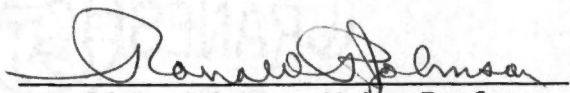
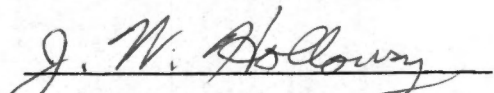
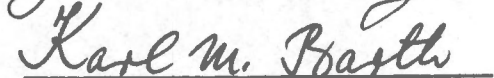


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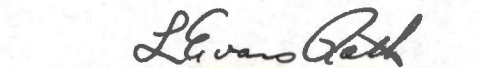
I am submitting herewith a thesis written by Natalie Wiest Shipman entitled "Influence of Tall Fescue-Legume Mixed Forages on Ruminant Volatile Fatty Acids of Angus Steers." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.


Ronald R. Johnson, Major Professor

We have read this thesis
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INFLUENCE OF TALL FESCUE-LEGUME MIXED FORAGES ON RUMINAL
VOLATILE FATTY ACIDS OF ANGUS STEERS

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Natalie Wiest Shipman

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ABSTRACT

Eight Angus steers involved in six standard digestion trials were used to evaluate the effects of variation in ratios of grass:legume forages, cutting date, dry matter intake, dry matter digestibility, and sampling time on ruminal production of volatile fatty acids (VFA) and ruminal pH. The grass portion of the forage was tall fescue (Festuca arundinacea, Schreb.), and legumes were crimson clover (Trifolium incarnatum) for Cutting Date 1, red clover (Trifolium pratense) for Cutting Dates 2,3, and 4, and Korean lespedeza (Lespedeza stipulacea) for Cutting Date 5. Samples were obtained via stomach tube and analyzed for volatile fatty acids by gas-liquid chromatography. Grass:legume ratios varied from 40:60 to 100:0, dry matter digestibility from 42% to 77%, and dry matter intake from 1.54 to 3.68 kg/day so there was wide variation that might affect VFA. Even with that diverse range of values, no more than 20% of the change in ruminal VFA could be explained by any one variable. In fact, many of the larger R^2 values were for relatively minor VFA. The lack of predictable variation in VFA ruled them out as important factors in animal performance differences between fescue-legume and pure fescue forages.

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CHAPTER I

INTRODUCTION

In Eastern and Central Tennessee much of the available farmland is rolling or hilly terrain more suitable to pasture land than cultivated crop land. Perennial grasses are the forage of choice of many Tennessee beef producers for reasons of erosion control and ease of maintenance. Of these grasses, tall fescue (Festuca arundinacea, Schreb.) is the most popular. Its advantage is its high yield: it grows well in the mild Tennessee climate and produces new growth when the mean weekly temperature is above 6.4 C (40 F) although its growth rate is curtailed in the hot summers when the temperature exceeds 27 C (81.2 F) during the nights (Allinson, 1971).

Fescue responds well to nitrogen fertilization, but because fertilizer costs are rising faster than the price of beef, many producers are looking for alternatives to the use of fertilizers. One possibility is the introduction of legumes into grass pastures. Grass-legume pasture systems require better management than all-grass systems (Heath, 1974). Legumes, however, are superior to grass nutritionally. The symbiotic relationship between legumes and Rhizobium bacteria fixes nitrogen from the atmosphere and makes it available to other plants (Vallis, 1978). Legumes increase the length of the grazing season, and may fix from 50 kg/ha to 200 kg/ha of nitrogen in the soil annually (Heath, 1974).

The energy derived from feedstuffs by ruminants is mostly in the form of rumen-derived volatile fatty acids (VFA). The concentrations

and molar percentages of these VFA have been widely studied in forage-only or forage-concentrate diets (Blaxter and Wainman, 1964) but variations due to ratios of grass to legume have not been widely reported. Variations of VFA levels of grass-legume mixtures may partly explain differences in nutritive quality as estimated by standard techniques such as rate of growth or digestion trials. The objective of this study was to determine the effects of varying grass-legume mixed forages on ruminal VFA concentrations in vivo.

CHAPTER II

LITERATURE REVIEW

I. VOLATILE FATTY ACIDS (VFA) FOUND IN THE RUMEN

In the digestion of feedstuffs by the ruminant animal, the microorganisms of the rumen secrete carbohydrases which hydrolyse a range of plant polysaccharides to simple sugars which are fermented to VFA, CO_2 , H_2 , and CH_4 . The quantities of VFA synthesized and bacterial cells produced in the rumen are closely related (Leng, 1973). Basic energetic processes take place at the cellular level of the animal, where VFA are absorbed and utilized. The VFA produced account for more than one half of the energy derived from feed by the ruminant (Blaxter, 1962; Wheaton, 1970; Leng, 1973).

It has been known since 1880 that ruminal fermentation produces mainly acetic, propionic, and butyric acids. El Shazly (1952) detected branched chain fatty acids considered to arise from microbial attack on protein in the rumen. Annison (1954) established the presence of isobutyric acid and the three naturally occurring isomers of valeric acid in the sheep rumen. He surmised that the amounts of branched-chain VFA in the rumen depend on the rate and extent of degradation of ingested and microbial protein.

Acetic acid is an important metabolic intermediate and a precursor of a wide variety of compounds in the body, including higher fatty acids and cholesterol. It can be converted to ketone bodies in the liver (Pennington, 1952).

Propionic acid is considered to be the major gluconeogenic

precursor in ruminants (Herbein et al., 1978). In ruminants, whose supply of absorbed carbohydrates as a source of blood glucose is otherwise restricted, this fact is of considerable importance (Pennington, 1952; Moir, 1965).

Labelled butyrate can be converted within the rumen to acetate, propionate, and valerate (McCarthy et al., 1958b). Butyrate plays an important role in the development of the rumen mucosa when fermentation is initiated in the rumen (Sutton et al., 1963; Sakata, 1979), and much of it is metabolized by the rumen wall during absorption.

The ratio of acetic acid:propionic acid has been used as an indicator of energetic efficiency. Jorgensen and Schultz (1963) indicated that it would be desirable to feed rations producing more propionic acid to increase efficiency, reduce heat production in warm climates, and to control ketosis. Diets listed by Jorgensen and Schultz (1963) that increased propionic acid production were pelleted forages, steam heated concentrates, high levels of beet pulp, and restricted forage.

II. NUTRITIVE VALUE OF GRASS-LEGUME MIXTURES

Steers grazing grass-legume mixed pastures outperformed steers grazing fescue grass in an experiment by High et al. (1965). During the summer grazing period, average daily gains (ADG) of steers were significantly higher on an orchardgrass-clover pasture than on fescue-clover, or fescue-clover-lespedeza pasture, but the fescue-clover-lespedeza pasture produced daily gains higher than the fescue-lespedeza pasture. Thompson and Holdaway (1954) found little difference in milk production between cows grazing an alfalfa-orchardgrass and a

ladino-orchardgrass mixture, whereas cows grazing a ladino-Kentucky 31 fescue mixture produced significantly less milk than those having access to either alfalfa-orchardgrass or ladino-orchardgrass. Wang (1968) found that ADG of steers grazing orchardgrass-clover pasture was higher than that of steers grazing fescue-lespedeza pasture. This was especially pronounced in the early part of the growing season.

Pratt and Haynes (1950) found ladino clover-fescue plots to be superior for milk yield over pure fescue pasture the first year of their study. They attributed inferior yields from this same plot in second and third years to a decline of the clover and an increased growth of fescue. However, Barth et al. (1972) found the values "percentage of fescue" and "legume index" (product of legume percentage in the pastures and legume height) were not significant predictors of ADG for steers grazing fescue-lespedeza pasture. Crowley (1975) investigated the virtues of red clover and recommended that it was best grown with grass, for the following reasons: 1) dry matter yields were 20-30% higher from the mixture than from pure clover, 2) it is easier to ensile as a mix, 3) grass reduces the problem of annual weeds in the crop, 4) grass gives some protection to clover plants from the passage of heavy machinery over the crop. Davies (1975) used red fescue (Festuca rubra L.) by itself and in combination with white clover as a forage under hill conditions. He observed differences between treatments in efficiency of conversion of dry matter intake (DMI) into liveweight gains but could not relate superior conversion ratios to any of the herbage quality measurements he recorded.

Holloway et al. (1979) compared the effect of grazing tall fescue-legume mixed pastures clipped at regular intervals to tall fescue

pastures allowed to mature. Angus cows grazed the pastures through two lactations and effects of the two pastures were compared for forage digestibility, intake, and animal performance. Forage digestibility and intake favored cows grazing the fescue-legume pastures, with differences most pronounced during the summer when fescue was dormant and legumes may have supplied more forage. Cows on the fescue-legume mixed pastures gained more weight, had more subcutaneous fat, and gave milk with higher butterfat percentage than cows on fescue. Calves raised with cows on fescue-legume pastures were heavier at weaning than calves with cows on fescue pastures.

