pasture had a better carbohydrate and protein fraction than KY31 E+ and KY31E- pasture.

Dry matter disappearances for *in situ* digested samples are shown in Figure 1².

²All figures are located in Appendix.

Percent disappearance of *In situ* digested KY31 E+ and KY31 E- masticate samples was similar. KY31 E+ and clover pasture masticate samples had higher ($P < 0.001$) percent disappearances than other masticate samples at all incubation time points. Chestnut et al. (1991) and Lusby et al. (1990) reported that animals had better performance on tall fescue E+ and clover pastures than on tall fescue E+ pastures. Our results indicated that KY31 E+ and clover samples were better digested, which could explain the improved animal performance on KY31 E+ and clover pastures. KY31 E+ and KY313E- did not differ in nutritional value or DM digestion. Therefore, differences in animal performance are associated with other factors (ergot alkaloids and toxins) rather than nutrient content of the forages. These other factors were not determined on the masticate samples used in this study. However, pastures were sampled for presence of endophytes and the results confirmed the designation of E+ and E- used in this study. Rumen parameters of E+ and E- tall fescue in this study would indicate that the problem of cattle grazing endophyte-infected pastures is related to the toxins in the forage rather than the nutrient content of the plants.

The CP values of *in situ* digested CP values are shown in Table 11. CP contents of *in situ* digested KY31 E+, KY31E- masticate samples were similar. *In situ* digested CP of KY31 E+ and clover masticate samples 10.04 and 6.34% and were higher ($P < 0.001$) at 24 and 72 h, respectively. The NDIP values for *in situ* digested samples are shown in Table 12. The NDIP values for *in situ* digested KY31 E+, KY31E-, and KY31 E+ and clover masticate samples were similar for at all incubation times. These results indicate that the rumen microbes are better supplied with available nitrogen when KY31

E+ pastures have clover present. This is important in developing supplementation strategies for cattle grazing tall fescue pastures. The greater digestion of CP at 24 h when clover is present could alter the animal's response to a supplement containing highly soluble protein sources. Protein sources that are not degraded rapidly in the rumen would probably result in better animal performance when clover is present. In addition, animals consuming tall fescue and clover pastures would respond better if a supplement containing more ruminally available carbohydrates were used.

Implications

KY31 E+ and clover pasture had better forage quality and digestion characteristics than KY31 E+ and KY31E- pastures. This difference may explain a portion of the improved performance reported for cattle grazing E+ tall fescue and clover pastures. However, the alkaloids affects associated with cattle grazing E+ tall fescue were not determined in this study. Performance differences between cattle grazing E+ and E- tall fescue could not be explained by the chemical analyses or ruminal digestion results obtained in this study. .

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Appendix

Table 1. Chemical analyses of feed ingredients and diet.

Chemical Analyses	Alfalfa Cubes (70% of Diet DM)	Corn-based Supplements ^a (30% of Diet DM)	Total Diet
DM, %	91.79	90.73	-
CP ^b , %	13.68	10.02	12.58
NDF, %	57.00	25.63	47.58
ADF, %	43.26	3.30	31.27
ASH, %	10.00	2.80	7.84

^aSupplement: 98.16% corn; 1% molasses; and 0.83% mineral mix (27 % Ca, 0.4.5% P, 19.2% NaCl, 0.3% Mg, 0.1% K, 24 ppm Co, 36 ppm I, 20 ppm Se, 1000 ppm Cu, 3000ppm Mn, 0.002 Se, 3500ppm Zn, 240,000 IU/lb Vitamin A, 45,000 IU/lb Vitamin D-3 and Vitamin E 140 IU/lb).

^bNutrients on a DM basis.

Table 2. Chemical analyses of KY31 E+ masticate samples^a.

