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Dry matter intake and methane emissions of beef cattle grazing tall fescue pastures

Aaron Eugene Fisher

To the Graduate Council:

I am submitting herewith a thesis written by Aaron Eugene Fisher entitled "Dry matter intake and methane emissions of beef cattle grazing tall fescue pastures." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

John C. Waller, Major Professor

We have read this thesis and recommend its acceptance:

John H. Reynolds, Henry A. Fribourg

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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DRY MATTER INTAKE AND METHANE EMISSIONS OF BEEF CATTLE GRAZING TALL FESCUE PASTURES

A Thesis
Presented for the
Master of Science
Degree
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Aaron Eugene Fisher
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This thesis is dedicated to my parents, Harold E. and Betty Jane Fisher, for without their constant love and support, my entire college experience would not have been possible.

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Abstract

A two-year study was conducted to use methane (CH_4) production as an indicator of beef cattle efficiency on tall fescue (*Festuca arundinacea*) pasture management systems and to evaluate the importance of dry matter intake (DMI) among different tall fescue systems At Blount Unit, two steers on two pastures each of endophyte (*Neotyphodium coenophialum*) infected (E+) tall fescue, of endophyte free (E-) tall fescue, of E+/E- (1:1 ratio), and of E+/clover (*Trifolium repens*) were used to determine CH_4 and DMI At Holston Unit, four steers and four cow/calf pairs on one pasture each of a best management practices (BMP) pasture system and of an unimproved pasture (UIP) system were used to determine CH_4 and DMI Grazing occurred from March to September in 1997 and 1998. At Blount Unit, steers on E+ pasture gained less ($P < 0.05$) weight than those on the E- and E+/clover pastures and consumed less ($P < 0.05$) forage than on all other treatments There were no differences in ADG and DMI, except that cows consumed more ($P < 0\ 05$) forage than steers at Holston Unit Animals on the BMP produced less ($P < 0.05$) CH_4 than the UIP. Cows produced more ($P < 0.05$) CH_4 than steers The E+/clover and BMP pasture systems were lower ($P < 0.05$) in ADF and NDF and higher ($P < 0\ 05$) in CP and IVDMD than the other pasture systems within their respective unit The presence of clover in E+ tall fescue increased forage quality, DMI, and ADG over that of E+ tall fescue This coupled with other management strategies may reduce CH_4 production.

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1. LITERATURE REVIEW

Ruminants depend heavily on the fermentation of ingested feed and fiber to meet their daily nutritional requirements. The eructation of methane (CH_4) is the result of inefficiencies in the microbial fermentation of feed and forages in the rumen of the animal. The sulfur hexafluoride (SF_6) tracer technique (Westberg et al 1996) to measure CH_4 has never been used with animals actively grazing tall fescue. The CH_4 production values could possibly be used as an indicator of beef cattle efficiency when animals graze in tall fescue pasture-based management systems.

However, the main component of this thesis is estimation of dry matter intakes (DMI), generated by using fecal output (FO) and dry matter digestibility estimates Dry matter intake measurements are key to our understanding better the tall fescue dominated pasture systems used in the mid-south region These CH_4 and DMI values will be used to ascertain the fescue system that maximizes animal productivity and efficiency while maintaining forage stand persistence

Methane

Concern for the environment and awareness of changes in the ecosystem has become increasingly more important over the past twenty years The phenomenon of global warming is one of the major environmental issues. Global warming is a popular term given to the increase in the Earth's surface temperature due to the influence of increasing concentrations of greenhouse gases (IPCC, 1990). Greenhouse gases absorb solar radiation and re-emit

infrared radiation thus warming the earth (IPCC, 1990) Carbon dioxide (CO_2), CH_4, nitrous oxides (N_2O), and chlorofluorocarbons (CFC) have been designated as the most influential greenhouse gases (IPCC, 1990, USEPA, 1993a) In 1993, President Clinton and Vice-President Gore drafted *The Climate Change Action Plan.* This document called for a reduction in total greenhouse gas emissions to their 1990 levels by the year 2000 This reduction by executive order translates to about a 6% decrease in emissions from the enteric fermentation of ruminants (Johnson and Johnson, 1995). The fact that CH_4 is a very potent greenhouse gas, coupled with its increasing atmospheric concentrations, has caused an evaluation of methods to reduce CH_4 emissions (USEPA, 1993a)

Methane and global warming

Methane, second only to CO_2 in total contribution to global warming, is a trace greenhouse gas that is radiatively and chemically active Methane is radiatively active by trapping infrared radiation and preventing it from escaping the atmosphere. Methane is chemically active by reacting in the atmosphere and increasing concentrations of itself and ozone (O_3) as well as stratospheric levels of water vapor, all of which are greenhouse gases (USEPA, 1993a) Methane also influences the level of hydroxyl radicals (OH^-), which are responsible for cleaning up the accumulation of almost all gases in the atmosphere (Crutzen, 1991). The CH_4 and OH^- reaction is the predominant CH_4 sink (Crutzen, 1991, USEPA, 1993a) The Global Warming Potential (GWP) is a measure of the relative, globally averaged warming effect arising from the emissions of a

particular greenhouse gas It is a relative measure because it conveys the warming effect in relation to a reference gas (CO_2) (Isaksen et al, 1992) Methane is a more potent greenhouse gas than CO_2, being 60 times greater after a 20-year period and 22 times greater after a 100-year period (USEPA, 1993a) Methane is also considered to be approximately 21 times more effective at increasing radiative forcing when compared on a molecule-for-molecule basis to CO_2 (IPCC, 1990)

Methane concentrations remained relatively constant in the pre-industrial age (Crutzen, 1991) With increasing human populations and more importantly the Industrial Revolution, CH_4 levels have increased dramatically (Crutzen, 1991, Steele et al , 1992; USEPA, 1993b). In the 1980s, 12% of the total addition to global warming was due to CH_4 alone (Crutzen, 1991) In 1990, the CH_4 contribution to global warming rose to roughly 18% (USEPA, 1993a) Methane concentrations have been found to increase 0.9 to 1 0% per year (Watson et al , 1990, Crutzen, 1991, Johnson et al , 1994) Due to the increasing CH_4 levels, it should account for approximately 15 to 17% of global warming over the next 50 years (Johnson and Johnson, 1995)

There are many different sources of CH_4 These sources can be classified into two main categories, natural and anthropogenic Natural sources account for roughly 30% of all CH_4 emissions (USEPA, 1993b) The natural sources include wetlands, termites, oceans and freshwater systems, gas hydrates, and permafrost, with the largest of these being wetlands (USEPA, 1993b)

Anthropogenic, or non-natural sources are responsible for the remaining 70% of all CH_4 emissions (USEPA, 1993b) The anthropogenic sources of CH_4 include landfills, coal mining, natural gas systems, burning of fossil fuels, rice cultivation, livestock manure, and the enteric fermentation of domesticated livestock (USEPA, 1993a). Domesticated livestock collectively account for about 21% of all CH_4 emissions, thus making them the second largest contributor They provide from 4 6 to 6 9 teragrams (Tg) of CH_4 per year Beef and dairy cattle are responsible for the largest portion of all livestock emissions in the US, 69 and 26% respectively (USEPA, 1993a)

Methane and rumen fermentation

Bacterial fermentation is the process by which microbial activities convert diet ingredients into usable and unusable products for the animal The beneficial products include volatile fatty acids (VFA), microbial protein, and B-vitamins. The unusable products are CH_4, CO_2 and/or formic acid (HCOOH) while the detrimental products are ammonia and nitrate (Van Soest, 1994, Owens and Goetsch, 1993). The principal substrates of rumen fermentation are derived primarily from plant origins. They are cellulose, hemicellulose, pectins, starches, dextrans, and soluble carbohydrates (Bergman, 1990) The major VFA produced via rumen fermentation are acetic acid, propionic acid, and butyric acid Production of VFA represents approximately 75% of the dietary carbohydrate energy content The remaining 25% are lost mainly as H_2 and CH_4, (Bergman, 1990). Carbon dioxide and/or HCOOH that are produced will react with hydrogen

(H_2) to produce CH_4 and water (H_2O) This conversion is illustrated by the following reactions

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

$$HCOOH + 3H_2 \rightarrow CH_4 + 2H_2O \quad \text{(Van Soest, 1994)}$$

Cattle will generally lose up to 6% of their intake energy through the eructation of CH_4 (Johnson and Johnson, 1995). The bacteria responsible for methanogenesis are *Methanobrevibacter ruminantium*, *Methanobacterium formicicum*, and *Methmierobium mobile* This set of bacteria regulates the overall fermentation by the removal of H_2 This reduction of CO_2 and H_2 is the primary way that CH_4 is formed in the rumen (Yokoyama and Johnson, 1993)

Methane sampling methods

Respiration calorimetry chambers have been historically the preferred technique utilized for estimation of CH_4 production. Such enclosures include whole animal chambers, head boxes, ventilated hoods, and face masks (Johnson and Johnson, 1995) This system uses total airflow, inspired, and expired air measurements to calculate CH_4 production. Prediction equations have been generated from data collected in chambers for use in estimating CH_4 production The equations take into account such parameters as dry matter intake and digestibility as well as feed characteristics (Blaxter and Clapperton, 1965, Moe and Tyrell, 1979; Crutzen et al., 1986) The major disadvantage associated with using generated equations is the lack of consideration given to

the environment of the animal The natural environment of the animal is one of active grazing rather than a respiration chamber Additionally, CH_4 emissions have been found to be somewhat of an overestimation when the Blaxter and Clapperton prediction equations are used (Johnson et al , 1994).

The SF_6 tracer technique was developed at Washington State University as a way of collecting CH_4 samples in a more natural setting for the animal (Johnson et al , 1994, Johnson and Johnson 1995, Westberg et al , 1996). This method utilizes a permeation tube with a known emission rate of SF_6, an inert gas tracer, in the reticulum of the animal (Johnson and Johnson, 1995). For SF_6 to be an effective marker, it must meet several criteria The conditions are the release rate of the permeation tube must be constant and predictable, the tracer must have no impact on ruminal fermentation, the tracer must be detectable at low concentrations; and the tracer must be inert and nontoxic Sulfur hexafluoride meets these qualifications as it is a colorless, odorless gas that is used in pulmonary function tests and is detectable at one part per trillion (PPT) (Westberg et al , 1996) The CH_4 emission rate is calculated with the known SF_6 permeation rate and measured SF_6 and CH_4 concentrations (Johnson et al., 1994). The formula used is

$$Q_{CH4} = Q_{SF6} * [CH_4] / [SF_6]$$

where Q_{CH4} = CH_4 emission rate (g min^{-1}); Q_{SF6} = SF_6 permeation rate (g min^{-1}), $[CH_4]$ = measured CH_4 concentration (g min^{-1}); $[SF_6]$ = measured SF_6 concentration (g min^{-1}) (Johnson et al , 1994, Johnson and Johnson, 1995,

Westberg et al , 1996). The SF_6 tracer technique dispels the need to restrain and completely enclose the animal (Johnson and Johnson, 1995). This technique accomplishes CH_4 sampling during active grazing This is thought to be a true and more accurate assessment of the contribution domesticated livestock make to global CH_4 budgets

Tall Fescue

Tall fescue is a cool-season, perennial grass that originated in Europe It is believed that tall fescue entered the United States as a contaminant in other seed that was imported into the country (Stuedemann and Hoveland, 1988) Kentucky-31 tall fescue was found on a farm in Kentucky in 1931 and in 1942 was released by the University of Kentucky as a new cultivar The popularity and acceptance of tall fescue stemmed from its ease of establishment, wide range of adaptation, long grazing season, tolerance to abuse, pest resistance, good seed production, and excellent appearance when utilized for non-forage purposes (Stuedemann and Hoveland, 1988)

Tall fescue is one of the most widely grown forage crops in the United States and Tennessee, with 14 2 million and 1.42 million hectares, respectively (Fribourg et al , 1991c) It is very important to Tennessee beef cattle production Tennessee currently ranks ninth in the US in total beef cow numbers, with approximately 1 03 million in the state with cash receipts in 1998 of just over $376 million (Tennessee Agriculture, 1999).

The endophyte

Despite the early acceptance of tall fescue, problems with animal performance began to appear (Pratt and Haynes, 1950, Pratt and Davis, 1954) This problem continued to baffle scientists until the mid 70s when it was proposed that an endophytic fungus was the cause of the adverse effects on animal performance (Stuedemann and Hoveland, 1988) Bacon et al. (1977) implicated the fungal endophyte *Epichloe typhina* as the cause of the reduction in animal performance. This endophyte was reclassified by Morgan-Jones and Gams (1982) to *Acremonium coenophialum* The endophytic fungus is currently known as *Neotyphodium coenophialum* after the reclassification by Glenn et al (1996)

The endophyte and the host plant live in a mutualistic association in which the plant is provided insect, nematode, drought, and disease resistance Additionally, the fungus could cause the plant to be less appealing to the grazing animal due to toxins that are produced (Latch 1993). The endophyte in turn receives vital nutrients and protection from the host plant (Latch, 1993, Fribourg et al , 1996) This symbiosis reveals no outward signs of endophyte infection because the fungus resides between the cell walls of the plant (Fribourg et al , 1991b) The predominant toxin present in endophyte infested (E+) tall fescue is ergovaline (Paterson et al , 1995). Ergovaline accounts for 84 to 97% of the total ergopeptide alkaloid portion (Lyons et al , 1986)

Tall fescue toxicosis

Tall fescue toxicosis is a condition in cattle that is triggered by the ingestion of tall fescue infected with *N. coenophialum*. This fungus affects animal performance by decreasing average daily gains (ADG), conception rates, milk production, as well as promoting an inability to dissipate heat (Fribourg et al., 1991c, Paterson et al , 1995) Signs of tall fescue toxicosis include slightly elevated body temperatures, rough haircoats (Fribourg et al., 1991c) and decreased blood serum prolactin levels (Paterson et al., 1995). The production losses associated with tall fescue toxicosis are estimated to cost the beef cattle industry annually over $1 billion nationwide and in excess of $100 million in Tennessee (Fribourg et al., 1996)

Several methods have been explored to reduce this loss in animal productivity. Reducing the level of endophyte infestation and the addition of a legume into the pasture have shown promise Fribourg et al (1991a) found that, as the level of endophytic infestation decreased, ADG increased and the incidence of tall fescue toxicosis decreased Crawford et al. (1989) reported that spring-summer gains were increased by .068 kg/head/day for every 10% decrease in endophyte infestation level This same linear increase in ADG was not seen for the fall season A problem associated with decreased endophyte levels is a shift to higher infestation percentages over time, thus canceling the benefits of lower endophyte infestation Shelby and Dalrymple (1993) noted that E+ level will increase an average of 4.1% per year, with the highest degree of

increase being in the low E+ treatment group Gwinn et al. (1998) reported that for medium and high stocking densities, E+ levels increased by 20 to 30% after two years. However, the E+ levels in high E+ pastures and pastures with low grazing pressures remained constant over the same two years Marsalis (2000) found that stocking density increased the percent endophyte infection of intermediate E+ level pastures (24-56%) the most Changes in endophyte levels of high and low E+ pastures (0 and >80%, respectively) were negligible. Bacon and Seigel (1998) reported that endophyte free (E-) tall fescue may possess less pest resistance than E+ tall fescue Findings by Bouton et al. (1993) indicate that removal of the endophytic fungus from tall fescue will severely compromise the survival and productivity of the plant.