Digestibility and Maturity of Forages

Digestibility and maturity are closely interrelated. There is widespread agreement among researchers that digestibility is the most significant criterion of forage quality governing the amount of basic feed intake and its conversion into milk and meat (Lampeter and Schmeiser, 1974).

Fescue digestibility, total sugar content, and palatability to livestock are lowest during summer, intermediate in early spring, and highest during the fall (Lassiter et al., 1956; Buckner et al., 1967; Jung et al., 1974; Kaiser, 1974). Beaty et al. (1978) studied the effect of clipping heights, clipping frequency, and nitrogen fertilization levels at maintaining the green forage and digestibility of fescue into the fall, desirable for the overall 70.9% in vitro dry matter digestibility (IVDMD) of the green forage as opposed to the 42.4% IVDMD of dead forage. None of the treatments were effective.

Terry and Tilley (1964) determined in vitro digestibilities of

leaf blade and leaf sheath of grasses including fescue and the legumes alfalfa and sainfoin. They showed that all parts of the plant have a high digestibility at early stages of growth. Leaf sheath digestibility declined with increasing maturity. The decline of the digestibility was associated with a reduction in the content of water-soluble and protein constituents of the plants. Figures 1 and 2 illustrate the growth characteristics and yield-quality-composition relationships of fescue and legume pastures (Blaser et al., 1975).

Level of Intake

The level of intake affects ration digestibility inversely (Bath and Rook, 1963). Animals confined to crates in digestion trials often decreased their intakes considerably, even when ad libitum amounts of forage were fed. In a forage feeding experiment by Bryan et al. (1970), grazing steers gained an average of 100 kg per head on fescue pastures. Another group of steers fed the same mixture of cut forages indoors gained only 25 kg per head.

III. PRODUCTION OF VOLATILE FATTY ACIDS FROM VARIOUS GRASS-LEGUME MIXTURES

Effect of Ration

Many investigators have found that the rumen fermentation of roughage rations is associated with the formation of VFA mixtures with higher proportions of acetic acid and lower proportions of propionic and butyric acids than fermentation of concentrate rations. Church (1976) cites many examples in his discussion of the effect of ration ingredients on rumen VFA.

High concentrations of total VFA in the rumen occurred on leafy

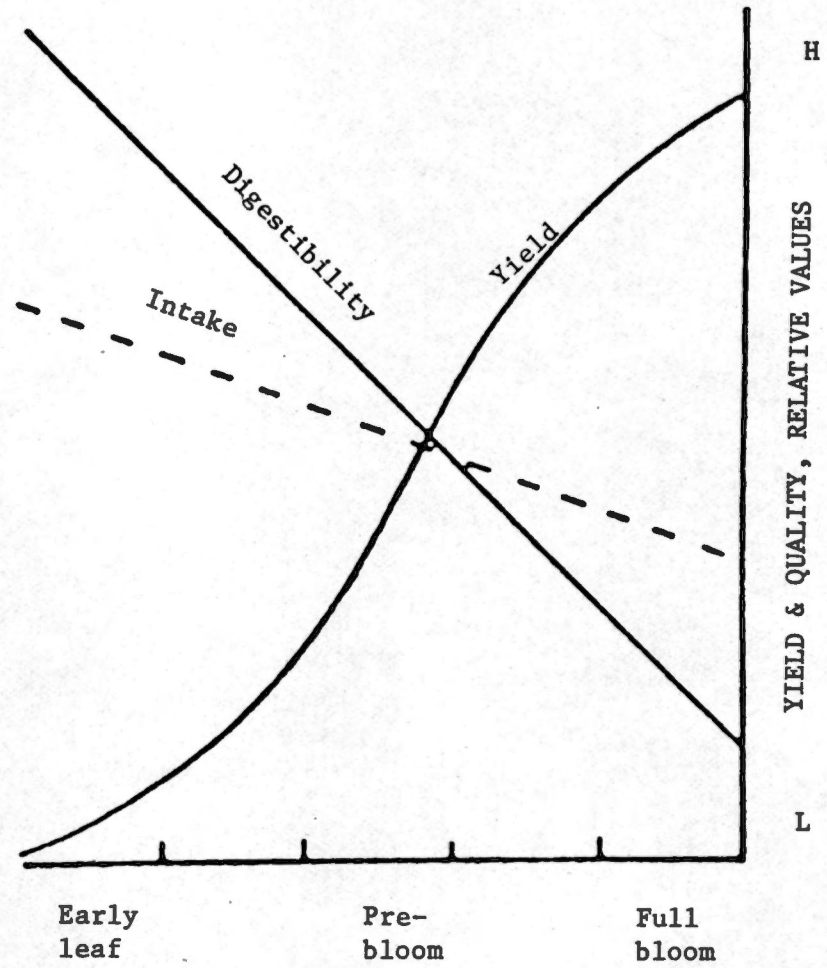


Figure 1. Growth stages of perennial grasses and legumes; yield and quality, relative values.

From Blaser et al., 1975

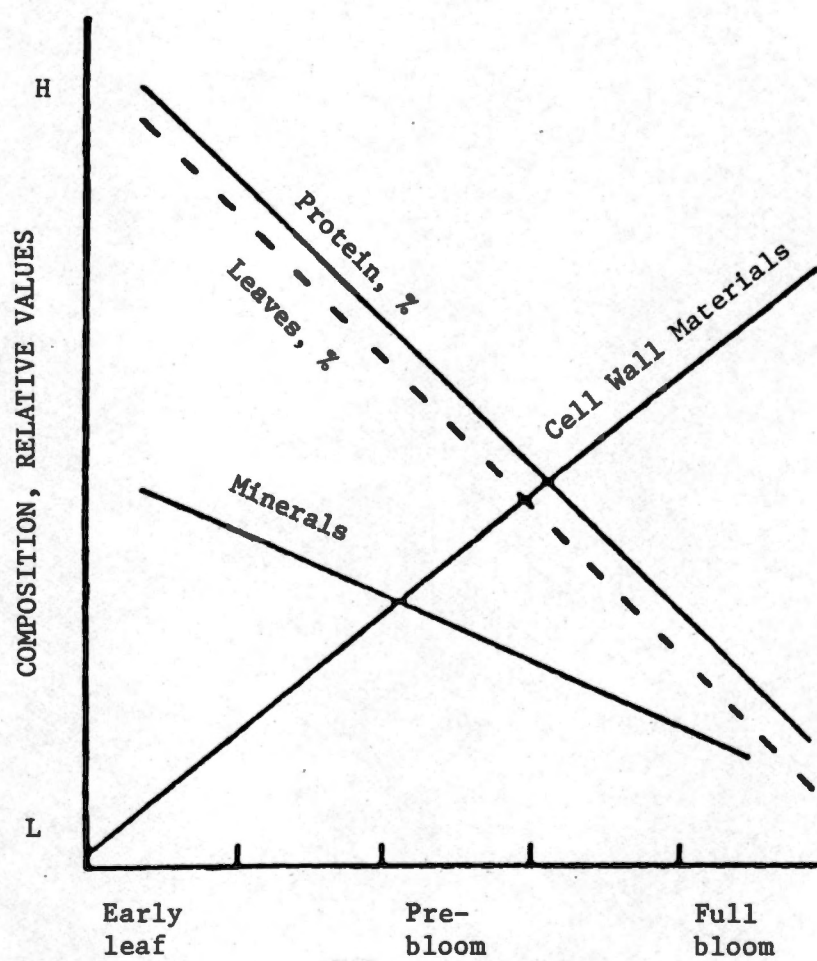


Figure 2. Growth stages of perennial grasses and legumes; composition, relative values.

From Blaser et al., 1975

herbage diets, particularly for grazing cows (Bath and Rook, 1965). Mackintosh and Ulyatt (1970) cite variations in VFA produced in the rumen as possible reasons for differences in animal production by different pasture species. They found, using isotope infusion and sampling technique on sheep, that Grasslands Manawa ryegrass produced the lowest VFA levels of the three species they studied, 1.78 moles/day, perennial ryegrass was highest with 3.33 moles/day and white clover intermediate with 2.99 moles/day.

Michell (1974) compared white clover with ryegrass and orchardgrass for sheep in Australia. Overall species and seasons means showed that sheep grazing white clover had the highest concentration of VFA, followed by those grazing ryegrasses and orchardgrass species. He found no differences between seasons. Wang (1968), studying steers grazing fescue-lespedeza or orchardgrass-clover pastures, found VFA concentration accounted for most of the variation in ADG.