Month	Year	DM, % AF	CP, % DM	NDF, % DM	Lignin, % NDF	SolP, % CP	NPN, % SolP	NDIP, % CP	ADIP, % CP	Fat, % DM	Ash, % DM
June	97	94.03	18.05	63.63	5.16	20.14	61.59	59.74	5.48	0.72	13.19
July	97	94.60	17.54	61.41	6.13	18.24	71.28	49.40	5.98	1.69	14.23
August	97	93.21	15.17	70.81	7.35	12.34	38.54	54.19	9.07	1.36	11.05
May	98	94.31	14.25	62.34	7.74	23.57	69.45	43.58	6.22	0.93	12.31
June	98	94.17	20.01	58.73	8.24	19.44	69.08	37.90	7.76	2.28	15.64
July	98	94.09	17.07	63.26	8.65	20.80	64.80	45.28	10.72	0.90	14.96
August	98	94.22	20.62	56.63	8.45	23.55	69.70	47.40	6.43	1.72	14.47

^aSolP = soluble protein; NPN = trichloroacetic acid (TCA) soluble nitrogen; NDIP = neutral detergent insoluble protein; and ADIP = acid detergent insoluble protein.

Table 3. Chemical analyses of KY31 E- masticate samples^a.

Month	Year	DM, % AF	CP, % DM	NDF, % DM	Lignin, % NDF	SolP, % CP	NPN, % SolP	NDIP, % CP	ADIP, % CP	Fat, % DM	Ash, % DM
June	97	94.04	19.73	66.01	7.23	18.56	62.25	81.20	8.90	1.45	12.69
July	97	93.70	18.85	66.94	7.97	27.99	57.20	51.61	7.95	1.95	14.59
August	97	93.13	16.47	67.37	4.71	16.40	57.61	52.90	7.14	2.63	11.38
May	98	93.60	14.86	64.44	6.42	23.46	53.60	41.30	7.71	1.69	11.58
June	98	94.28	18.65	56.39	6.44	23.27	70.50	35.17	6.15	1.60	14.18
July	98	94.60	15.76	60.16	7.12	26.82	61.15	35.43	7.18	2.38	12.64
August	98	94.69	19.67	57.98	5.96	24.96	66.98	36.93	5.93	2.01	12.41

^aSolP = soluble protein; NPN = trichloroacetic acid (TCA) soluble nitrogen; NDIP = neutral detergent insoluble protein, and ADIP = acid detergent insoluble protein.

Table 4. Chemical analyses of KY31 E+ and clover masticate samples^a.

Month	Year	DM, % AF	CP, % DM	NDF, % DM	Lignin, % NDF	SolP, % CP	NPN, % SolP	NDIP, % CP	ADIP, % CP	Fat, % DM	Ash, % DM
June	97	95.69	29.37	42.58	6.40	22.88	69.79	29.15	5.55	1.81	11.58
July	97	95.33	24.70	40.74	7.04	23.87	73.29	26.08	5.11	1.14	11.76
August	97	95.26	25.78	34.58	8.76	19.86	78.61	25.46	7.87	1.54	12.06
May	98	95.28	25.30	42.90	7.67	24.01	61.25	25.74	5.72	2.54	14.04
June	98	95.27	24.03	41.85	7.06	23.01	63.26	22.81	4.94	1.90	12.54
July	98	94.86	20.83	51.50	6.48	27.25	70.43	30.21	5.41	2.07	10.97
August	98	95.07	22.88	48.17	6.76	25.97	73.07	27.23	17.28	1.80	11.97

^aSolP = soluble protein; NPN = trichloroacetic acid (TCA) soluble nitrogen; NDIP = neutral detergent insoluble protein; ADIP = acid detergent insoluble protein.

Table 5. Calculated carbohydrate fractions based on chemical values of KY31 E+ masticate samples^a.

Month	Year	Carbohydrate Fractions ^{b,c} , % DM			
		A	B1	B2	C
June	97	-2.43	-0.02	62.63	7.87
July	97	-2.85	-0.03	60.35	9.08
August	97	-9.41	-0.10	69.44	12.49
May	98	-0.48	0.00	61.48	11.52
June	98	-6.66	-0.07	57.17	11.64
July	98	-7.42	-0.07	61.43	13.13
August	98	-3.63	-0.04	55.32	11.53

^aBased on chemical analyses, carbohydrate fraction equations were used for calculations (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = sugars; fraction B1 = starch and pectin; fraction B2 = hemi cellulose; fraction C = cellulose and lignin.

^cStarch value were accepted as zero because these forages contains little starch. In the calculation starch = 1 was used to eliminate mathematical error while multiplying numbers to calculate fraction B1 instead number zero.

Table 6. Calculated carbohydrate fractions based on chemical values of KY31 E-masticate samples^a.