Pasture renovation is another method of reducing the severity of tall fescue toxicosis. Renovation is the addition of a legume, such as white ladino and/or red clover (*Trifolium pratense*) into pastures that have been properly limed and fertilized However, only approximately 10% of the tall fescue pastures in Tennessee have clover present (Fribourg et al , 1991c) Tall fescue/clover pasture systems produced the highest ADG and beef gain per hectare (Hoveland et al , 1981) Annual gains per steer were 45.4 kg higher on the fescue/clover pastures. Waller et al (1989) stated that percent of cows calving on renovated pastures of E+ tall fescue was higher than cows on non-renovated pastures They also saw an increase in ADG and subsequent weaning weights of calves grazing renovated E+ pastures. Thompson et al. (1993) and Paterson et al

(1995) reported that the addition of clover to E+ pastures decreased the effects of tall fescue toxicosis.

It has been suggested that the reduction in animal productivity can be attributed in part to decreased dry matter intake (DMI) of cattle grazing E+ tall fescue Waller et al. (1993) stated that steers grazing high E+ pastures had approximately 20% lower DMI than those on E- pastures. This is within the range reported by Fribourg et al. (1991c) of 10 to 50%. Goetsch et al. (1987) found that DMI decreased linearly by 0.0055% of body weight as the amount of E+ in the diet increased by 1%. This translates to a 28% higher DMI for the typical E- diet.

High environmental temperature has been shown to enhance the effects of tall fescue toxicosis Hemken et al (1981) demonstrated that at high ambient temperatures animals grazing E+ tall fescue would consistently consume less forage. Peters et al (1992) reported no differences in DMI during June 1988, but in August 1988, DMI was reduced by 18%.

Dry Matter Intake

Feed intake is a fundamental aspect of nutrition It influences animal response and function by setting the input level of all nutrients. Digestibility and utilization of nutrients are only qualitative descriptions of DMI Dry matter intake is governed by the requirements of the physiology and metabolism of the animal (Van Soest, 1994) With knowledge of DMD and FO, DMI can be estimated (Pond et al., 1987). The formula for estimating DMI is as follows.

$$DMI\ (g\ d^{-1}) = \frac{FO\ (g\ d\text{-}1)}{1 - (DMD / 100)}$$

(Pond et al., 1987, Burns et al , 1994)

Dry Matter Digestibility

Digestibility is simply the fraction of a dietary constituent that is lost during passage through the digestive tract of an animal, expressed as a percent of total constituent Digestibility is determined by measuring the quantity of feed that is consumed and the amount of feces that is eliminated by the animal after sufficient time for adaptation to the test diet (Cochran and Galyean, 1994). There are several different techniques that can be utilized for determining dry matter digestibility (DMD) of a forage or feedstuff Some of them include *in vivo*, *in situ*, and *in vitro* systems *In vitro* can be subdivided into enzyme based fermentation and microbial fermentation through the use of rumen fluid from a donor animal (Owens and Goetsch, 1993),

In vivo System

In vivo digestibility (Griffiths et al., 1993) is the conventional method for estimating DMD. It generates reliable digestibility estimates for harvested forages that are predominantly hand-fed. Confined animals are fed a diet for several days After this time period, feed consumption and fecal production are measured According to the purpose of the experiment, DMD is either calculated by subtracting the total amount of feces from the total quantity of forage that was fed or from the total forage consumption (Streeter, 1969)

The *in vivo* technique has several constraints and limitations Housing, animal restraint, and selection are some of the major constraints to be considered. The most ideal setting for this system is in a totally confined environment where such factors as temperature and photoperiod are completely controlled, with temperature being the most critical (Cochran and Gaylean, 1994) Animal restraint is also a very important issue The manner in which excreta are to be collected will dictate the method of restraint. Animal movement and exercise are very important concerns in that their presence tends to minimize animal behavior problems and soreness in the feet and legs. Animals that are selected for the experiment must possess a relatively docile disposition. They must also be trained in order to minimize disturbance during the digestibility trial This training can last anywhere from only a few days to several weeks (Cochran and Galyean, 1994).

The main limitation of the *in vivo* system is that animals in confinement are not able to graze in their natural setting Nelson and Furr (1966) found that animals graze forage throughout the day and night, whereas confined animals are fed in equal amounts at regular intervals according to the protocol of a given experiment Weir and Torrell (1959) reported that sheep consistently select forage that is higher in crude protein and lower in crude fiber than that of hand-clipped forage They also stated that it is not feasible to estimate forage consumption of a sheep from hand-clipping a pasture Streeter (1969) found that the conventional hand-fed method is of limited value in grazing studies This is

due to differences in selectivity and digestive processes that occur between grazing animals and hand-fed animals. The rate and consumption of forage can cause differences in passage rate and thus can influence digestibility (Balch, 1961)

In all other systems for determining DMD, the animals are allowed to graze actively. They do require the use of fistulated animals for collection of forage samples (Weiss, 1994)

In situ System

In situ disappearance (Griffiths et al., 1993) is measured by placing forages and feedstuffs into a fabric bag. The bags are then incubated in the rumen of the animal (Weiss, 1994). Microbes, fluids, and digestive end products move in and out of the sac through the pores. Material that disappears is considered digested (Owens and Groetsch, 1993) This is the best way to simulate the complete rumen environment (temperature, pH, buffer substrate, enzymes) The major weakness associated with the *in situ* technique is that processes such as mastication, rumination, and passage are not part of the system (Nocek, 1988). There are few data available on the accuracy of the *in situ* system, therefore precision is used as the determinant of the proper analytical technique (Weiss, 1994).

There are many areas that need to be addressed before effectively initiating an *in situ* digestibility trial Parameters such as bag porosity, particle size, sample size to bag surface ratio, dietary effects, animal effects, and

microbial contamination can affect *in situ* results (Nocek, 1988). Pores must be large enough to allow free movement of fluid and microorganisms between the sample bag and the rumen, but small enough to prevent loss of indigestible particles or the entry of other feed constituents (Weiss, 1994) Lindberg et al (1984) reported that nylon bags should be used with pore sizes no smaller than 10 μm in order to mimic more closely the *in vivo* microbial conditions. The most ideal particle size currently is not known The debate is whether the size of the particle should emulate pre or post mastication (Nocek, 1988, Weiss, 1994). Weiss (1994) stated that until more is known, samples should be milled to less than or equal to 5 mm in order to increase precision

The bag size has the least independent effect on *in situ* data, but sample size to bag surface ratio has a great influence on the results (Weiss, 1994). The optimum sample size is one that will provide enough residue for chemical analysis after incubation without overfilling the bag. This will delay bacterial attachment, increase lag time, and underestimate the rate of digestion (Nocek, 1988). Diet of the animal is the major factor that will determine the quantity and type of microbes that are present (Nocek, 1988) Weiss (1994) reported that the forage to concentrate ratio is the most important dietary effect. Species and biological type of the animal can also influence the results of the *in situ* system. Numerous species of animals have been used in *in situ* studies Sheep and cattle are the most commonly used (Nocek, 1988) Finally, microbial contamination can be a major source of variation when estimating true nutrient digestibility. This is

due to the intimate contact between the test feed and microbes within the rumen (Nocek, 1988)

Standards for various technical phases of the *in situ* assay have not yet been developed This lack of standards produces a large source of variation when comparing results of the assay between different laboratories (Weiss, 1994) *In situ* results are also subject to influx and efflux errors. This is because of the ability of some small components to leave the bag without being digested and the possibility that microbes enter the bag during the fermentation process (Owens and Goetsch, 1993) Additionally, Neathery (1969) stated that the higher the fiber content of roughages, the greater the difference between 72-hour disappearance values and published total digestible nutrients (TDN) values There are very few data available on the ability of the *in situ* technique to estimate total tract digestibility (Weiss, 1994).

Enzymatic *In vitro* System

Traditional *in vitro* systems (Griffiths et al , 1993) have been associated with uncontrollable variation, such as differences in rumen fluid collected from different animals on different diets Additionally, they do require access to a ruminally cannulated animal One way these problems can be overcome is by substituting enzymes for rumen fluid The mixtures generally consist of cellulase and/or pepsin (Weiss, 1994)

The initial enzymatic systems used were one-stage methods. The test feed was ground to pass through a 1-mm screen, then incubated in a cellulase

solution for 48 to 72 hours (Weiss, 1994) The DMD of the one-stage method was substantially less than that obtained from *in vitro* and *in vivo* systems (Jones and Hayward, 1973) A two-stage technique was developed which involves pre-treating the sample with pepsin in a HCl solution. In this technique, the sample is incubated for 24 to 48 hours at 40 to 50°C in pepsin/HCl solution. The pepsin is then removed and a cellulase solution is added (Weiss, 1994) Jones and Hayward (1975) reported much improved correlations with *in vivo* and *in vitro* data when the pepsin/HCl pretreatment was used Another enzymatic method utilizes a neutral detergent (ND) solution before treatment with cellulase Bugharar and Sleper (1986) stated that pretreatment with ND solution yielded higher DMD values than that of no pretreatment Both of these pretreatment methods produce DMD values that were more closely correlated to *in vivo* and *in vitro* digestibility estimates (Bughara and Sleper, 1986)

The use of enzymatic digestion as a method of determining DMD holds great promise Enzymatic digestion is a relatively inexpensive technique that utilizes commercially available enzymes (Weiss, 1994) The reliability of this method has been questioned (Owens and Goetsch, 1993) and many more studies are needed before it can be widely recommended It is best suited for a relative ranking of feeds rather than for producing accurate measures of digestibility (Weiss, 1994)

Rumen Fluid *In vitro* System

In vitro DMD is determined by incubating a test feed in rumen fluid and a buffer solution (Weiss, 1994). In 1919, Waentig and Gierisch were the first to utilize this technique for the evaluation of the digestibility of a feed Their results were 50% of the values seen in feeding trials This was due to too high a concentration of feed, which resulted in abnormally high acidity (Hungate, 1966). This inability to control pH was the major limitation of early *in vitro* systems. It confined incubation times to approximately 8 hours (Weiss, 1994) McDougall (1948) described the mineral composition of sheep saliva. Using this report, McDougall's buffer was developed and has made long term *in vitro* studies possible (Weiss, 1994) Several attempts were then made to imitate ruminal processes (Johnson, 1963) Warner (1956) established a set of criteria for evaluating different *in vitro* systems The standards include· the maintenance of numbers and normal appearance of the bacteria, selenomonads, and protozoa of the rumen, the maintenance of normal rates of digestion of cellulose, starch, and protein, and of normal interactions between these, the ability to predict quantitative results *in vivo* *In vivo-in vitro* relationships were developed for predicting DMD of forages (Baumgardt et al., 1958; Walker, 1959) The advent of the two-stage *in vitro* technique (Tilley and Terry, 1963) was the most important advancement in *in vitro* methodology. With some modification, this technique is still in practice on a widespread basis (Weiss, 1994)

A representative sample of the forage to be tested is dried and ground It is then incubated in rumen fluid that was collected from a fistulated animal. McDougall's buffer is added to the solution in order to maintain the pH level within the limits of what is usually found in the rumen of the animal This incubation is kept at 38°C in the dark for 48 hours The second stage begins with the tubes being centrifuged and the supernatant discarded. A HCl/pepsin solution is added to the residue of each tube It is important that both solutions be gassed with CO_2 in order to sustain anaerobic conditions The tubes are incubated again at 38°C for 48 hours. The weight of the undigested residue is determined and used with the initial weight to calculate DMD Modifications to the Tilley and Terry two-stage technique were made in order to increase laboratory throughput and efficiency (Alexander and McGowan, 1966, Minson and McLeod, 1972). The possibility of variation associated with *in vivo* methods is much larger than the controlled environment of *in vitro* digestion (Tilley and Terry, 1963) Thus, it is preferable to report *in vitro* data as opposed to *in vivo* (Tilley and Terry, 1963, Alexander and McGowan, 1966)

There are several factors that can influence *in vitro* results These include donor animal effects, run effects, pre-collection fasting interval, and basal ration effects (Ayres, 1991). The source of inoculum has a major influence on in vitro DMD values (Weiss, 1994) Bezeau (1965) found a highly significant difference in inoculum activity between two donor animals. Ayres (1991) also reported significant differences in inoculum between donor sheep that were fed the same

diet. Run-to-run variation within a single laboratory also can occur. Ayres (1991) found significant variation among runs as well as a significant run x test feed interaction

Increasing the pre-collection fasting interval will cause a decrease in the activity of microbial inoculum (Ayres, 1991) When animals are fed once daily, rumen fluid should be collected at a specific time post-feeding and kept constant throughout the experiment (Weiss, 1994) Feeding the animals three times daily will virtually remove this effect (Alexander and McGowan, 1966). The basal ration will also have an effect on inoculum activity (Ayres, 1991). The diet fed to the donor animal is the most influential factor affecting the microbial inoculum (Weiss, 1994). Alexander and McGowan (1966) stated that feeding coarsely chopped, medium quality hay would ensure uniformity.