Effect of Selectivity of Grazing

The grazing behavior of animals effects what forage species are selected in a pasture and the level of intake. In addition, grazing has an effect on VFA levels in the rumen when compared to forages cut and fed from the same pasture. Forbes and Garrigus (1958) found decreased feed values for cut and fed forages. Rook (1964) found that values obtained for grazing tended to show a lower proportion of acetic acid and a higher proportion of butyric acid than those for animals fed the same forage cut and offered fresh twice daily in the stall. He conjectured that this could have been the result of selective grazing and ingestion of more nutritious forages, of a

greater forage intake by grazing animals, or of continuous access to the pasture.

Coleman and Barth (1973) found that animals selected forage that contained more crude protein and less acid detergent fiber and soluble carbohydrates than was observed in the available forage samples. Forage selected by the animals was also more digestible. Coleman and Barth concluded that animals grazed selectively and that hand sampling pasture forage was inaccurate in representing the diet of grazing animals.

Effect of Forage Maturity

Barnett and Reid (1956) and Deinem and Dirven (1971) correlated seasonal changes in grasses with VFA production in vitro. They found that the main acid produced in early growth stages was acetic acid, but as the season progressed, propionic became the acid of major production.

Effect of Feed Intake Level

Fenner et al. (1967) showed that the pH increased and concentration of total VFA decreased in direct proportion to lowering the level of hay intake when measured by hay/100 kg body weight. Ad libitum feeding significantly increased the concentration of total VFA and the molar percent of butyric plus higher acids and lowered acetic acid. Steers on exclusively concentrate rations exhibited decreased ruminal pH and the acetic:propionic ratio also decreased. Total VFA concentration increased as the level of intake increased. Fenner et al. (1967), feeding alfalfa hay, found a significant decrease in isovaleric acid with increasing hay/100 kg body weight feed intake. Rumsey et al. (1970)

also fed an alfalfa hay diet for comparison but the extent to which changes occurred when the intake was varied appeared to be most affected by the concentrate diet. A higher feed intake also increased rumen volume and passage rate and had a lowering effect on total VFA concentration (Fenner et al., 1967).

Effect of Protein Level of Ration

Davis et al. (1957) found the proportion of total VFA represented by acetic acid decreased with increasing levels of protein in the diet. Total amounts of propionic acid increased in the presence of protein above the standards set by Morrison in 1948. Butyric acid levels increased significantly with both medium and high protein rations as opposed to maintenance, or low, rations. Statistically significant increases in the quantities of valeric acid present were observed for each change in protein intake. There was an increase in the total VFA present in the rumen fluid in response to each increase in protein feeding. There was a tendency for the proportion of the branched-chain higher acids to be greatest with diets rich in protein (Bath and Rook, 1963; Rook, 1964). Hart et al. (1973) fed molasses and urea during the grazing season to steers on fescue pastures. The urea supplement plus molasses decreased the pH of the rumen, and molasses fed with or without urea depressed the concentrations of isobutyric and isovaleric acid.

Effect of Energy Level of Ration

The energy level of the diet influences VFA levels. In the Hart et al. (1973) fescue pasture study, molasses fed without urea supplement

decreased the total VFA level. ADG was not increased and the authors hypothesized this may have been because molasses served as a substitute for the less palatable forage rather than as a supplement to it.

Putnam et al. (1966) reported that percent of butyric plus higher acids was greatest and the percentage of acetic acid was lowest at higher energy level intakes. The acetate:propionate ratio decreased with increasing energy in the diet.

Effect of Rumen Microbes

Though the chemical composition of the diet is clearly an important factor affecting the proportions of VFA in the rumen fluid, one must keep in mind the fact that it is the rumen microflora that both mediate the chemical changes and that they are themselves influenced by those changes. It is apparent that the cellulolytic organisms that develop on high forage diets are characterized in mixed culture by a high proportion of acetic acid in the end products of their metabolism and that, with the addition of concentrates there can be a preferential development of organisms producing a high proportion of propionic acid or of organisms producing a high proportion of butyric acid (Rook, 1964; Hungate, 1966).

Effect of Frequency of Feeding

Frequency of feeding and rate of VFA production after feeding have important effects on volatile fatty acid levels in the rumen. Knox and Ward (1961) found that cattle fed twice daily at 12 hour intervals showed slightly lower average VFA concentrations than animals fed eight

times daily at 3 hour intervals and animals consumed more feed with the more frequent feeding times. The relative proportion of propionate and butyrate were higher with the more frequent feedings. Feeding ten times daily instead of twice resulted in a 25% increased gain in heifer calves. In this experiment there was no significant difference in the VFA in the rumen of the two groups, but the number of protozoa was significantly greater in the frequently-fed animals.

Effect of Rate of VFA Production

Many workers (Reid et al., 1957; Stewart et al., 1958; Knox and Ward, 1961; Bath and Rook, 1963; Fenner et al., 1967; Jensen, 1975) have reported wide variations in VFA levels at varying times after feeding. In general, VFA levels increase rapidly shortly after feeding and fall to a minimum before the next feeding. Stewart et al. (1958) found the peak of VFA production was 4 to 6 hours after feeding, after which the VFA concentration decreased rapidly. In general, the relative proportions of the VFA in the rumen stayed remarkably constant. pH also shows wide variation after feeding (Fenner et al., 1967; Jensen, 1975).

Effect of In Vivo vs. In Vitro Studies

In vivo experiments with ruminant animals are costly in terms of animal upkeep and housing, and time for the experimenter. Several in vitro methods for studying rumen fermentation and VFA production have been developed but there are certain biases in the results. Stewart et al. (1958) showed that there were differences between these two methods of study. The Stewart and Schultz (1958) in vitro experiment gave much higher proportions of propionic acid and lower proportions

of acetic acid than were produced in vivo. Urea added to a hay and grain ration did not change VFA levels in vivo. In vitro there were significant changes. Stewart and Schultz surmised that the metabolic pathways taken by the long term in vitro incubations were different from those occurring in vivo, where the microorganisms were effected by the continuous absorptive and secretive processes of the host.

CHAPTER III

MATERIALS AND METHODS

Description of Trials

This study was conducted in conjunction with a series of six in vivo digestion trials by Estell (1979). Eight Angus steers were used for each trial throughout the experiment except in Trial 6 when there was insufficient forage for eight steers, so only four were used. The steers were fed ad libitum amounts of clipped forages. Between trials, the steers grazed pasture consisting predominately of red clover, orchardgrass, and tall fescue.

Each trial consisted of a seven-day preliminary period. On the eighth day rumen fluid samples were collected before the once-daily feeding in the morning, and this first collection period was designated SN1. The animals continued on the trials for another five days, during which fecal samples were taken, and on day twelve rumen samples were again collected before the morning feeding. This second collection period within the trial was designated SN2.

Date of cutting the fescue was also recorded as an independent variable. Date of cutting 1 (DC1) was the clipping of May 6 (animals were on trial May 2 but no record was kept), DC2 was May 24 and May 28, DC3 was July 3 and July 7, DC4 was August 14 and August 18, DC5 was September 18 and 22, and DC6 was not included in the study.

The steers were confined to digestion crates and randomly allotted to either a tall fescue (Festuca arundinacea, Schreb.) diet or an array of tall fescue-legume mixtures which are presented in Table 1. Crimson clover (Trifolium incarnatum), first, second, or third cutting

red clover (Trifolium pratense), and Korean lespedeza (Lespedeza stipulacea) served as the legume fraction of the mixtures in Trials 1, 2, 3, 4, and 5 respectively. No legumes were fed in Trial 6 because that late in the season all the clovers and lespedeza had died out of the experimental plots. The legumes and tall fescue obtained for each trial were characterized in terms of estimated mean height and maturity by Estell (1979) as shown in Table 1.

Digestion Trial Management

Throughout the trials the steers were fed ad libitum amounts of the forage at approximately 0900 and were given water twice daily. All steers had access to trace mineralized salt. Fescue used in the trials was harvested within an hour immediately preceeding feeding time using a lawnmower equipped with a grass catcher.

Clovers used in trials 1 through 4 had been collected and frozen prior to use. The lespedeza for trial 5 was collected immediately prior to feeding each day. Trial 6 was without legumes and by this time of the season only enough fescue from the experimental plot remained to feed four steers.

Data Collection

Rumen fluid samples were obtained by stomach tube under suction. Immediately upon collection of the samples their pH was determined with a portable pH meter with a glass electrode. A 70 ml aliquot was sealed in a plastic bag, and cooled and frozen within 15 minutes of collection to await volatile fatty acid analysis.