Month	Year	Carbohydrate Fractions ^{b,c} , % DM			
		A	B1	B2	C
June	97	-9.47	-0.10	64.22	11.48
July	97	-13.46	-0.14	65.45	12.75
August	97	-4.24	-0.04	66.19	7.61
May	98	-1.38	-0.01	63.30	9.97
June	98	1.59	0.02	55.25	8.71
July	98	-0.14	0.00	59.03	10.32
August	98	0.80	0.01	56.81	8.30

^aBased on chemical analyses, carbohydrate fraction equations were used for calculations (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = sugars; fraction B1 = starch and pectin; fraction B2 = hemi cellulose; and fraction C = cellulose and lignin.

^cStarch value were accepted as zero because these forages contains little starch. In the calculation starch =1 was used to eliminate mathematical error while multiplying numbers to calculate fraction B1 instead number zero.

Table 7. Calculated carbohydrate fractions based on chemical values of KY31 E+ and clover masticate samples^a.

Month	Year	Carbohydrate Fractions ^{b,c} , % DM			
		A	B1	B2	C
June	97	9.65	0.10	40.95	6.55
July	97	15.87	0.16	39.49	6.89
August	97	20.60	0.21	32.55	7.27
May	98	8.65	0.09	41.45	7.93
June	98	13.68	0.14	40.66	7.05
July	98	7.58	0.08	50.38	8.10
August	98	11.32	0.11	44.08	7.83

^aBased on chemical analyses, carbohydrate fraction equations were used for calculations (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = sugars; fraction B1 = starch and pectin; fraction B2 = hemi cellulose; and fraction C = cellulose and lignin.

^cStarch value were accepted as zero because these forages contains little starch. In the calculation starch = 1 was used to eliminate mathematical error while multiplying numbers to calculate fraction B1 instead number zero.

Table 8. Calculated protein fractions based on chemical values of KY31 E+ masticate samples^a.

		Protein Fractions ^b , % DM				
Month	Year	A	B1	B2	B3	C
June	97	2.24	1.40	3.63	9.79	0.99
July	97	2.28	0.92	5.68	7.62	1.05
August	97	0.72	1.15	5.08	6.84	1.38
May	98	2.33	1.03	4.68	5.32	0.89
June	98	2.69	1.20	8.54	6.03	1.55
July	98	2.30	1.25	5.79	5.90	1.83
August	98	3.38	1.47	5.99	8.45	1.33

^aBased on chemical analyses, protein fraction equations were used for calculations. (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = non-protein N; fraction B1 = Soluble CP in borate phosphate buffer; fraction B2 = insoluble in borate phosphate buffer but soluble in neutral detergent; fraction B3 = insoluble in neutral detergent but soluble in acid detergent; and fraction C = acid detergent insoluble CP.

Table 9. Calculated protein fractions based on chemical values of KY31 E- masticate samples^a.

Month	Year	Protein Fractions ^b , % DM				
		A	B1	B2	B3	C
June	97	2.28	1.38	0.05	14.26	1.76
July	97	3.02	2.26	3.85	8.23	1.50
August	97	1.56	1.15	5.06	7.54	1.18
May	98	1.87	1.62	5.24	4.99	1.15
June	98	3.06	1.28	7.75	5.41	1.15
July	98	2.59	1.64	5.95	4.45	1.13
August	98	3.29	1.62	7.50	6.10	1.17

^aBased on chemical analyses, protein fraction equations were used for calculations. (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = non-protein N; fraction B1 = Soluble CP in borate phosphate buffer; fraction B2 = insoluble in borate phosphate buffer but soluble in neutral detergent; fraction B3 = insoluble in neutral detergent but soluble in acid detergent; and fraction C = acid detergent insoluble CP.

Table 10. Calculated protein fractions based on chemical values of KY31 E+ and clover masticate samples^a.

Month	Year	Protein Fractions ^b , % DM				
		A	B1	B2	B3	C
Jun	97	4.69	2.03	14.09	6.93	1.63
July	97	4.32	1.57	12.36	5.18	1.26
August	97	4.02	1.09	14.09	4.53	2.03
May	98	3.72	2.35	12.71	5.06	1.45
Jun	98	3.50	2.03	13.02	4.29	1.19
July	98	4.00	1.68	8.86	5.17	1.13
August	98	4.34	1.60	10.70	2.28	3.95

^aBased on chemical analyses, protein fraction equations were used for calculations. (Nutrient Requirements of Beef Cattle, 1996).