Precision of the digestibility technique is important for reducing the replications needed High precision does not always mean high accuracy It is possible for a technique to be precise but not very accurate. Digestibility estimates need to be accurate to balance rations, determine economic value of forages and feedstuffs, and project animal growth and performance (Weiss, 1994) Although an important feature of the two-stage method is its accuracy, it does have disadvantages This method does require access to a ruminally cannulated animal Additionally, it does have a relatively long assay time Variation sources within this system have been identified and reduced so that

precision has become adequate. The rumen fluid *in vitro* technique is the best available laboratory method for estimating DMD (Weiss, 1994).

Fecal Output

Markers have been widely used to estimate such things as forage digestibility, FO, DMI, rate of passage, and fill of undigested residues Markers are classified as internal or external. Internal markers consist of natural constituents that are not digested or absorbed by the test animal. External markers consist of unnatural constituents that also cannot be digested nor absorbed Examples of external markers are chromic oxide (Cr_2O_3), ferric oxide, silver sulfide, and polyethylene glycol (Pond et al., 1987). Chromic oxide is the most commonly used marker It is insoluble in H_2O and is neither associated with the solid or liquid portions of the digesta (Burns et al , 1994) The major problem related with the use of Cr_2O_3 is diurnal variation in its excretion by ruminants This causes variation in Cr_2O_3 concentration within the feces (Hardison and Reid, 1953)

The controlled release device (CRD) has been developed in recent years to overcome the problems associated with once or twice daily dosing as well as circumventing the diurnal variation in fecal output of Cr_2O_3. It has been marketed under such names as Captec, Nufarm, Auckland, and New Zealand (Burns et al , 1994) The CRD bolus is administered the week prior to the start of sampling Fecal grab samples are collected for 5 consecutive days and then are combined for a composite sample. These weekly fecal combinations are then freeze-dried

and ground. Atomic absorption spectophotometry is used to analyze the fecal samples for Cr_2O_3 Fecal output is calculated using the following equation:

$$FO\ (g\ d^{-1}) = \frac{\text{marker administered } (\mu g\ d^{-1})}{\text{fecal marker concentration } (\mu g\ d^{-1})}$$

(Pond et al., 1987)

Adams et al (1991) found that the CRD method was a good and reliable technique for measuring FO Compositing of grab samples from 5 consecutive daily collections is a reliable way to predict total FO when Cr_2O_3 is utilized (Momont et al., 1994).

Summary and Objectives

Methane is a byproduct of the microbial fermentation that occurs within the rumen and represents inefficiencies in this enteric fermentation Methane has also been implicated in contributing to the global warming of the planet. The SF_6 tracer technique can be used to estimate CH_4 production from cattle during active grazing of tall fescue pasture systems In the mid-south region, this is important because tall fescue pasture systems support a multi-billion dollar cow/calf industry

Measuring DMI is critical for making inferences about forages and subsequent animal responses Accurate DMI values are the basis for the application of nutritional requirements in formulating rations to achieve desired animal responses (Burns et al , 1994). Estimating the DMD of tall fescue is also important to the region The "rumen fluid *in vitro* system" is currently the best

available method for determining DMD. Along with FO, DMD values can be used to calculate DMI.

The objectives of this study were (1) to use CH_4 production as an indicator of beef cattle efficiency on tall fescue pasture management systems, (2) to evaluate the difference of DMI among different tall fescue systems, and (3) to contribute to a cooperative regional database on forage and beef cattle productivity

2. MATERIAL AND METHODS

Treatments

Eight 1.2 ha pastures that were part of an existing grazing study at the Blount Unit (35° 49′N, 83° 13′W) of the Knoxville Experiment Station were used There were two replications of four pasture systems (1) E+ tall fescue, (2) E- tall fescue, (3) E+ tall fescue/clover, and (4) alternating groups of four 20-cm drill rows of E+ and E- tall fescues

Two unreplicated pastures of approximately 4 ha each at the Holston Unit (35° 57′N, 83° 51′W) of the Knoxville Experiment Station also were used The pasture systems were (1) an unimproved pasture (UIP) typical of the region (tall fescue, bermudagrass (*Cynodon dactylon*), Kentucky bluegrass (*Poa pratensis*), other grasses and weeds), and (2) a best management practices (BMP) pasture typical of well managed farms in the region (E+ tall fescue/clover).

Phosphorus (P) and potassium (K) fertilizers were applied to all the pastures (except for the UIP at the Holston Unit) in winter or early spring of each year to maintain a medium soil test level of fertility All pastures except the pastures containing clover and the UIP at the Holston Unit received 56 kg nitrogen (N) ha^{-1} applied as ammonium nitrate in early spring and early September of each year

The UIP at the Holston Unit has received no inputs in pasture management, such as fertilization, seeding of improved species, and mowing, in the recent past All other pastures were managed so they provided between 900

and 1500 kg ha^{-1} of available dry matter forage at all times, as estimated every 21 days with 53 3 x 304 cm clipped forage strips. This should have provided enough forage to allow adequate voluntary intake by the cattle. Within each pasture, artificial shade, fresh water, and mineralized salt were provided to the experimental animals.

Experimental Animals

At the Blount Unit, two Angus steers (*Bos taurus*) were selected from each experimental pasture These pastures were already part of a larger existing grazing study At the Holston Unit, four Angus steers and four Angus cow/calf pairs (*Bos taurus*) were placed on each of the 2 experimental pastures.

The steers used in the first year of this study were weaned stockers selected from the Knoxville and the Plateau Experiment Stations from the spring 1996 calf crop, and the steers used in the second year of the study were weaned stockers from the spring 1997 calf crop The mature (>3-yr old) cows from the Knoxville Experiment Station spring calving herd were pregnancy checked in fall 1996 and again in fall 1997 Two of the eight cows used in 1997 were not pregnant before the start of the 1998 grazing year, and were replaced with two pregnant cows of similar age and body condition All cattle used were selected on the basis of age, weight, and body condition

The experimental animals were weighed every 21 days while on pasture The weights were determined to calculate ADG for each individual animal Regression analysis of the 21-d weights was performed to obtain ADG

measurements The slope of the regression line was found to be the ADG Gains were expressed as yearly ADG (g d^{-1}). Body condition scores (BCS) on a 9-point scale were recorded for cows at the beginning of the spring 1997 and at the end of the summer 1997 grazing season.

Grazing Seasons

The grazing seasons were spring/summer of 1997 and 1998 At the Blount Unit, the 1997 grazing season began on March 26 and went through September 9 The 1998 season began on March 26 and went until August 26 At the Holston Unit, the 1997 grazing season began on May 5 and finished on September 8 The 1998 season began on May 4 and went through September 5.

Methane Production

Methane was collected only during the summer 1998 grazing season for this experiment. In order to facilitate a seasonal comparison of CH_4 production, CH_4 data for spring and summer 1997 and spring 1998 were taken from Pavao-Zuckerman et al (1999) Methane data were expressed as season CH_4 (g d^{-1}), CH_4 per unit of ADG (g kg^{-1} d^{-1}), and CH_4 per unit of metabolic weight (MW) (g $kg^{-0.75}$ d^{-1})

The SF_6 tracer gas method developed at Washington State University was used to measure the CH_4 emissions from the steers and cows on the experimental pastures The same protocol explained in detail by Pavao-Zuckerman et al. (1999) was followed for this study, and thus a brief description

is provided. The SF_6 tracer technique involves placing a permeation tube, with a known permeation rate of SF_6 (ng min^{-1}), in the reticulum of the animal Eructated gases (SF_6, CH_4, and CO_2) were constantly sampled through a collection device worn by the animal. Knowing the rate of SF_6 permeation from the tube, and measuring concentrations of CH_4 and SF_6 in the collection canister, the CH_4 emission rates from each animal were calculated

Collection system

Each permeation tube was a 5 08-cm long brass capsule, fitted with a swagged nut, stainless steel frit, and a thin piece of Teflon, through which SF_6 was emitted. Each tube was filled with approximately 1 0 g of SF_6. The emission rates for all permeation tubes were calibrated by weighing the tubes each week over a two-month period while they were incubating in a 39°C water bath Permeation tubes with emission rates greater than 800 ng*min^{-1} were utilized in the experiment

Collection canisters were made from 50 8-cm lengths of 5 08-cm diameter white PVC tubing The canisters were heated until flexible enough to be bent into an ox-bow shape. A valve connected by Teflon tubing to a quick-connect was attached to the top of the canisters The quick-connect was connected to halters for sample collection and later used to connect to the injection port on the gas chromatograph (GC) (SRI Instruments, Model 8610C, Torrance, CA 90503-2162) for analysis The software used was PeakSimple for Windows Velcro straps, swivel hooks, and cable ties were used to secure the canisters to the collection

halters worn by the experimental animals. A vacuum was created inside the canister such that it would take in at least 27-hr of exhaled air by the animal

The collection halters were large, adjustable horse halters, fitted with a leather patch sewn on top of muzzle to secure the filter end of the tubing system to the halter. The tubing system used on the halters consisted of a 35 56-cm length of 0.127-mm inside diameter stainless steel capillary tubing The filter was placed in an appropriate length of 2 54-cm diameter PVC pipe for protection and was attached to the leather patch with cable ties The patch with filter was placed on top of the muzzle, between the nostrils of the animals

Methane sampling periods

Methane sampling periods of 6 days were conducted on the eight pastures at the Blount Unit and on the two pastures at the Holston Unit in July and August of 1998. In this study, CH_4 sampling was done only in the summer because it is the completion of the work done by Pavao-Zuckerman et al (1999). Sampling periods began on Monday morning and ended on the following Saturday morning Five 24-hr CH_4 samples per animal were taken during each sampling period

At the beginning of the spring grazing season, a permeation tube was administered via a balling gun to each experimental animal At the end of the experiment, the permeation tubes were removed surgically by rumenotomy The Knoxville Experiment Station held all animals for a minimum of 120 days after

removal of the tubes in accordance to the Department of Health and Human Services Investigational New Animal Drug (INAD) file number 9542.

Canisters also were placed near the experimental pastures to monitor background levels of CH_4 and SF_6 daily during each sampling period Background CH_4 and SF_6 were not considered significant (data not shown) enough to warrant inclusion into the calculation of daily cattle CH_4 emissions.

Laboratories used

Sulfur hexafluoride molecules tend to reside in plastics and other materials to which they are exposed. In order to prevent contamination with SF_6, and to provide better laboratory space for the GC, three separate laboratories were established at The University of Tennessee

Two laboratories were utilized in the Brehm Animal Science Building The first Animal Science laboratory was used as a workspace. In this location, collection halters and canisters were constructed and repaired, tools and replacement equipment were stored, and collection canisters were prepared for both collection and GC analysis The second one housed only the GC and supporting equipment in an attempt to reduce contaminants that would interfere with GC analysis.

A separate laboratory was established in the Ellington Plant Sciences Building In this laboratory, the permeation tubes were filled with SF_6, incubated in a 39°C water bath, and weighed weekly to calibrate permeation rate

Methane calculation

The following equation was used to estimate the emission from each animal

$$Q_{CH4} = Q_{SF6} * [CH_4] / [SF_6]$$

where Q_{CH4} = CH_4 emission rate (g min^{-1}); Q_{SF6} = SF_6 permeation rate (g min^{-1}), $[CH_4]$ = measured CH_4 concentration (g min^{-1}), $[SF_6]$ = measured SF_6 concentration (g min^{-1}) (Johnson et al., 1994, Johnson and Johnson, 1995, Westberg et al., 1996) The SF_6 emission rate was determined from the decay rate derived from laboratory calibration of the permeation tube in the animal The sample CH_4 and SF_6 concentrations were obtained through analysis of the daily sample with the GC

Dry Matter Intake Estimation

Dry matter intake was estimated by using the FO and DMD data. The equation used is as follows

$$DMI\ (g\ d^{-1}) = \frac{FO\ (g\ d^{-1})}{1 - (DMD / 100)}$$

(Pond et al , 1987, Burns et al , 1994)

Fecal Output Determination

The week prior to collection, a Captec bolus (Captec (NZ) LTD, Auckland, New Zealand) with a known emission rate of Cr_2O_3 was administered to each experimental animal via a balling gun All animals at the Blount and the Holston

Units in 1997 as well as the cows at the Holston Unit in 1998 received boluses with an emission rate of 1.5 $\mu g\ d^{-1}$ All steers at the Blount and the Holston Units in 1998 were administered boluses with an emission rate of 1 46 $\mu g\ d^{-1}$ Fecal grab samples subsequently were collected from the animals on the same six days that CH_4 samples were taken Enough feces to fill two small plastic cups were taken per animal The fecal samples were put into a plastic cup and immediately placed on ice for transport to the laboratory They were frozen for storage The fecal samples were freeze-dried and ground using a coffee grinder (Brahn, Inc , Model KSM 2B, Woburn, MA 01801-3376) Once ground, the weekly samples were combined on a per animal basis and placed in Ziploc freezer bags. The composited samples were then shipped to the University of Georgia Forage Laboratory for Cr_2O_3 analysis via atomic absorption spectophotometer Fecal output was calculated using the following equation

$$FO\ (g\ d^{-1}) = \frac{\text{marker administered } (\mu g\ d^{-1})}{\text{fecal marker concentration } (\mu g\ g^{-1})}$$

(Pond et al , 1987)

Dry Matter Digestibility Evaluation

Dry matter digestibility was determined using the Moore modification of the Tilley and Terry (1963) two stage *in vitro* technique (Moore and Dunham, 1971) Ruminally fistulated steers were used for the collection of pasture samples from the 8 experimental pastures at the Blount Unit and from the 2 experimental pastures at the Holston Unit The steers were allowed to graze each

experimental pasture for approximately 20 minutes The rumen contents were then evacuated and transported to the laboratory and frozen The samples were air dried in a 60°C forced air oven and ground to pass through a 2-mm Wiley Mill screen The samples were analyzed via near-infrared technology (NIR) (FOSS NIRSystems, Model 5000, Silver Spring, MD 20904) for the estimation of dry matter (DM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) concentrations After NIR analysis, the samples were ground to pass a 1-mm Wiley mill screen for *in vitro* DMD determination.