Estell (1979) collected and analyzed daily random forage and

TABLE 1. FORAGES FED TO STEERS IN DIGESTIBILITY TRIALS^{ab}

Trial number	Date initiated (1978)	Legume source	Ratios of Fescue:Legume						Est. mean height of fescue mat.	Est. mean height of legume mat.
			100:0	90:10	80:20	70:30	60:40	50:50		
1	April 27	Crimson clover	2		2		2		2	13 Prehead 20 Prebloom
2	May 17	1st cut Red clover	2	1	1	1	1	1	1	8 Midhead 25 Prebloom
3	June 26	2nd cut Red clover	1	1	1				1	16 Vegetative 17 Prebloom, early blm
4	August 7	3rd cut Red clover	2	1	1	1	1	1	1	15 Dry/dormant 16 Early blm, midbloom
5	Sept. 11	Korean lespedeza	4	1	1	1	1			10 Vegetative/ 4 Vegetative/ tough dry
6	Oct. 16	None	3							11 Vegetative

^aFrom Estell (1979)

^bNumbers indicate number of animals allotted to particular forage ratios

fecal samples. Forage samples were dried in duplicate in a 60 C forced air draft oven, ground and later analyzed further. Fecal samples were dried in a 60 C forced air draft oven to determine dry matter content and to calculate dry matter digestibility (DMD). Dry matter intake (DMI) was calculated for the forages and chemical analysis was performed for crude protein (CP), crude fiber (CF), nitrogen free extract (NFE), ether extract (EE), and ash (Estell, 1979).

Volatile Fatty Acid Analysis

VFA analysis was performed in duplicate on a Bendix 2500 gas-liquid chromatograph equipped with an Infotronics CRS-309 digital integrator. The method of sample preparation is similar to that of Erwin et al. (1961). Samples were prepared for analysis by first thawing and transferring 40 ml of the supernate to a 45 ml tube followed by centrifuging under refrigeration at 10,000 g for 5 minutes. Two 5 ml aliquots of the supernate were acidified with 1 ml of 25 percent meta-phosphoric acid solution that contained 2-ethyl butyric acid as an internal standard. The acidified sample was refrigerated for 30 minutes or longer, then centrifuged again in the refrigerated centrifuge at 10,000 g for 10 minutes. With the coagulated protein thus removed, a 4 to 5 ml aliquot of the supernate was frozen and stored until the sample was thawed again and analyzed by gas-liquid chromatography.

The sample was injected into the gas-liquid chromatograph in 1 μ l amounts from a microliter syringe¹. A 1.8 meter glass column (2.0 mm i.d.) was packed with 10% SP1200/1 percent H₃PO₄ on 80/100 mesh chromosorb W AW² for separation of the VFA. Peak areas were compared

¹Hamilton Company, Reno, Nevada

²Supelco Incorporated, Bellefonte, Pennsylvania

to the standard VFA solutions and adjusted using the 2-ethyl butyric acid standard peaks for final quantification of each acid.

Statistical Analysis

The Statistical Analysis System (SAS) developed by Barr et al. (1979) was utilized to determine if differences existed among the different forages and maturities of forages for ruminal pH and levels of ruminal VFA production in the steers. The MEANS procedure of SAS was utilized to produce simple descriptive statistics of the pH and VFA levels. Stepwise procedures (Barr et al., 1979) were used to produce regressions of dependent variables pH and VFA on independent date of cutting (DC), sampling time (SN), percent grass (PG), dry matter digestibility (DMD), and dry matter intake (DMI) to produce indices which best ($P < .1$) predicted the dependent variables.

CHAPTER IV

RESULTS AND DISCUSSION

I. RESULTS

The results of chemical composition determination on fescue and legume portions of the diet appear, respectively, as Tables A1 and A2.

Effects of Diet Composition

Arithmetic means of the effect of diet composition on volatile fatty acids appear in Table 2. Unequal sample numbers (N) for valerate occurred because the gas-liquid chromatograph was not always sensitive enough to detect the low levels of valerate in rumen fluid. Unequal sample numbers between percentages of grass were from the random distribution of experimental diets. The 100% grass ration was by far the most common one. No outstanding trends were apparent from the arithmetic means.

The relationships between the various VFA and diet composition are shown in Table 3. Variations in propionate, pH, and percent isovalerate were related to ($P < .1$) percent grass and percent grass². The graphic representation for propionate, pH, and percent isovalerate are found, respectively, on Figures 3, 4, and 5. For propionate, the equation explaining the variation is $\hat{Y} = 16.63 - .24(\text{percent grass}) + .002(\text{percent grass})^2$. As the percent grass increased from 40% to 100%, the concentration of propionate fell from 9.6 μ M/ml at 40% to 8.0 μ M/ml at 70% and then rose again to 9.1 μ M/ml at 100% grass rations. Percent grass explained only 4.6% of the variation in propionate.

TABLE 2. ARITHMETIC MEANS OF EFFECT OF PERCENT GRASS ON VOLATILE FATTY ACIDS AND pH^{ab}

Variable	Overall		Percent Grass												100	
			40		50		60		70		80		90			
	N	Avg	N	Avg	N	Avg	N	Avg	N	Avg	N	Avg	N	Avg	N	Avg
Total volatile fatty acids	64	55.86	8	53.43	4	48.12	8	63.32	6	53.84	10	55.45	8	53.25	20	57.24
Acetate	64	43.03	8	40.69	4	35.77	8	46.80	6	39.73	10	41.13	8	44.71	20	45.17
Propionate	64	8.85	8	7.48	4	6.94	8	9.45	6	8.85	10	8.89	8	9.20	20	9.39
Isobutyrate	64	.64	8	.68	4	1.34	8	.69	6	.62	10	.64	8	.52	20	.52
Butyrate	64	3.73	8	3.74	4	3.07	8	5.02	6	3.59	10	3.67	8	3.44	20	3.53
Isovalerate	64	.91	8	1.05	4	.82	8	1.08	6	.96	10	1.01	8	.74	20	.80
Valerate	31	.34	5	.49	3	.26	5	.49	2	.26	5	.32	4	.18	9	.22
pH	64	7.0	8	7.0	4	7.1	8	6.9	6	7.1	10	7.0	8	7.1	20	7.0
Percent acetate	64	74.57	8	76.56	4	74.53	8	73.68	6	73.44	10	74.06	8	73.93	20	74.98
Percent propionate	64	15.88	8	14.19	4	14.59	8	15.04	6	16.71	10	15.98	8	16.98	20	16.42
Percent isobutyrate	64	1.16	8	1.31	4	2.28	8	1.11	6	1.17	10	1.22	8	.97	20	.92
Percent butyrate	64	6.65	8	6.65	4	6.45	8	8.04	6	6.68	10	6.69	8	6.54	20	6.15
Percent isovalerate	64	1.66	8	2.00	4	1.82	8	1.75	6	1.81	10	1.86	8	1.40	20	1.41
Percent valerate	31	.54	5	.75	3	.43	5	.69	2	.60	5	.48	4	.37	9	.35
Acetate:Propionate ratio		4.88	8	5.82	4	5.34	8	4.98	6	4.48	10	4.80	8	4.54	20	4.65

^aThe gas liquid chromatograph could not detect all levels of valerate and percent valerate

TABLE 3. MODELS FOR RELATING VOLATILE FATTY ACIDS, PERCENT VOLATILE FATTY ACIDS, AND pH TO PERCENT GRASS^a

Variable	Intercept	Percent grass	Percent grass ²	R ²	Residual standard deviation
Total volatile fatty acids	90.35**	-1.08	.0074	.033	13.51
Acetate	68.39**	-.84	.006	.037	9.98
Propionate	16.63**	-.24 ⁺	.002 ⁺	.046	2.50
Isobutyrate	.79	.0001	0	.0087	.48
Butyrate	6.99**	-.09	.0005	.025	1.30
Isovalerate	.63	.013	-.0001	.050	.33
Valerate	.87 ⁺	-.02	.0001	.043	.23
pH	6.01**	.02*	.0001 ⁺	.136	.20
Percent isovalerate	.067	.058 ⁺	-.0004*	.113	.548
Percent valerate	1.33	-.03	.0001	.054	.354

^aOnly significant models are presented for percentage figures, percent isovalerate and percent isovalerate
⁺ P < .1
^{*} P < .05
^{**} P < .01