^bFraction A = non-protein N; fraction B1 = Soluble CP in borate phosphate buffer; fraction B2 = insoluble in borate phosphate buffer but soluble in neutral detergent; fraction B3 = insoluble in neutral detergent but soluble in acid detergent; and fraction C = acid detergent insoluble CP.

Table 11. Crude protein of *in situ* digested masticate samples after different times of incubation.

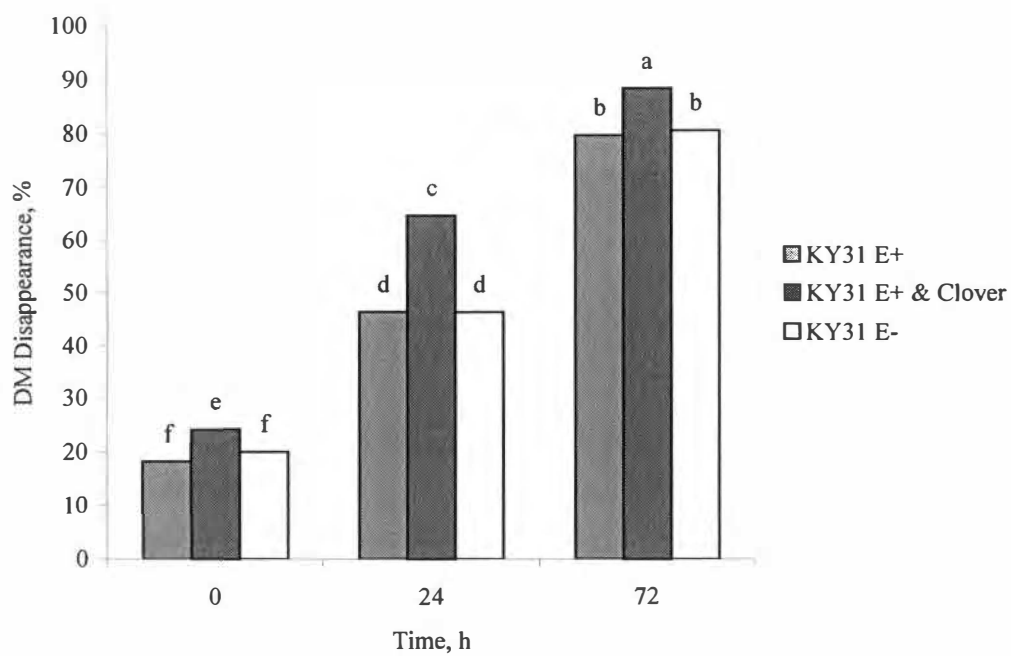
Residual CP as % of Initial Dry Sample Weight			
Time, h	KY31 E-	KY31 E- & Clover	KY31 E+
24	6.91 ± 0.8 ^b	10.04 ± 0.8 ^a	6.53 ± 0.8 ^{b,c}
72	5.18 ± 0.8 ^{d,e}	6.34 ± 0.8 ^{c,d}	4.64 ± 0.8 ^e

^{a,b,c,d,e}Least square means having different letters differ ($P < 0.05$).

Table 12. NDIP of *in situ* digested masticate samples after different times of incubation.

Residual NDIP as % of Initial Dry Sample Weight			
Time, h	KY31 E-	KY31 E- & Clover	KY31 E+
12	3.72 ± 0.6 ^a	3.57 ± 0.6 ^a	3.52 ± 0.6 ^a
24	1.17 ± 0.6 ^b	0.78 ± 0.4 ^b	0.86 ± 0.6 ^b

^{a,b}Least square means having different letters differ ($P < 0.05$).



a,b,c,d,e,f, Least square means having different letters differ ($P < 0.001$).

Figure 1. In situ dry matter disappearance of KY31 E+, KY31 E-, and KY31 E+ and clover masticate samples after different times of incubation.

Vita

Musa Yavuz is born in Senirkent, Isparta, Turkey on November 1, 1970. He was raised in Isparta, Turkey. He graduated from University of Cukurova, Adana, Turkey and received a B.A. in animal science in 1993. He went to the University of Tuskegee, Tuskegee and received a M.S. in animal science in 1997. From there, he went to the University of Tennessee, Knoxville and received Ph.D. in animal science in 2003.