Rumen fluid inoculum

A donor animal was maintained on a medium quality alfalfa (*Medicago sativa*) diet with supplementation of 0.454 kg d^{-1} of 48% soybean meal The supplementation was given at approximately 7.00 AM with collection being 2-hr post-feeding Whole ingesta from the top half of the rumen were removed and transferred to a trashcan lined with a plastic trash bag Enough of the remaining contents to fill three insulated containers were squeezed and strained through four layers of cheesecloth Fluid was transported from the Dairy Farm to the nutrition laboratory (about 15 minutes) where it was flushed with CO_2 and then placed into a large glass bottle in the water bath containing the McDougall's saliva (McDougall, 1948) An appropriate quantity of McDougall's saliva was prepared prior (40 mL per tube plus 200 mL extra) and placed into a 13 25 liter bottle One ml of 4% $CaCl_2$ per liter of McDougall's saliva was added to the bottle and then it was put into a 39°C water bath and CO_2 was bubbled gently through

the bottle The pH of the buffer was adjusted to between 6.9-7 0 in order to mimic the natural buffer in the animal more closely One part rumen fluid was added to 4 parts buffer and was allowed to mix for 10 min by the action of the bubbling CO_2.

Dry matter determination

The initial dry matter (DM) was determined by placing approximately 0.5 g of the pasture sample into a dry, tared, numbered crucible and weighed to 1/10,000th of a gram on a laboratory balance The crucibles containing the samples were then placed into a drying oven overnight at 105°C. The following morning, the samples were removed from the drying oven and cooled in a desiccator for approximately 1 hr and weighed again to 1/10,000th of a gram. Dry matter of the samples were calculated by the following formula:

$$\text{DM (\%)} = \frac{\text{dry sample weight (g)}}{\text{wet sample weight (g)}} * 100$$

First stage of digestion

Approximately 0.5 g of each sample were weighed to 1/10,000th of a gram and placed into a numbered centrifuge tube The number of the tube corresponded to the number on the crucible used for dry matter determination Four extra tubes were used as blanks, in which only media (rumen fluid, McDougall's saliva, and distilled water) was placed in these tubes Two mL of distilled water were added to each centrifuge tube to moisten the sample and then the tubes were agitated with a test tube mixer

Upon thorough mixing, 50 mL of media were added to each centrifuge tube gently using a automatic pump. The tubes were placed into a water bath immediately after adding media and flushed with CO_2 for 15 seconds. The tubes were sealed quickly with a rubber stopper and fitted with a Bunsen valve The tubes were later transferred to a 39°C water bath and were swirled 1 hour later to ensure that all forage particles were soaked with media This swirling was repeated twice the first day and thrice the second day

Second stage of digestion

After 48 hours of incubation, the rubber stoppers were removed and the forage particles adhering to the stoppers were washed with distilled water One mL of 20% HCl was added to each tube and swirled and then repeated (total two ml per tube) Finally, four mL of 20% HCl were added to each tube and then swirled. A total of six mL of 20% HCl were added Two mL of 5% pepsin were then added and swirled thoroughly. The rubber stoppers were replaced and the tubes returned to the 39°C incubator The swirling was repeated twice the first day and thrice the second day.

Gooch crucibles were then prepared by forming a glass wool mat in the bottom of the crucible. After 46 hours of pepsin digestion, the contents of the centrifuge tubes were transferred to the Gooch crucibles and held in place by a crucible holder All residual material from the tubes was rinsed out with hot distilled water and the Gooch crucibles were rinsed with hot distilled water. The

Gooch crucibles were placed into a drying oven overnight at 105°C. The following day they were removed from the drying oven, cooled in a desiccator, and weighed to 1/10,000th of a gram

Calculations

The % dry matter in the samples was calculated using the following equation:

$$DM\ (\%) = \frac{\text{dry regular crucible plus sample wt - regular crucible wt}}{\text{initial regular crucible plus sample wt - regular crucible wt}} * 100$$

The IVDMD was then calculated using the equation

$$IVDMD\ (\%) = \frac{\text{initial DM - (residual DM - blank DM)}}{\text{initial DM}} * 100$$

Statistical analysis

A completely random design (CRD) factorial arrangement was applied to all data from the Blount Unit and animal performance and forage quality data from the Holston Unit. A CRD split plot with a whole plot factorial was applied to DMI and CH_4 data at the Holston Unit. Forage and year were placed in the whole plot and biological type (BT) was placed in the subplot in both split plot arrangements All data were analyzed by analysis of variance (ANOVA) using the MIXED procedure of SAS (1999). Least square means were obtained and separated using least significance difference (LSD) Differences were determined at $P < 0.05$

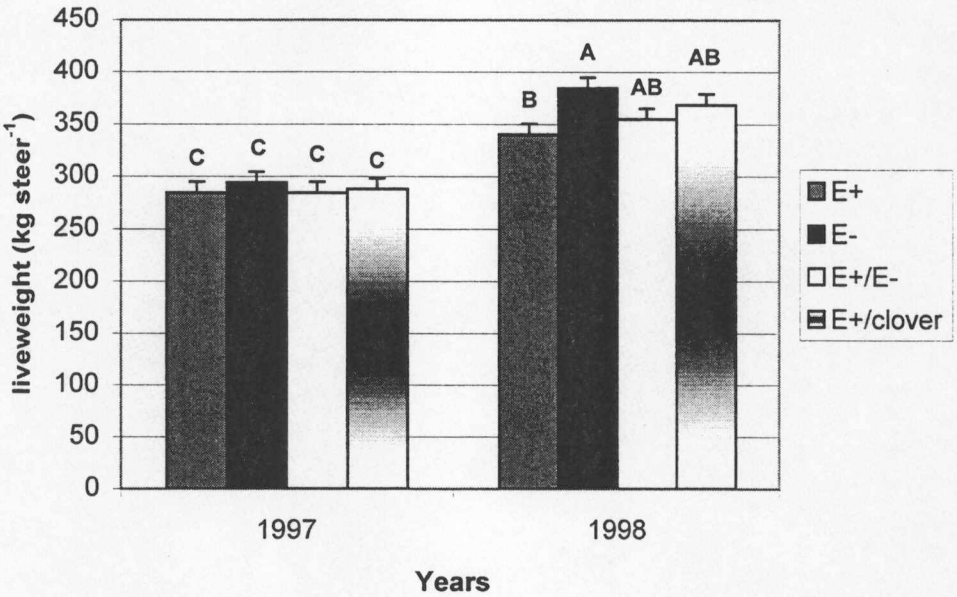
3. RESULTS AND DISCUSSION

Blount unit

Animal performance

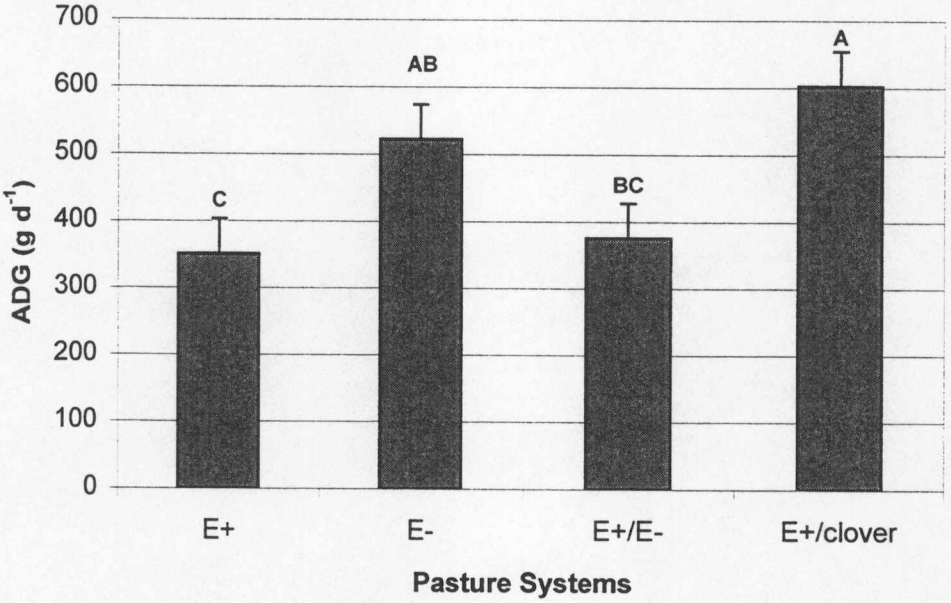
There were no differences in initial steer weights across treatments in 1997. In 1998 initial steer weights between E+ and E- treatments were different ($P < 0.05$). Initial steer weights were higher ($P < 0.05$) in 1998 than in 1997 (Figure 1). This may have been the result of a milder, less stressful fall/winter 1997-98. Steers may have gained more weight while on pasture during this time period than in the previous fall/winter. Steers on the E+/clover system had higher ($P < 0.05$) ADG than steers on E+ and E+/E- pasture systems. The E- steers gained more ($P < 0.05$) weight than the E+ steers (Figure 2). Steers in 1997 had higher ($P < 0.05$) ADG than steers in 1998 (Figure 3).

The higher ADG on the E- pasture system compared to the E+ pasture system was similar to findings by Fribourg et al. (1991a) and Crawford et al. (1989). They reported that as endophytic infestation decreased, ADG increased. The animal performance response to the addition of clover into E+ tall fescue pasture systems was similar to those reported by Thompson et al. (1993), Waller et al. (1989), and Hoveland et al. (1981). There were no differences in ADG on the E+/clover and the E- pasture systems. This is important because the persistence of E- tall fescue is severely diminished by the absence of the endophyte (Bouton et al., 1993). The addition of clover to E+ tall fescue will result



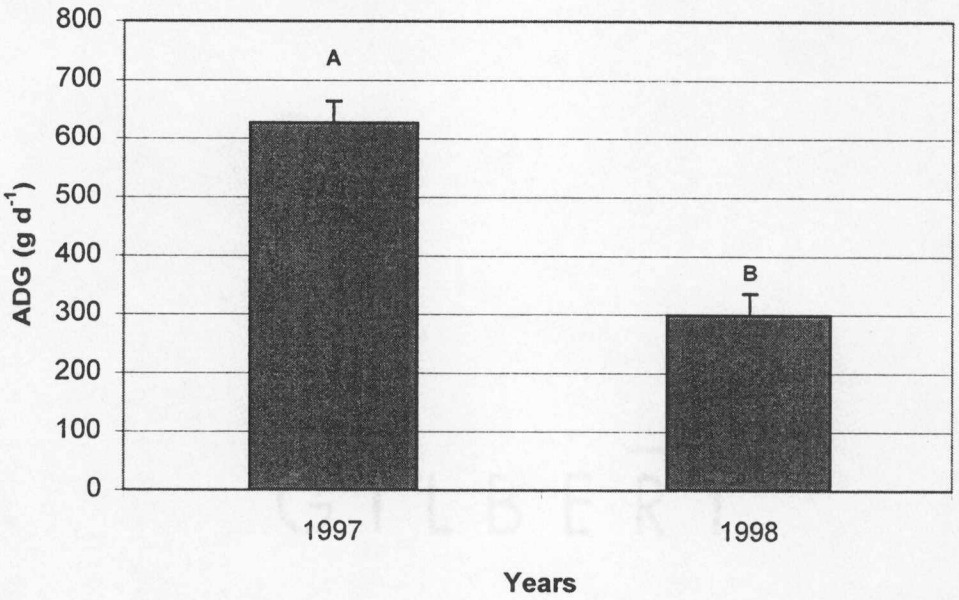
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 1. Least squares means and associated standard errors for initial weights of steers grazing four pasture systems at the Blount Unit in 1997 and 1998.



Pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 2. Least squares means and associated standard errors for average daily gain (ADG) of steers grazing four pasture systems at the Blount Unit in 1997 and 1998.



Years not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 3. Yearly least squares means and associated standard errors for average daily gain (ADG) of steers grazing four pasture systems at the Blount Unit in 1997 and 1998.

in productivity equal to E- tall fescue while retaining the resilience of E+ tall fescue

The ADG of steers on the E+/E- pasture system was intermediate, but not different from the ADG of the E+ and E- systems The mixture of E+ and E- tall fescues in the E+/E- pasture system was not as effective in reducing the endophyte toxicosis as the inclusion of clover in the E+/clover system

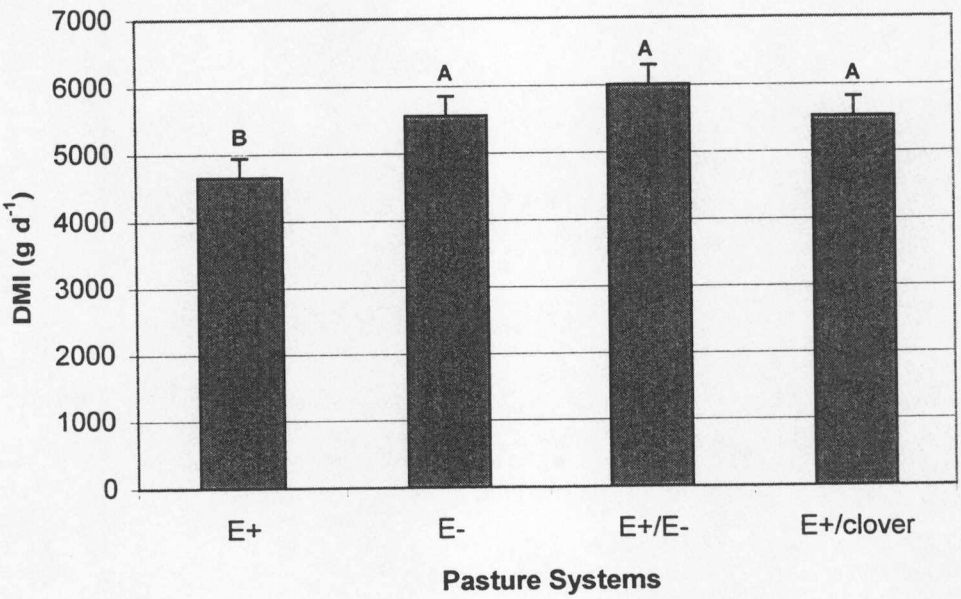
Dry matter intake

Steers on the E+ pasture system consumed less ($P < 0.05$) forage than steers on all other treatments (Figure 4) Waller et al (1993) and Goetsch et al (1987) reported that animals on E- tall fescue consumed more forage than animals on E+ tall fescue Peters et al (1992) reported higher DMI estimates for animals grazing E- tall fescue than for those grazing E+ tall fescue in August 1988. They reported no differences between the two treatments in June 1988, June 1989, and August 1989

Waller et al (1993) and Goetsch et al. (1987) also reported an increase in DMI when clover was added to the E+ pasture system. This could be the product of the animal ingesting less of the endophyte The uneven distribution of precipitation during the two years of this experiment (Figure 5) could have influenced the amount of available forage for consumption which may have altered steer ADG

Methane production

There were no differences in daily CH_4 production among treatments



Pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 4. Least squares means and associated standard errors for estimated dry matter intake (DMI) of steers grazing four pasture systems at the Blount Unit in 1997 and 1998.