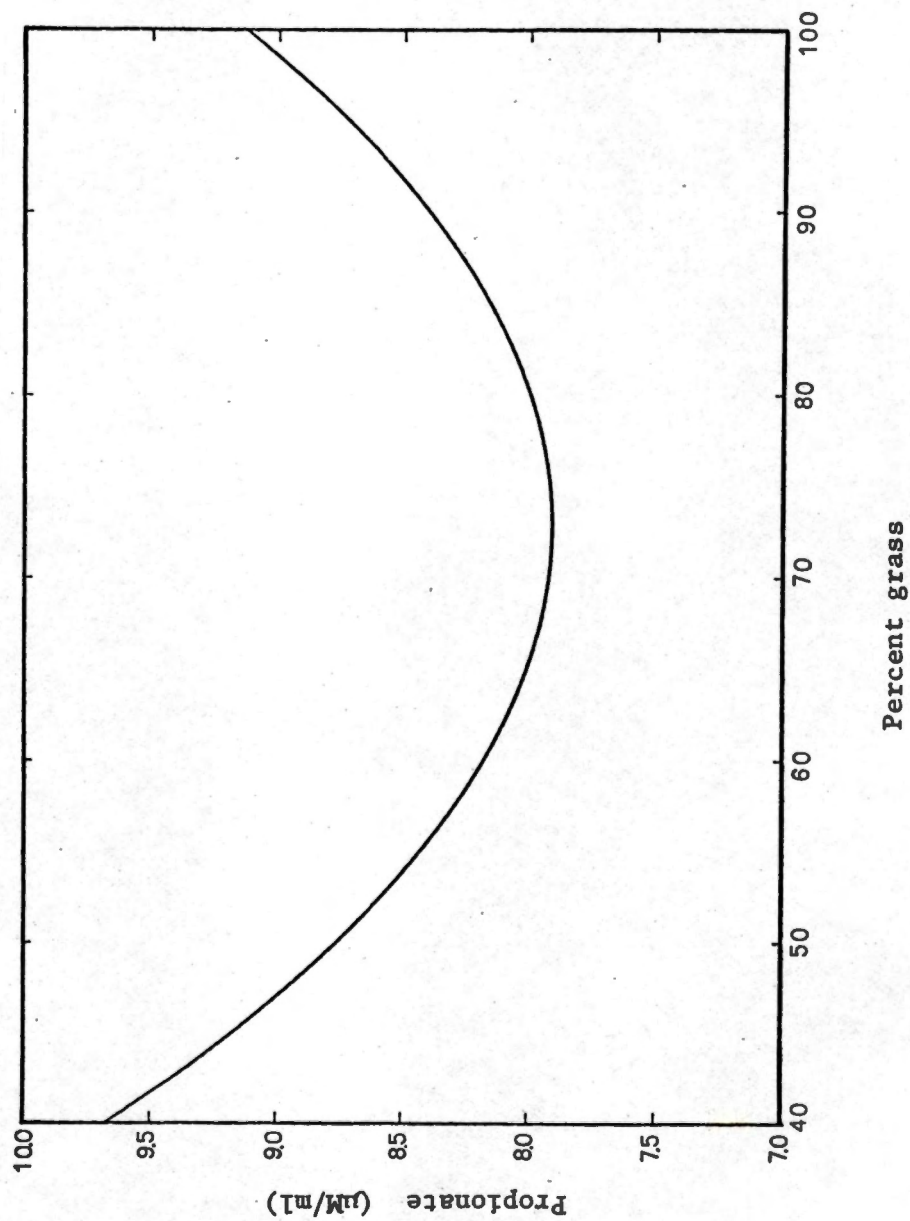


Figure 3. Effect of percent grass on propionate.
Model $\hat{Y} = 16.63 - .24PG + .002(PG)^2$

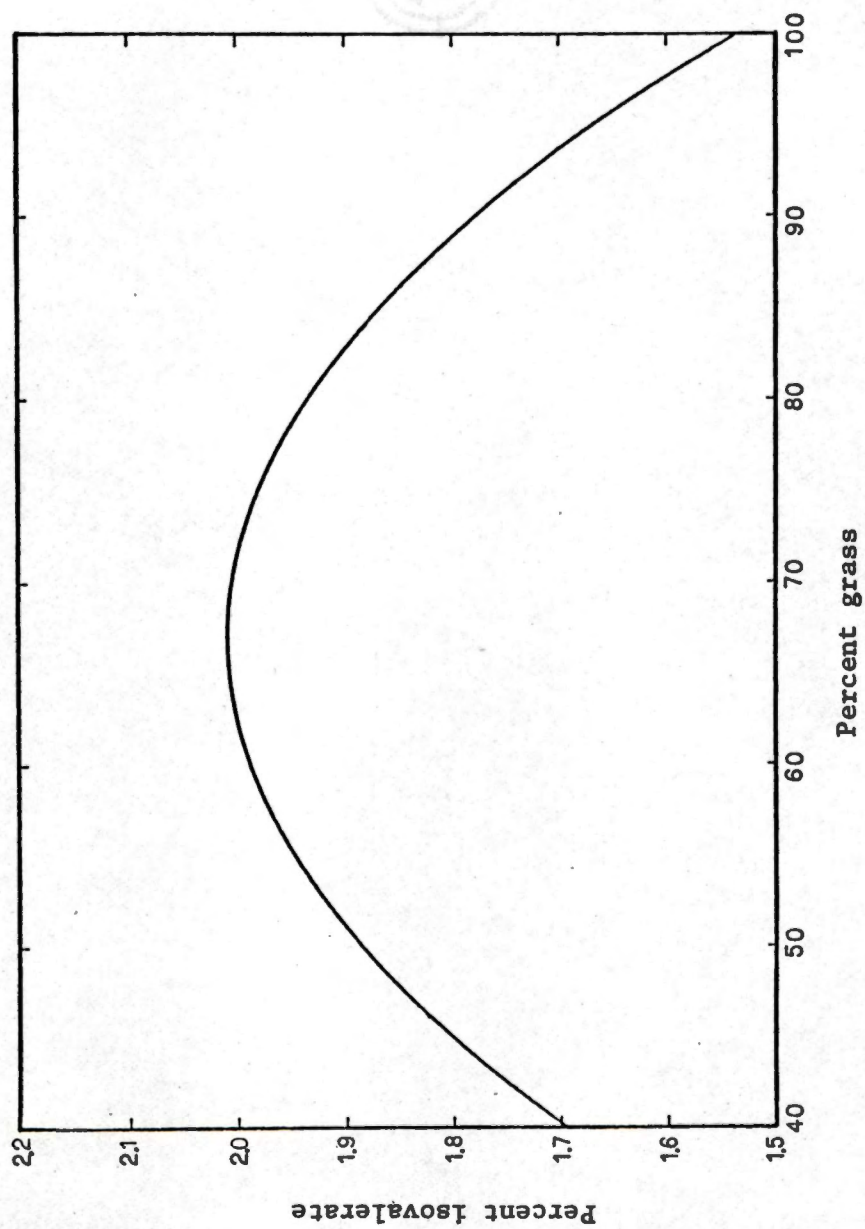


Figure 4. Effect of percent grass on percent isovalerate.
Model $\hat{Y} = .63 + .013PG - .0001(PG)^2$

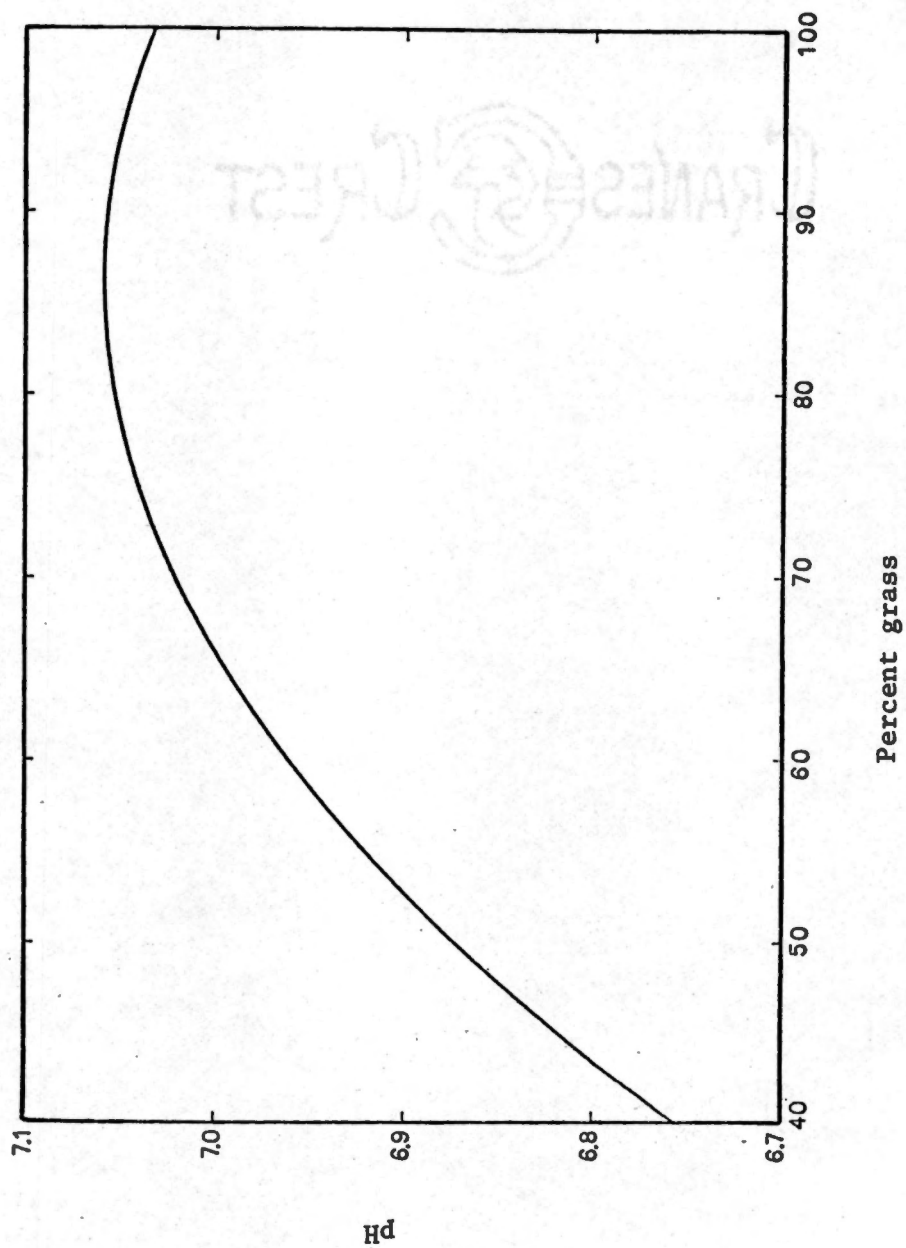


Figure 5. Effect of percent grass on pH
Model $\hat{Y} = 6.01 + .02PG - .001(PG)^2$