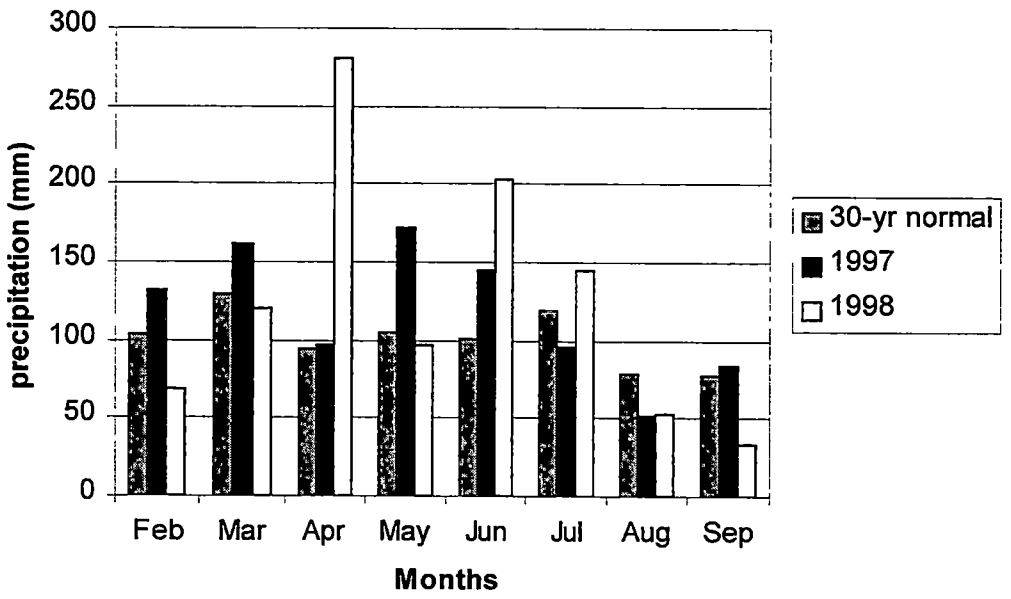


Figure 5. Monthly spring precipitation for Knoxville, TN; in 1997, 1998, and the 30-year norm

(Table 1) The E+/clover treatment tended to have the highest numerical amount in daily CH_4 production, followed by E+, E-, and E+/E-. This numerical trend was similar to summer 1997 daily CH_4 production reported by Pavao-Zuckerman et al. (1999) on the same tall fescue system There were no treatment differences in CH_4 production per unit of ADG (Table 1). This conflicts with summer 1997 data reported by Pavao-Zuckerman et al. (1999), in which the E+/clover system had a much lower CH_4 production per unit of ADG than all other pasture systems The summer ADG between years was comparable, but CH_4 production was higher in 1998, thus causing 1998 steers to be considered less efficient than 1997 steers There were no differences in CH_4 production per unit of MW among treatments (Table 1). The lack of difference in summer 1998 was due to animals of similar weight producing similar levels of CH_4 daily Pavao-Zuckerman et al (1999) reported the highest and lowest CH_4 production per unit of MW on the E+/clover and the E+ pasture systems in summer 1997, respectively.

Forage quality

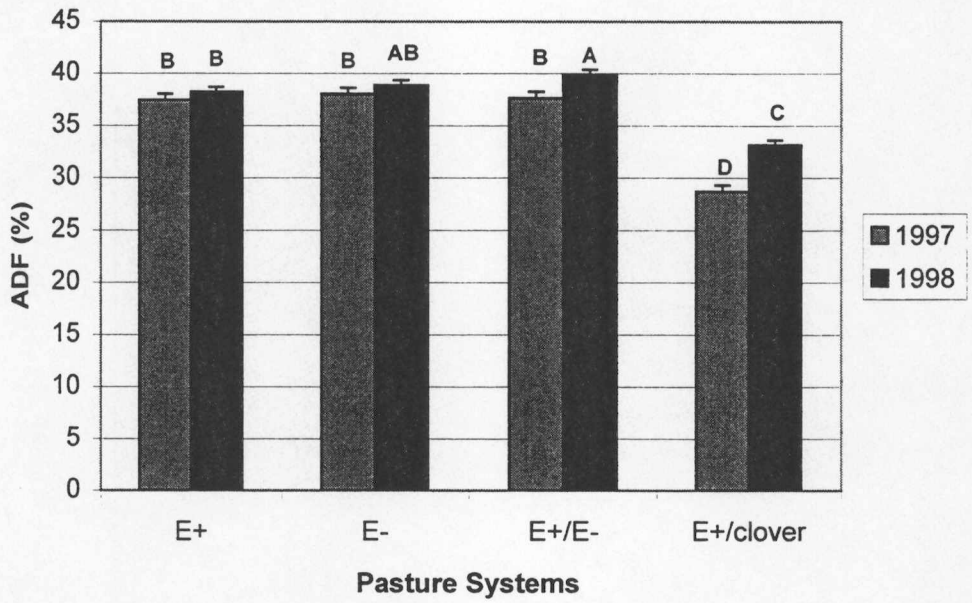
The E+/clover pasture system was lower ($P < 0.05$) in ADF and NDF than all other treatments in 1997 and 1998 (Figures 6 and 7). The E+/clover pasture system was higher ($P < 0.05$) in CP and IVDMD than all other treatments in 1997 and 1998 (Figures 8 and 9).

Johnson and Johnson (1995) stated that the type of carbohydrate (CHO), whether soluble or non-soluble, would have an impact on ruminal pH and

Table 1. Least squares means and associated standard errors for CH_4 emission estimates of steers grazing four pasture systems at the Blount Unit in summer 1998

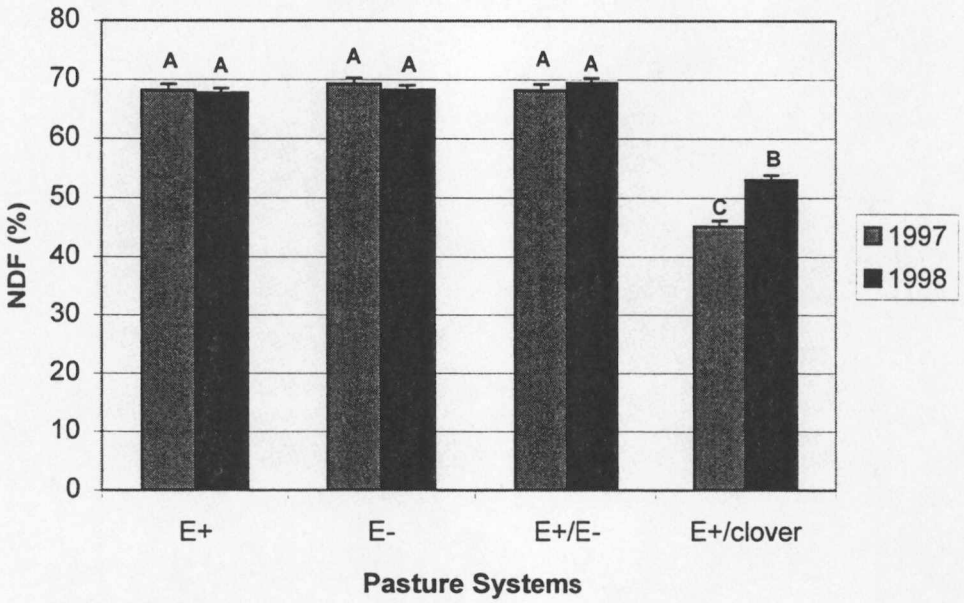
Pasture System	Daily CH_4		CH_4 per unit of ADG		CH_4 per unit of MW	
	July	August	July	August	July	August
	g d^{-1}		g kg^{-1} d^{-1}		g $kg^{-0.75}$ d^{-1}	
E+	167	182	616	573	1 99	2 17
E-	159	154	505	483	1 67	1 61
E+/E-	136	150	475	471	1 57	1 72
E+/clover	163	193	602	607	1 78	2 10
Std. Error	30	30	137	137	0 38	0.38

No significant differences were found at $P < 0.05$.



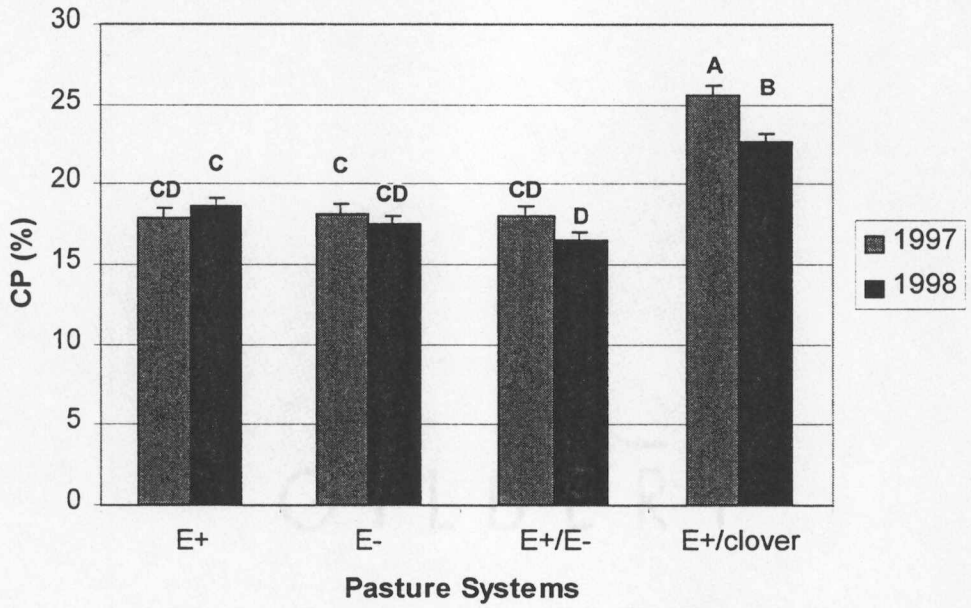
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 6. Least squares means and associated standard errors for acid detergent fiber (ADF) of forage from four pasture systems at the Blount Unit in 1997 and 1998.



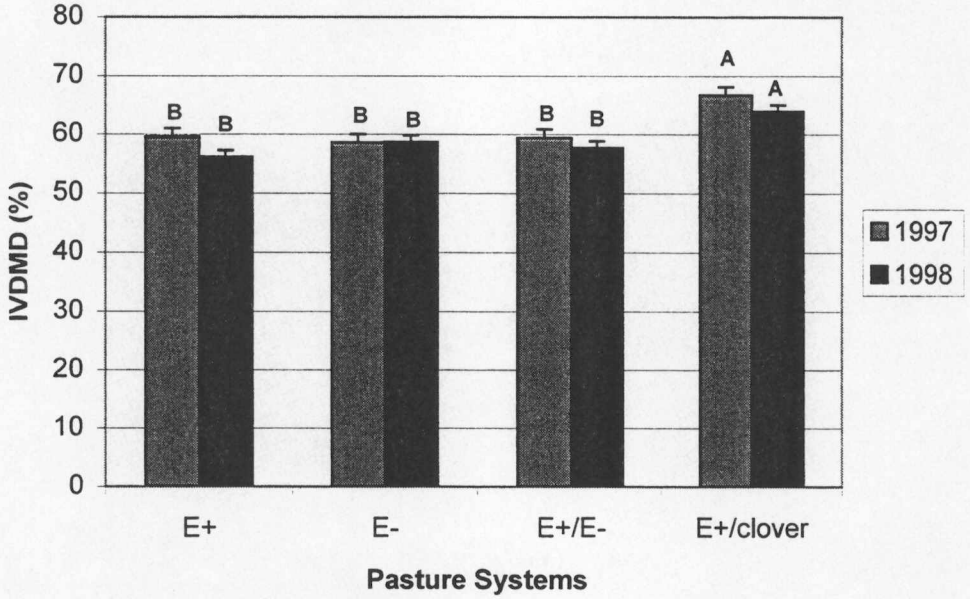
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 7. Least squares means and associated standard errors for neutral detergent fiber (NDF) for forage from four pasture systems at the Blount Unit in 1997 and 1998.



Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 8. Least squares means and associated standard errors for crude protein (CP) of forage from four pasture systems at the Blount Unit in 1997 and 1998.



Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 9. Least squares means and associated standard errors for *in vitro* dry matter digestibility (IVDMD) for forage from four pasture systems at the Blount Unit in 1997 and 1998.

microbial populations, which would in turn influence CH_4 production. Moe and Tyrrell (1979) concluded that the fermentation of non-soluble CHO would produce more CH_4 than that of soluble CHO. These findings are in contrast to the data reported above where the E+/clover system was lowest in ADF and NDF and highest in CP and IVDMD, but was also highest in CH_4 production One possible explanation for this difference is that steers used in sampling the pasture systems may have preferentially grazed more clover than grass/clover areas of the pastures In addition, steers used to collect CH_4 were not the same animals that were used to collect forage quality samples.