The relationship between percent grass and pH is more of an increasing function. This relationship is described by the equation $\hat{Y} = 6.01 + .02(\text{percent grass}) - .0001(\text{percent grass})^2$. The pH of rumen liquor on a 40% grass diet was 6.76 and increased to a high of 7.05 at 85% grass, after which it slowly decreased to 7.03 for 100% grass rations. Percent grass explained 13.6% of the variation in pH.

Effects of Date of Cutting

The arithmetic means and least square means of volatile fatty acids and pH related to dates of cutting are on Table 4 and for percent volatile fatty acids are on Table 5. No clear trends were apparent from the means.

Effects of Sampling Time

The values of Tables 4 and 5 also reflect variation due to Sampling Time (SN). In many cases, values increased from SN1 to SN2 within the Dates of Cutting.

Effects of Dry Matter Digestibility

Table 6 reports Dry Matter Digestibility (DMD) values by cutting dates and sampling times. DMD tended to decrease from the start of the trials until the conclusion. A curvilinear relationship was detected between DMD and the following VFA: butyrate, valerate, percent butyrate, percent isovalerate, and percent valerate (Table 7). Butyrate was effected ($P < .01$) most and the graph of this effect appears as Figure 6. At 40% DMD the predicted value of percent butyrate was 6.1%, and decreased slightly to a low of 5.8% butyrate at 50% DMD, then increased to 8.4% butyrate at 80% grass and would

TABLE 4. MEANS OF VOLATILE FATTY ACIDS AND pH
BY DATE OF CUTTING AND SAMPLING TIME^{abcde}

Variable	SN	Date of cutting				
			May 24 and May 28	July 3 and July 7	Aug 14 and Aug 18	Sep 18 and Sep 22
		May 6	(%)			
Percent acetate ^b	1		75.66	74.70	75.48	73.66
	2	72.85	73.25	74.41	76.32	74.54
Percent propionate ^b	1		14.47	15.00	15.52	17.18
	2	15.63	16.67	16.65	15.73	16.05
Percent isobutyrate ^b	1		1.48	1.82	1.34	1.08
	2	1.04	.99	1.28	1.10	1.07
Percent butyrate ^c	1		7.45	5.98	5.95	6.26
	2	8.27	6.87	6.21	5.77	6.37
Percent isovalerate ^d	1		1.64	2.09	1.76	1.55
	2	1.75	1.72	1.32	1.57	1.84
Percent valerate ^b	1		.39	.37	.03	.32
	2	.49	.46	.09	.06	.24
Acetate:propionate ratio ^b	1		5.32	5.33	5.17	4.41
	2	4.78	4.49	4.64	5.35	4.71

^aSN refers to sampling time

^bArithmetic means unless models tested were significant

^cLeast squares mean from model $\hat{Y} = \mu + DC + SN + DC*SN + \text{error}$
DC is date of cutting

^dLeast squares mean from model $\hat{Y} = \mu + DC + SN + DC*SN + PG + PG^2 + \text{error}$
PG is percent grass

TABLE 5. MEANS OF PERCENT VOLATILE FATTY ACIDS AND pH
BY DATE OF CUTTING AND SAMPLING TIME^{abcd}

Variable	SN	Date of cutting				
			May 24 and May 28	July 3 and July 7	Aug 14 and Aug 18	Sep 18 and Sep 22
		May 6	(uM/ml)			
Total volatile fatty acids ^c	1		57.39	46.36	44.65	59.86
	2	58.41	65.66	56.17	50.66	58.26
Acetate ^c	1		43.60	34.67	33.65	44.23
	2	42.60	48.16	41.71	38.45	43.46
Propionate ^d	1		8.44	7.72	9.75	10.69
	2	9.84	11.17	9.80	10.65	9.93
Isobutyrate ^b	1		.94	.87	.58	.63
	2	.59	.65	.69	.55	.62
Butyrate ^c	1		4.23	2.72	2.64	3.84
	2	4.72	4.50	3.46	2.92	3.71
Isovalerate ^b	1		.99	1.00	.72	.86
	2	1.06	1.11	.70	.73	1.03
Valerate ^e	1		.24	.20	.004	.14
	2	.32	.35	.05	.02	.09
pH ^d	1		6.65	7.08	7.17	6.92
	2	6.94	7.00	7.04	7.12	7.10

^aSN refers to sampling time

^bArithmetic mean, employed when no independent variables in the complete model were significant (P .05)

^cLeast squares mean from model $\hat{Y} = \mu + DC + SN + DC*SN + PG + PG^2 + \text{error}$ PG is percent grass, DC is date of cutting

^dLeast squares mean from model $\hat{Y} = \mu + DC + SN + DC*SN + PG + PG^2 + PG*DC + \text{error}$

^eLeast squares mean from model $\hat{Y} = \mu + DC + SN + DC*SN + DC*PG + \text{error}$

TABLE 6. DRY MATTER INTAKE AND DRY MATTER DIGESTIBILITY BY TRIAL^a

Variable	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5						
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD					
DMI (kg)	3.68	.83	2.67	2.32	.61	1.84	2.07	.09	.22	3.06	.43	1.54	3.36	.48	1.61
DMD (%)	77.10	2.53	6.44	63.70	5.86	18.90	42.01	3.46	8.43	64.18	4.39	13.99	67.37	1.92	4.93

^a Estell (1979)

TABLE 7. MODELS FOR RELATING VOLATILE FATTY ACIDS AND pH
TO DRY MATTER DIGESTIBILITY AND DRY MATTER DIGESTIBILITY²

Variable	Intercept	Dry matter digestibility	Dry matter digestibility ²	R ²	Residual standard deviation
Total volatile fatty acids	70.33	-.70	.007	.013	13.65
Acetate	50.11	-.41	.004	.008	10.13
Propionate	7.82	-.002	.0003	.016	2.54
Isobutyrate	.37	.012	-.0001	.001	.48
Butyrate	9.09*	-.234*	.002*	.104	1.25
Isovalerate	2.39	-.055+	.0005+	.049	.33
Valerate	1.21	-.041*	.0004*	.059	.23
pH	6.85	.008	-.00008	.010	.22
Percent acetate	71.53	.174	-.002	.041	2.79
Percent propionate	8.42	.237	-.002	.042	2.12
Percent isobutyrate	1.06	.012	-.0001	.004	.77
Percent butyrate	13.61**	-.310**	.003**	.218	1.11
Percent isovalerate	4.35+	-.091+	.0008+	.056	.57
Percent valerate	1.90+	-.064*	.0006*	.063	.35
Acetate:propionate ratio	7.84	.086	.0006	.052	.94