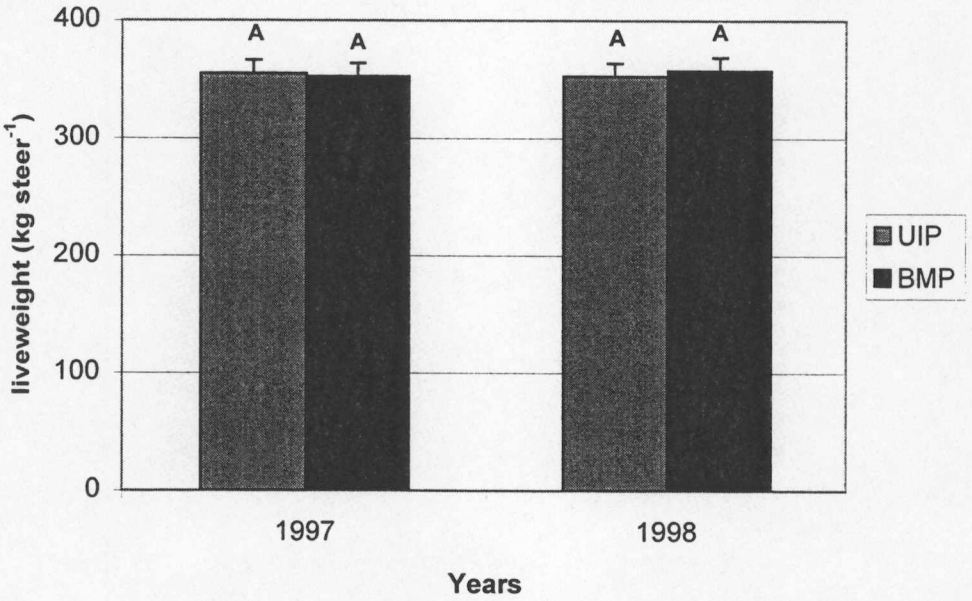
Holston unit

Animal performance

There were no differences in initial steer weights across treatments and years (Figure 10) The steers on the BMP system had a higher ($P < 0.05$) ADG than steers on the UIP system in 1997 This difference was not seen in the 1998 grazing year (Figure 11) In 1997, the presence of clover in the BMP pasture system resulted in an increase in ADG, similar to that observed by Thompson et al (1993) and Hoveland et al (1981)

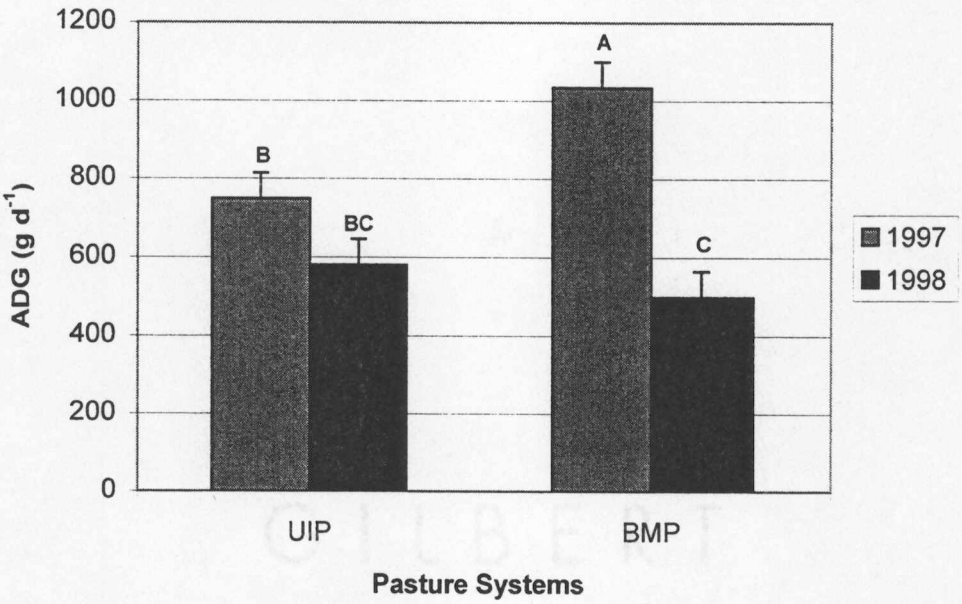
Dry matter intake

There were no differences in estimated DMI across years and treatments (Figure 12) Cows on both treatments consumed more ($P < 0.05$) forage than steers (Figure 13) The lack of differences in DMI between the BMP and the



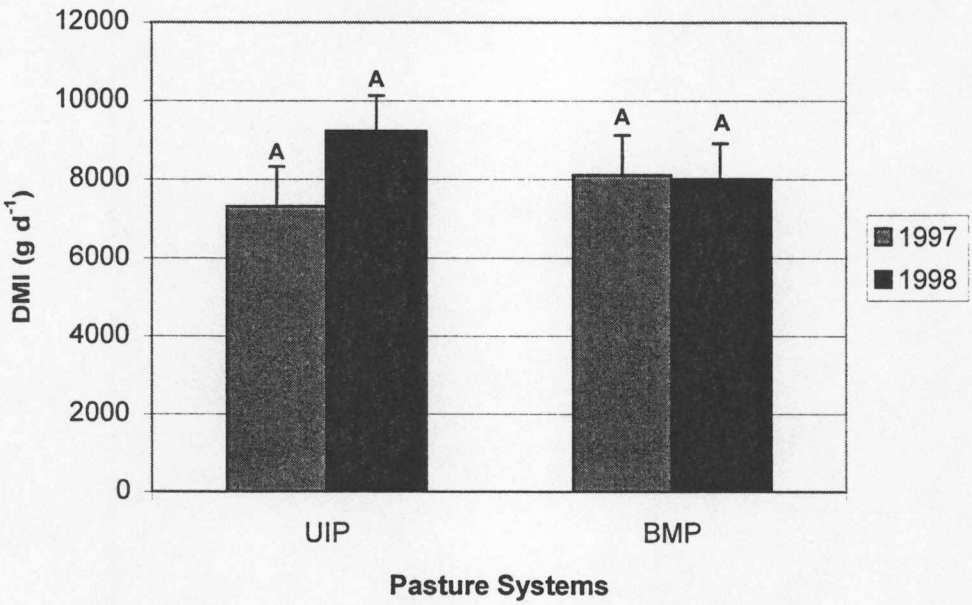
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure10. Least squares means and associated standard errors for initial weight of steers grazing either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.



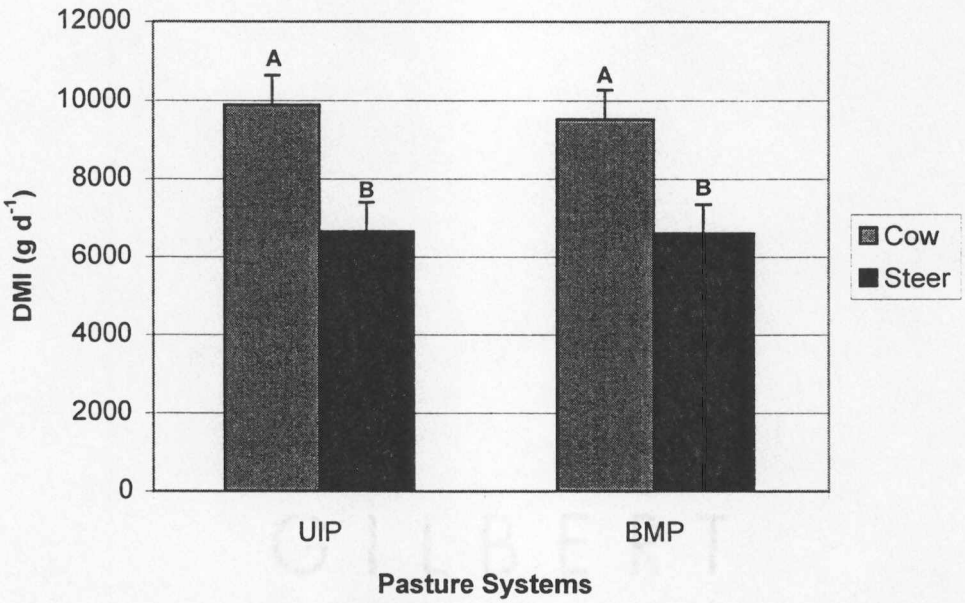
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 11. Least squares means and associated standard errors for average daily gain (ADG) of steers grazing either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.



Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 12. Least squares means and associated standard errors for estimated dry matter intake (DMI) of animals grazing either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.



Animals and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 13. Least squares means and associated standard errors for estimated dry matter intake (DMI) of steers and cows grazing either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.

UIP were in contrast to findings by Goetsch et al (1987), who found that the inclusion of clover into E+ tall fescue stands would increase forage intake. Clover was established in the BMP pasture system of this study, but the UIP pasture system contained some volunteer clover plants as a result of the wetter than normal spring and summer. The excessively wet springs (Figure 5) may have produced an excess of other grasses available for consumption during late spring and summer

Methane production

Animals produced less ($P < 0.05$) CH_4 in September than in July and in August The UIP system had more ($P < 0.05$) CH_4 production in July, August, and September than the BMP system. Cows produced a higher ($P < 0\ 05$) amount of CH_4 than steers on the UIP and an insignificantly higher amount of CH_4 on the BMP (Table 2) Pavao-Zuckerman et al (1999) reported low CH_4 production from cows and high CH_4 production from steers on the BMP compared to the UIP. The BMP system had more ($P < 0\ 05$) CH_4 production per unit of ADG than the UIP system This was inconsistent also with summer 1997 data from Pavao-Zuckerman et al (1999) The ADG of steers on the BMP system in 1998 were much lower ($P < 0\ 05$) than steers on the BMP system in 1997 (Figure 11) and probably caused them to appear to be less efficient than steers on the UIP system Methane production per unit of ADG was not an acceptable expression of efficiency when steers had unusually low ADG or even a loss of weight during the time period in which CH_4 emissions were measured

Table 2. Least squares means and associated standard errors for daily CH_4 production estimates of steers and cows grazing either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in summer 1998

Pasture System	Animal Class	Daily CH_4		
		July	August	September
			g d^{-1}	
BMP	Steer	142_{CDE}	141_{CDE}	127_{E}
	Cow	163_{ABC}	156_{BCD}	134_{DE}
UIP	Steer	155_{BCD}	157_{BCD}	146_{CDE}
	Cow	187_{A}	183_{A}	172_{AB}
Std. Error		9	9	9

Methane production estimates not sharing the same superscripts are significantly different at $P < 0.05$.

Animals in September had lower ($P < 0.05$) CH_4 per unit of MW than in July and August. The CH_4 per unit of MW for animals on the UIP system was insignificantly higher in July and August and significantly higher ($P < 0.05$) in September than the BMP system. Cows were numerically lower in CH_4 per unit of MW on the UIP and significantly lower ($P < 0.05$) on the BMP than steers (Table 3). This conflicts with data from Pavao-Zuckerman et al. (1999).

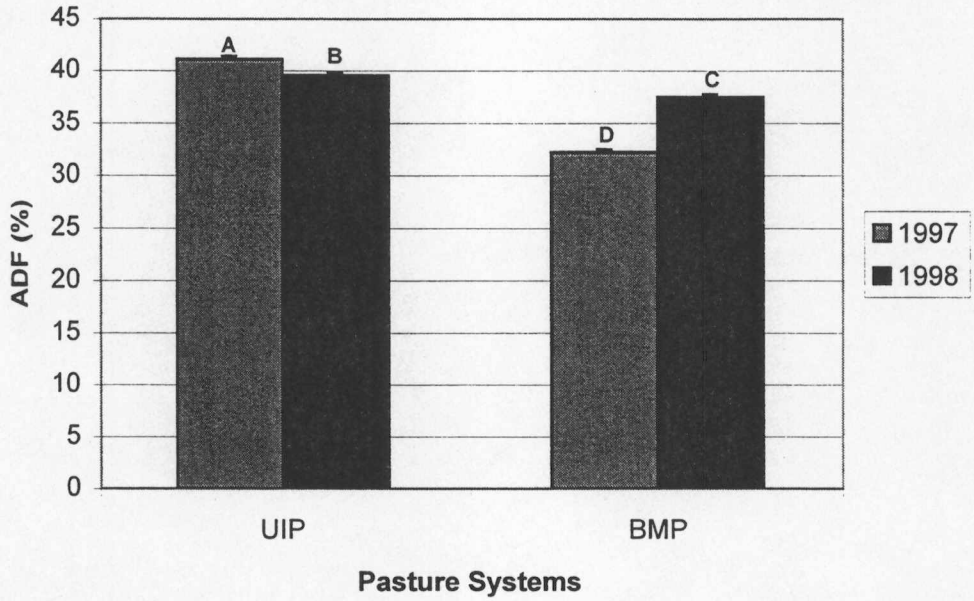
Forage quality

The BMP system was lower ($P < 0.05$) than the UIP system in ADF in 1997 and 1998 (Figure 14). Percent NDF in the BMP system in 1997 and 1998 was lower ($P < 0.05$) than the UIP system (Figure 15). The BMP was higher ($P < 0.05$) than the UIP in CP in 1997 and 1998 (Figure 16). The BMP system had a higher ($P < 0.05$) IVDMD than the UIP system in 1997 and 1998 (Figure 17). The samples used for determination of forage quality were collected via ruminally fistulated steers, which tended to be of higher quality than hand-clipped samples (Appendix). This was due to the selection of high quality forage by the animals (Weir and Torrell, 1959, Streeter, 1969). The Holston Unit data support the work of Moe and Tyrrell (1979). The BMP system was lowest in ADF and NDF and highest in CP and IVDMD. This may have resulted in lower CH_4 production than the UIP system.

Table 3 Least squares means and associated standard errors for CH_4 emission estimates for steers and cows grazing either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in summer 1998

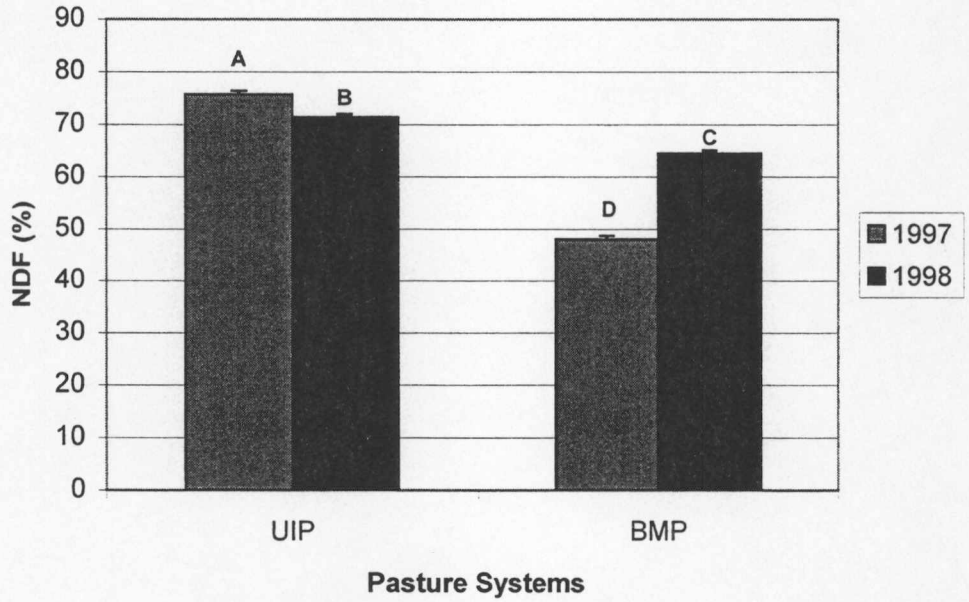
Pasture System	Animal Class	CH_4 per unit of ADG			CH_4 per unit of MW		
		July	August	September	July	August	September
		g kg^{-1} d^{-1}			g $kg^{-0.75}$ d^{-1}		
BMP	Steer	733_{A}	793_{A}	674_{A}	$1\ 58_{ABC}$	$1\ 57_{ABC}$	$1\ 42_{CD}$
	Cow				$1\ 46_{C}$	$1\ 39_{CD}$	$1\ 20_{D}$
UIP	Steer	307_{A}	301_{A}	278_{A}	$1\ 71_{AB}$	1.73_{A}	1.61_{ABC}
	Cow				$1\ 60_{ABC}$	1.57_{ABC}	$1\ 47_{BC}$
Std. Error		205	205	205	0 09	0 09	0 09

Methane emission estimates for the same variables not sharing the same superscripts are significantly different at $P < 0.05$.



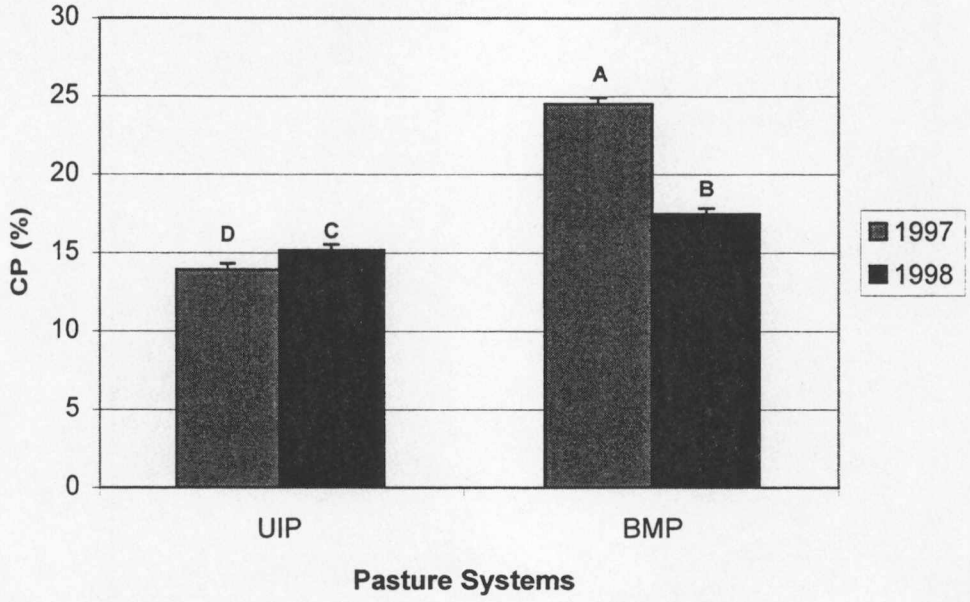
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 14. Least squares means and associated standard errors for acid detergent fiber (ADF) of forage from either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.



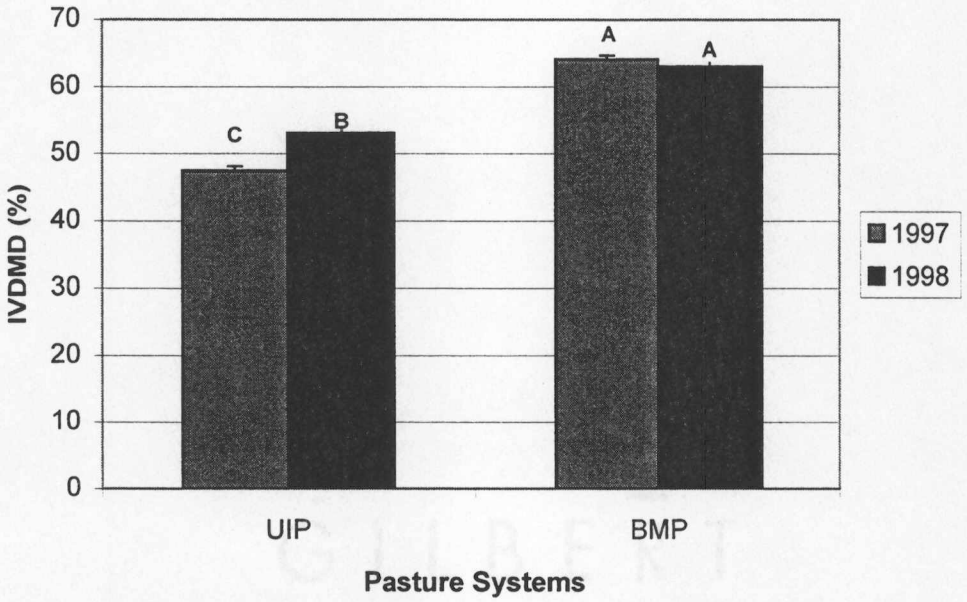
Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 15. Least squares means and associated standard errors for neutral detergent fiber (NDF) of forage from either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.



Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 16. Least squares means and associated standard errors for crude protein (CP) of forage from either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.



Years and pasture systems not sharing the same superscripts are significantly different at $P < 0.05$.

Figure 17. Least squares means and associated standard errors for *in vitro* dry matter digestibility (IVDMD) of forage from either a best management practices (BMP) pasture or an unimproved pasture (UIP) at the Holston Unit in 1997 and 1998.

4. CONCLUSIONS

The presence of clover in E+ tall fescue pasture systems did improve the quality of the forage system by decreasing ADF and NDF and by increasing CP and IVDMD Animal performance was also enhanced over that of E+ tall fescue There was no difference in the forage quality of E+ and E- tall fescue systems The use of regression analysis was a more effective way of calculating ADG since it takes into account all of the 21-d weights rather than using only the initial and final weights In addition, there was a depression of DMI of animals grazing E+ tall fescue. Steers on the E- and E+/clover pasture systems did consume more forage than steers on the E+ pasture system.

It is possible that with improved management strategies of a pasture system, the rate of daily CH_4 production can be decreased Methane production per unit of ADG is an acceptable method of defining efficiency of production in most cases If the animals exhibit abnormally low ADG or a loss of weight then CH_4 production per unit of ADG is not an appropriate expression of efficiency. The renovation of existing E+ tall fescue pastures will increase the productivity of these systems as well as potentially making the system more environmentally sound

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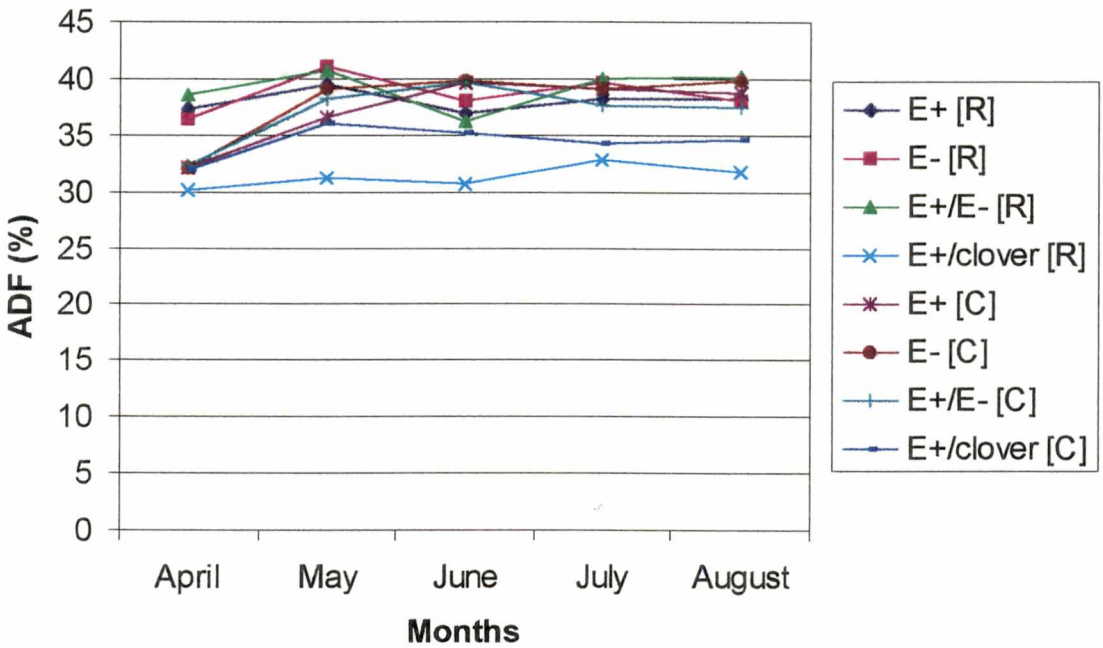
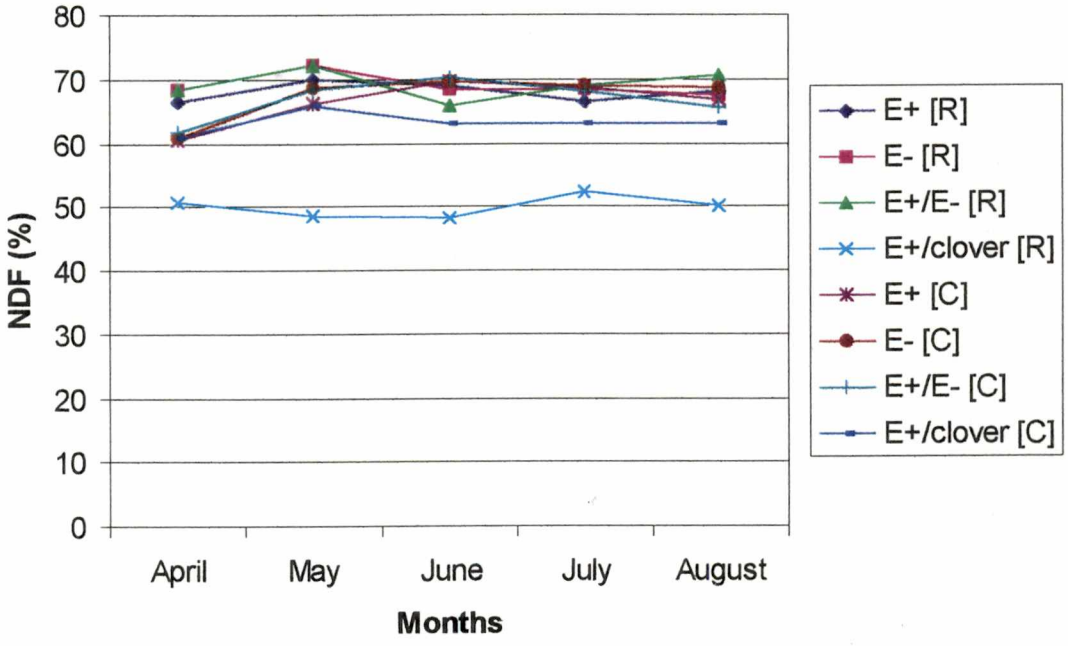
Appendix