+ P < .1

* P < .05

** P < .01

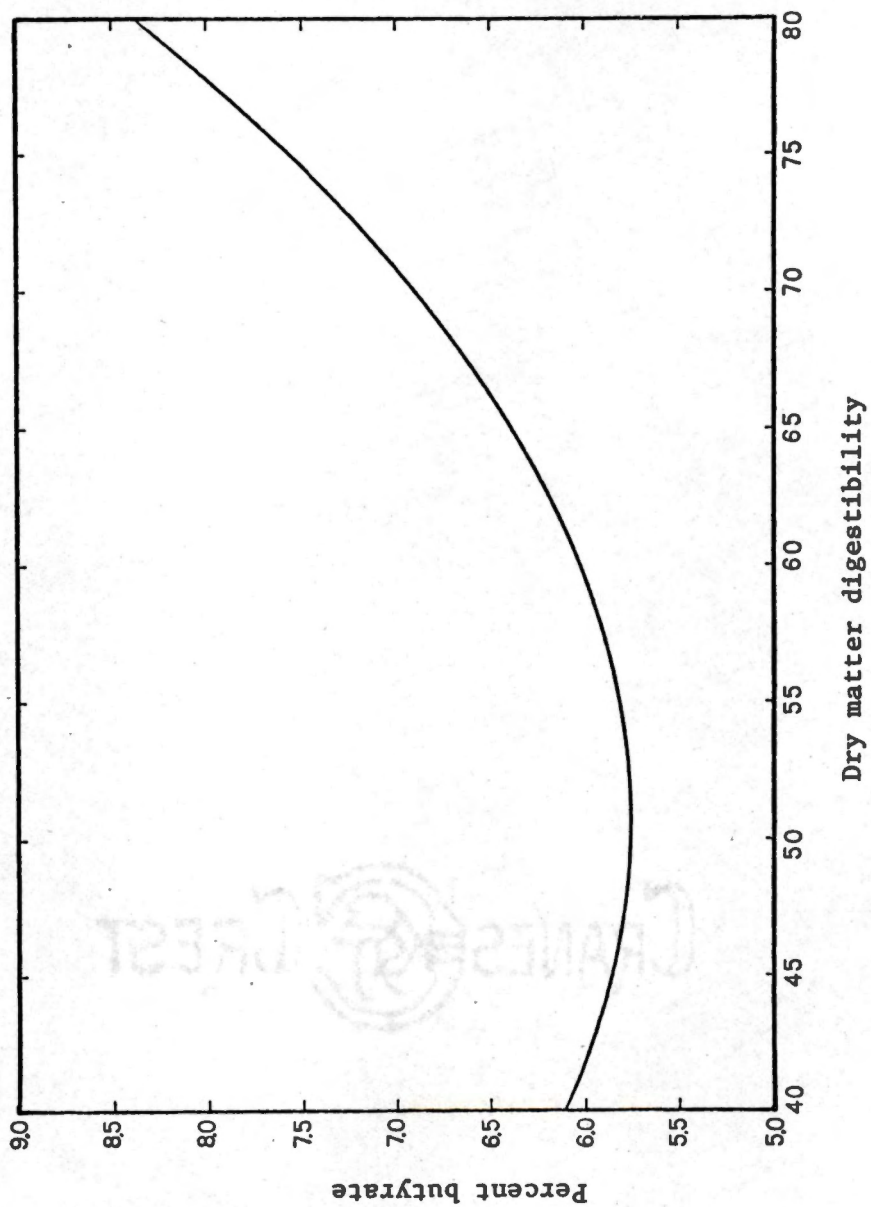


Figure 6. Effect of dry matter digestibility on percent butyrate
Model $\hat{Y} = 13.61 - .31DMD + .003(DMD)^2$

continue to increase to 100% grass. DMD explained 21.8% of the variation in percent butyrate, but only 10.4% for butyrate concentration, 4.9% for isovalerate concentration, 5.9% for valerate concentration, 5.6% for percent isovalerate, and 6.3% for percent valerate.

Effects of Dry Matter Intake

Table 6 also includes data for dry matter intake (DMI) values of the forages by cutting dates. Dry matter intake decreased for the first four trials but increased on the fifth trial. DMI and DMI^2 explained ($P < .01$) variation for pH, and for percent butyrate ($P < .05$) (Table 8). The prediction model for pH explained 16.9% of the variation. The graph of pH and dry matter intake relationship appears as Figure 7. The predicted pH peaked at 7.07 when dry matter intake was 3.0 kg/day. Its lowest points on the graph were at the end points, pH 6.88 at 2 kg/day DMI and 6.95 at 4.0 kg/day DMI.

II. DISCUSSION

Diet Composition

The emphasis of the study was on the effect of percent grass on volatile fatty acids and pH of the rumen fluid.

The parameter best explained by the prediction model $\hat{Y} = \mu + \text{percent grass} + (\text{percent grass})^2$ was pH. Other workers (Church, 1976) have cited variations for pH due to ration differences. Percent grass in the model accounted for only 13.6% of the total variation in pH. Therefore, although percent grass was helpful in explaining pH changes, it was not a major factor.

Percent grass explained 11.3% of the variation in percent isovalerate.

TABLE 8 . MODELS FOR RELATING VOLATILE FATTY ACIDS AND pH
TO DRY MATTER INTAKE AND DRY MATTER INTAKE²

Variable	Intercept	Dry matter intake	Dry matter intake ²	R ²	Residual standard deviation
Total volatile fatty acids	48.53	2.96	-.22	.006	13.69
Acetate	33.53	4.42	-.60	.004	10.15
Propionate	8.11	.008	.072	.013	2.54
Isobutyrate	-.48	.78	-.13	.023	.47
Butyrate	5.12	-1.31	.26	.028	1.30
Isovalerate	1.12	-.23	.05	.024	.33
Valerate	.17	-.05	.016	.017	.24
pH	5.59*	.95**	-.15**	.169	.20
Percent acetate	68.70*	4.86	-.93*	.057	2.77
Percent propionate	16.80	-1.13	.26	.020	2.14
Percent isobutyrate	-.74	1.35	-.22	.026	.76
Percent butyrate	11.29*	-3.54*	.63*	.085	1.20
Percent isovalerate	2.10	-.36	.071	.010	.58
Percent valerate	.46	-.22	.051	.030	.36
Acetate:propionate ratio	3.48	1.21	-.23	.034	.95

+ P .1
 * P .05
 ** P .01

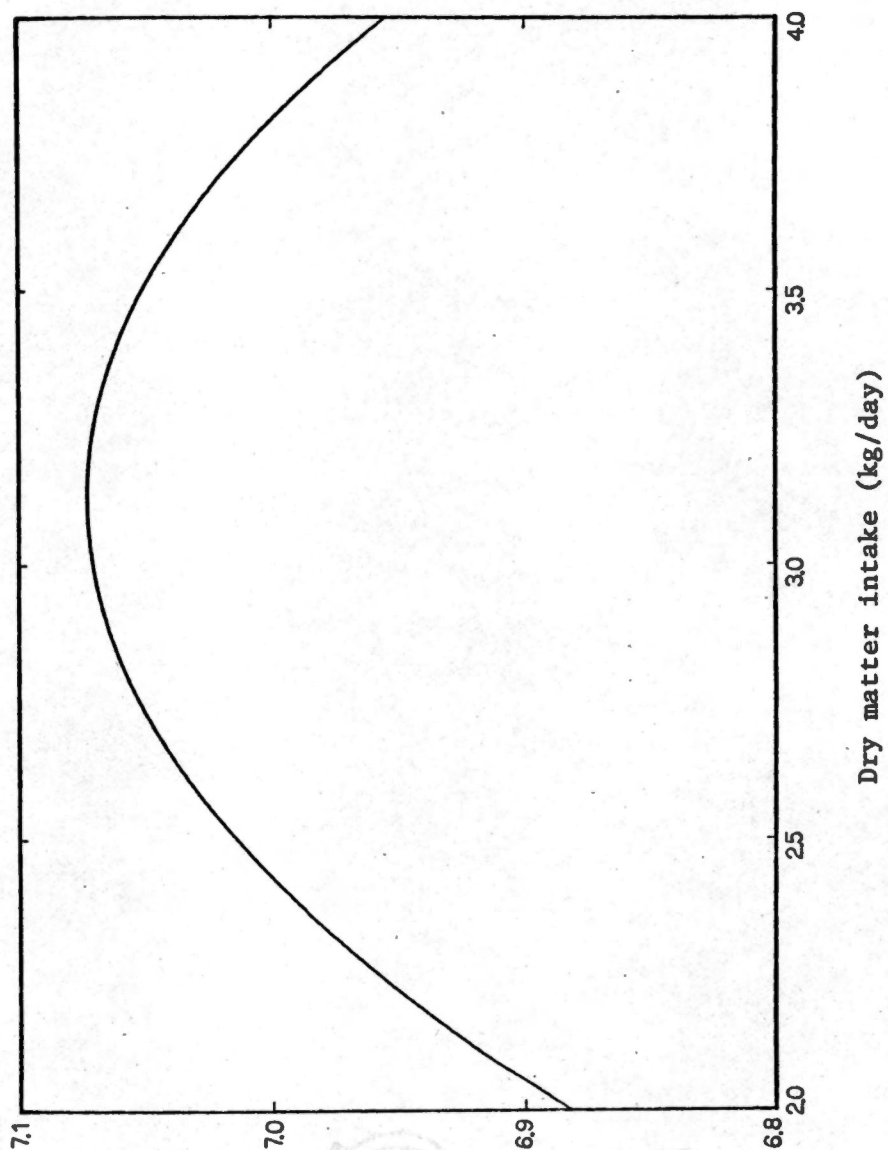


Figure 7. Effect of dry matter intake on pH
Model $\hat{Y} = 5.86 + .95DMI - .15 (DMI)^2$

Date of Cutting

Date of Cutting (DC) was only a partial measure of season and of maturity for fescue grass. DC does not measure season or maturity for legumes, most of which had been cut and frozen ahead of time, although in trials 2 through 4 it did reflect the progression from first through third cuttings for red clover (Table 1 page 18).