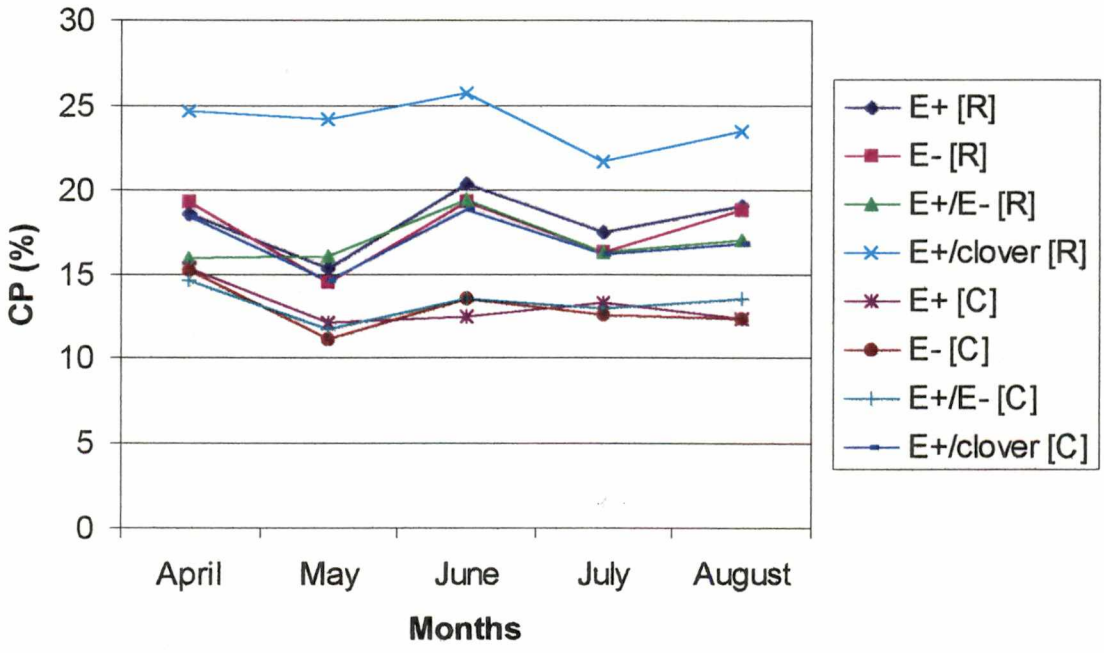
Forage quality data



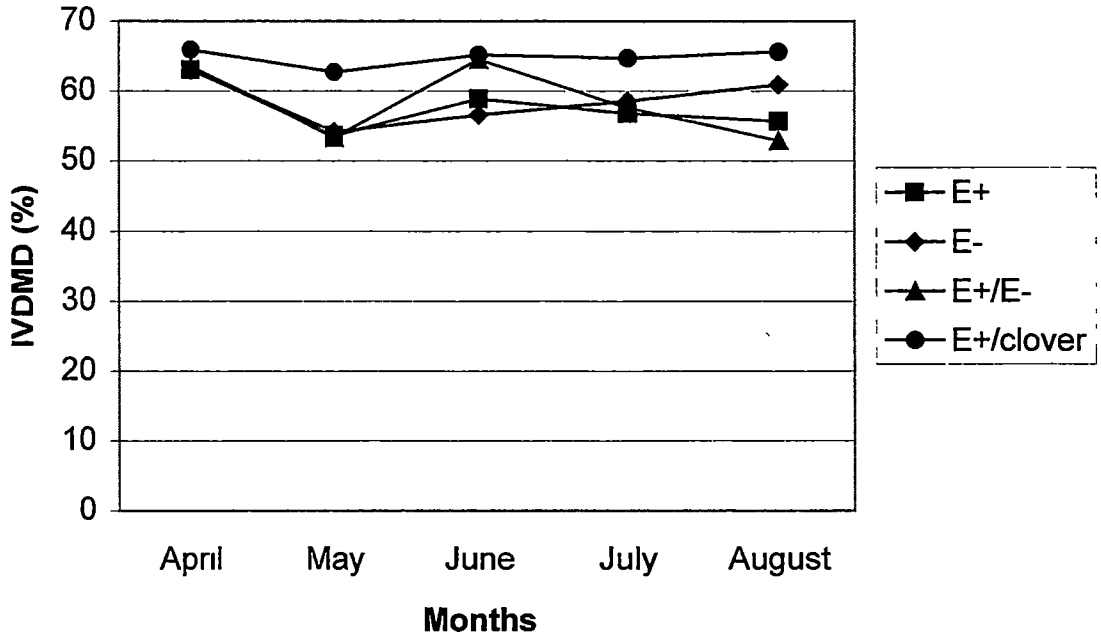
A1. Monthly means for acid detergent fiber (ADF) of forage from four pasture systems and two collection methods, rumen fistulated steers [R] and hand-clipped [C] at the Blount Unit in 1997 and 1998.



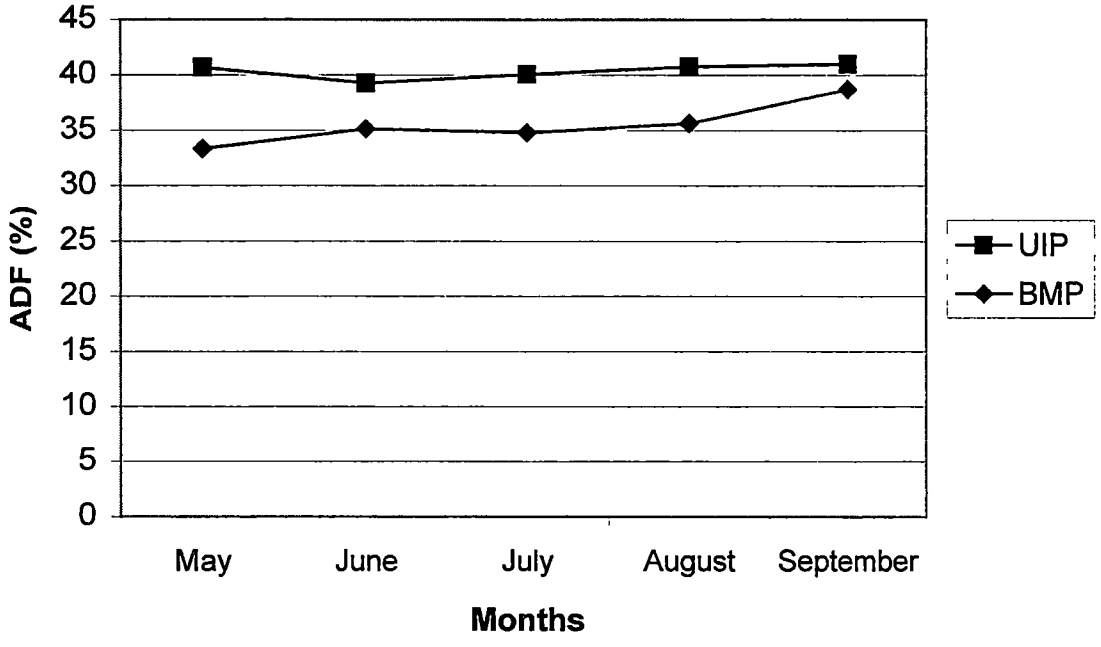
A2. Monthly means for neutral detergent fiber (NDF) of forage from four pasture systems and two collection methods, rumen fistulated steers [R] and hand-clipped [C] at the Blount Unit in 1997 and 1998.



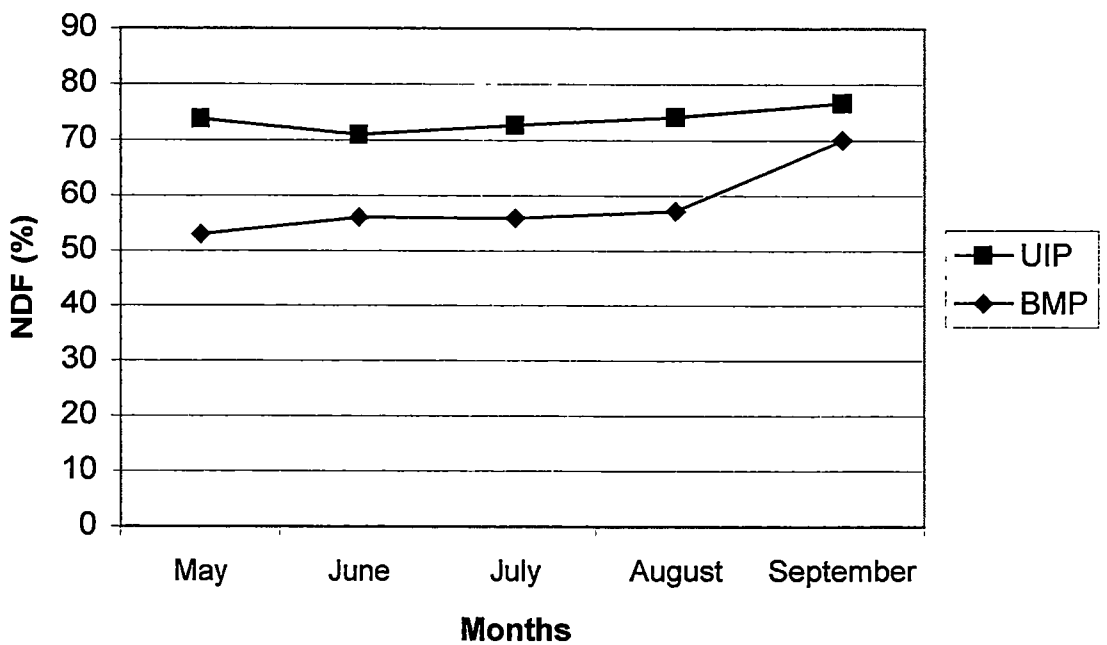
A3. Monthly means for crude protein (CP) of forage from four pasture systems and two collection methods, rumen fistulated steers [R] and hand-clipped [C] at the Blount Unit in 1997 and 1998.



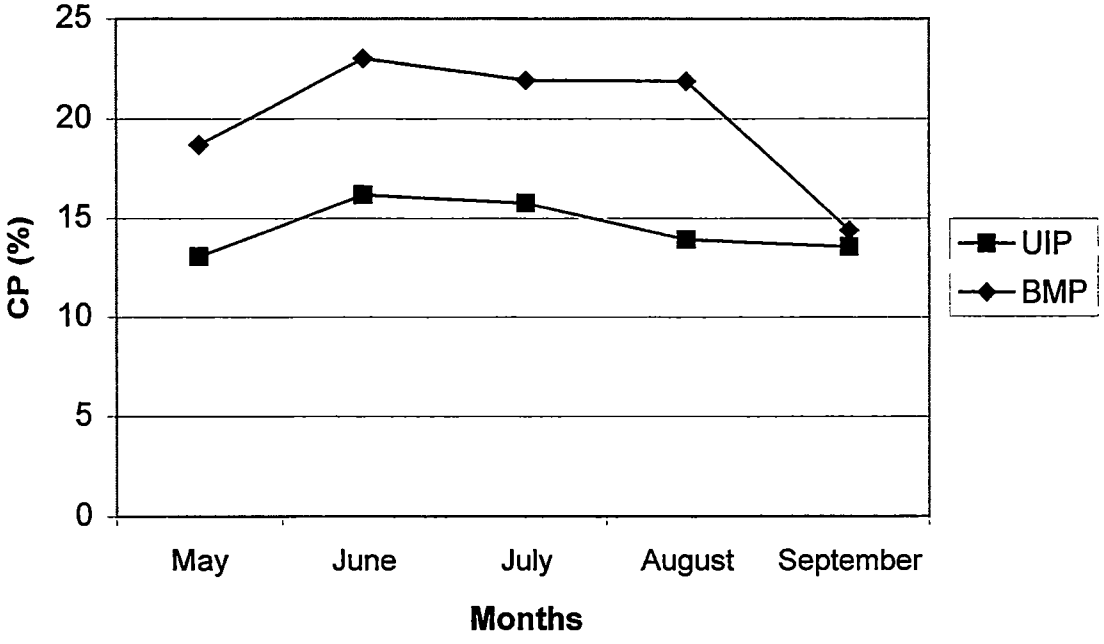
A4 Monthly means for *in vitro* dry matter digestibility (IVDMD) for forage from four pasture systems at the Blount Unit in 1997 and 1998.



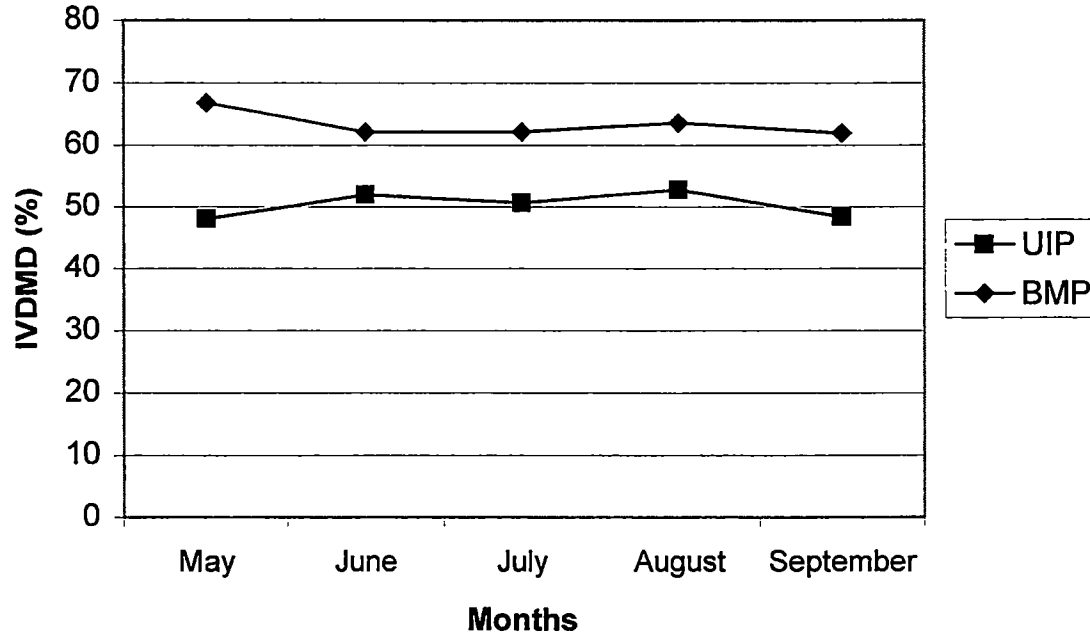
A5. Monthly means for acid detergent fiber (ADF) of forage from two pasture systems at the Holston Unit in 1997 and 1998



A6 Monthly means for neutral detergent fiber (NDF) of forage from two pasture systems at the Holston Unit in 1997 and 1998.



A7 Monthly means for crude protein (CP) of forage from two pasture systems at the Holston Unit in 1997 and 1998.



A8. Monthly means for *in vitro* dry matter digestibility (IVDMD) of forage from two pasture systems at the Holston Unit in 1997 and 1998.

Vita

Aaron Eugene Fisher was born to Harold E and Betty Jane Fisher in Spring City, TN, on August 12, 1976 He received an Honors Diploma from Rhea County High School in May 1994 He then entered The University of Tennessee majoring in Animal Science in August 1994 Mr Fisher received a Bachelor of Science in Agriculture in May 1998 He began his graduate career in the Department of Animal Science in August 1998, concentrating in beef cattle nutrition. He received a Master of Science degree in Animal Science in December 2000. Mr. Fisher remained at The University of Tennessee until May 2001 to complete work on developing a graduate level course in Advanced Beef Management to be offered via Internet technology.