Means for VFA and pH showed no consistent trends across DC. Therefore, season and maturity of fescue (legume, as explained earlier, did not necessarily reflect season and maturity as well as fescue) were not major factors for VFA levels or percentages.

Sampling Time

Sampling Time (SN) measures some of the adaptation of the steers to the digestion trial although at the time of the first sampling steers already were in their stalls for one week.

General numerical trends of Tables and showed slight increases of values from SN1 to SN2 within trials. These increasing trends were especially evident for trials 1 through 4 and showed an adaptation effect of the steers' rumen digestion to metabolism stall conditions. The increasing trends indicated that the seven day adaptation period was not long enough, but by trial 5 it was not a factor. This may have been because the lespedeza used in trial 5 was the only legume fresh cut and fed, as the fescue was for all trials.

Dry Matter Intake

Dry Matter Intake (DMI) as an absolute value was low for the steers on digestion trials (Table). It ranged from a low of 2.0 kg/day to a

high of 3.8 kg/day. DMI and DMI^2 , however, were predictors ($P < .05$) for pH and percent butyrate. Of the total variation in pH, 16.9% was explained ($P < .01$) by DMI and DMI^2 . The effect of DMI and DMI^2 explained only 8.5% of total variation in percent butyrate, and was therefore not a good predictor.

General Discussion

The anticipated results of this study were variations in ruminal VFA levels that could be correlated to the mixture of fescue-legume forage, changes of season, changes in dry matter digestibility, or changes in dry matter intake.

The legume portion of the diet was confounded, particularly by varieties of legume and from seasonal variation in availability. However, even within trials 2 through 4 where only red clover of successive first, second, and third cuttings was fed, there were not significant differences that may have been due to the legume fraction of the forage.

The fescue grass reflected a range of maturity from prehead to dry and dormant, and a dry matter digestibility that varied from 42% to 77%. The growing season varied from mid-spring to early fall. Even with that diverse range of values, no more than 20% of the change in ruminal VFA could be explained by any one variable. In fact, many of the larger R^2 values were for relatively minor VFA.

This study has indicated that varying rates of performance for cattle raised on fescue-legume pastures was due to some factor other than changes in ruminal VFA. One could speculate that factors involved might be selective grazing, even beyond the effect allowed for by varying the legume portion of the diet by 60%, or greatly increased dry matter intake.

CHAPTER V

SUMMARY

The objective of this study was to determine the effect of grass-only or mixed grass-legume diets on ruminal VFA levels and pH of all-forage rations for beef steers.

The experimental animals, yearling Angus steers, underwent six digestion trials. Forage DMI, DMD, and percent grass were recorded for each trial, and the series was spread at intervals from early May until mid-October. Between the one week sampling periods the steers grazed pastures of similar makeup to the digestion trial forages. In all cases the grass portion of the rations was tall fescue. The legume portion varied according to availability. It included crimson clover, red clover, and Korean lespedeza.

The steers were brought into their metabolism stalls one week prior to data collection. On days 8 and 12 rumen samples were taken by stomach tube. pH was determined immediately by portable pH meter, then samples were frozen for later VFA analysis by gas-liquid chromatography.

The independent variables considered were percent grass, date of cutting, sampling time, dry matter digestibility (DMD), and dry matter intake (DMI). The range of percent grass was 40% grass:60% legume to 100% grass in the ration. Date of cutting encompassed early May to mid-September and included at least partial effects of season, maturity, and adaptation to the digestion trial. DMD ranged from 42% to 77%. DMI was generally low but ranged from 1.54 to 3.68 kg/day.

The General Linear Models program of the Statistical Analysis System (Barr et al., 1979) analyzed the effects of cutting date,

sampling time, percent grass, DMD and DMI on the VFA and pH and developed prediction models. Percent grass and percent grass² partially predicted levels of propionate, percent isovalerate, and pH, but explained only 4.6%, 11.3%, and 13.6%, respectively, of the variation.

The variations of VFA were therefore characterized by low correlation with the independent variables percent grass, date of cutting, sampling time, DMD, and DMI. Observations of animal performance that favored fescue-legume forages over pure fescue apparently were due to factors other than ruminal VFA levels.

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APPENDIX



TABLE A1. FESCUE COMPOSITION BY TRIAL^{abc}

Variable	Trial 2			Trial 3			Trial 4			Trial 5			Trial 6							
	N	Mean	S.D.	Range	N	Mean	S.D.	Range	N	Mean	S.D.	Range	N	Mean	S.D.	Range				
Z DM	6	21.06	2.00	5.15	6	20.50	4.18	12.00	6	23.28	3.73	8.50	6	29.18	6.89	17.20	6	42.46	5.08	14.10
CP	6	11.92	1.57	4.10	6	11.56	1.43	4.02	6	12.12	1.34	3.53	6	15.30	1.10	3.05	6	11.39	1.87	5.19
EE	4	1.71	.12	.25	6	2.12	.52	1.25	5	2.63	.18	.42	6	2.55	.16	.39	6	2.57	.19	.55
NFE	0	--	--	--	6	49.26	1.04	2.54	5	48.05	1.03	2.63	5	46.38	1.84	4.76	6	53.69	1.17	3.29
CF	0	--	--	--	6	26.96	.76	2.04	5	27.25	1.61	4.50	5	27.48	1.49	3.89	6	25.24	.88	1.95
Ash	5	8.21	.63	1.46	6	10.09	.47	1.35	6	9.71	.51	1.06	6	8.33	.41	1.11	6	7.12	.39	.81
CNC	6	65.62	2.45	6.54	6	64.32	.74	2.24	6	67.43	1.96	5.63	6	64.35	1.20	3.21	6	65.75	1.30	4.03
HEMIC	4	28.63	1.13	2.52	6	28.23	.67	1.57	5	31.07	1.01	2.67	6	27.00	2.83	6.69	6	31.05	1.79	4.79
ADF	4	37.85	1.93	4.34	6	36.09	.56	1.51	5	36.04	1.42	3.52	6	37.35	2.30	5.66	6	34.70	1.68	4.63
CELLU	4	33.41	1.40	3.24	6	31.54	.71	1.67	5	32.20	1.17	3.22	6	31.40	1.15	3.10	6	29.77	1.40	3.41
ADL	4	4.44	.85	1.91	6	4.55	.39	1.07	5	3.85	.44	1.17	6	5.95	1.51	3.70	6	4.93	.49	1.22
IVDM	3	65.46	7.11	14.20	6	55.11	1.67	4.28	5	59.98	3.76	9.68	6	54.81	3.90	10.17	6	56.31	3.43	9.22
IVOMD	3	61.08	6.25	12.13	6	48.63	1.78	4.52	5	53.07	3.64	9.11	6	48.50	3.58	8.69	6	51.41	3.49	9.80

^aFrom Estell (1979)

^bAll analyses (excluding ZDM) are expressed on a dry matter basis

^cN indicates number of samples analyzed for each variable

VITA

Natalie Wiest Shipman was born December 11, 1948 in Lancaster, Pennsylvania, the only daughter and first of five children of Hiram List Wiest and Susanne Habecker Wiest. She attended school in Hempfield Union School District and moved with her family to Hershey, Pennsylvania in 1966. She was graduated from Hershey Senior High School in 1967. She attended the Pennsylvania State University, from which she was awarded the Bachelor of Arts degree in General Arts and Sciences in 1971. Two days after her Penn State graduation she was in Philadelphia, Pennsylvania attending Drexel University and was awarded the Master of Science degree in 1973 in Library Science. She is a member of Beta Phi Mu, national library science honorary society.

From September 1972 until August 1975 she was employed as Science Librarian by Juniata College, a small protestant denominational college in Huntingdon, Pennsylvania. She was attracted to employment opportunities at the University of Tennessee, Knoxville, which would allow, and in fact encouraged, her interest in doing graduate work in animal science. She was hired for one year by the UTK library system as Reference Librarian, Health Sciences Specialist from September 1975 to August 1976. She began at the Instructor level as Librarian of Agriculture and Veterinary Medicine in September 1976 and had been with that library until September 1980. In July of 1979 she was promoted to the rank of Assistant Professor and granted tenure.

She started on the animal science track when she first arrived in Knoxville, first making up a long list of prerequisites and then completing graduate level animal science coursework Spring Quarter

1980. She received her M. S. degree in Animal Science in December 1980.

She married Loyd Wayde Shipman on December 22, 1979. He accepted employment with the University of New Hampshire in Durham, N.H. beginning August 1980 and they left the University of Tennessee for that reason.