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To the Graduate Council:

I am submitting herewith a thesis written by Peter Eldon Shumway entitled "Volatile Fatty Acid Production After Different Lengths of In Vitro Fermentation and Its Value As A Predictor of the Performance of Grazing Steers." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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VOLATILE FATTY ACID PRODUCTION AFTER DIFFERENT LENGTHS OF *IN VITRO* FERMENTATION AND ITS VALUE AS A PREDICTOR OF THE PERFORMANCE OF GRAZING STEERS

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Peter Eldon Shumway

March 1970

Dedicated to my lovely wife, Pam, for her inspiration, her loyalty and her unending love, without which this experience could not have been possible; and to my family.

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ABSTRACT

The objective of this experiment was to determine which pasture quality measurements would be most accurate and most practical in combination with *in vitro* volatile fatty acid production after three specific periods of fermentation time in predicting body weight gains of beef animals grazing two types of pasture.

A grazing trial had been conducted previously with steers on orchardgrass-clover and fescue-lespedeza pastures to determine changes in body weight gains throughout the growing season. Chemical composition (AIL, ADF, cellulose and crude protein content, and lignin:fiber ratio), visual estimates of forage species composition (percent grass, percent legume and legume index) and *in vitro* digestible dry matter of pasture samples had also been determined at 28-day intervals during the spring-summer grazing season.

In the present study, an *in vitro* fermentation trial to determine VFA production was conducted with forage samples from these pastures, using 12, 24 and 36 hours of fermentation.

Multiple regression equations were developed using measurements of pasture quality and *in vitro* VFA production.

It was concluded that:

1. There is no combination of variables which can be used with 12-hour VFA production data which will accurately predict ADG of beef animals grazing either pasture species.

2. The combination of variables which explains the most variation in ADG of animals grazing OC pastures includes 24-hour molar percentage

of butyric acid, acid insoluble lignin, percentage of fescue in the OC pasture, lignin:fiber ratio, percent cellulose and percent crude protein. The significant R^2 ($P<.01$) indicates that this combination of variables accounts for 94 percent of the variation in ADG. For animals grazing FL pastures, the 24-hour equation using the combination of variables which accounted for the most variation in ADG yielded an R^2 of .80 ($P>.05$), which was not as good as results following 36 hours of fermentation.

3. The combination of variables which most accurately estimates variation in ADG of animals grazing FL pastures includes 36-hour molar percentage butyric acid in combination with percent cellulose, legume index, percent fescue, in vitro digestible dry matter and percent crude protein. The significant R^2 ($P<.01$) associated with this equation indicates that 91 percent of the variation in ADG of the cattle grazing FL pastures is accounted for by this combination of variables. For animals grazing OC pastures the 36-hour equation using the combination of variables which accounted for the greatest amount of variation in ADG yielded an R^2 of .93 ($P<.01$) which was not as accurate as results from 24 hours of fermentation.

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CHAPTER I

INTRODUCTION

The production of beef cattle in the southeastern United States is based primarily on forage feeding systems. Permanent pastures provide the majority of this forage. Two major forage species grown in Tennessee are Kentucky-31 tall fescue and native orchardgrass, both of which are often grown in combination with a legume, such as Ladino clover or Korean or Kobe lespedeza. Primarily through the use of grazing trials, past research has shown that animals respond differently to these two grass species. However, since grazing trials are both expensive and time consuming, other research methods have been employed to predict the performance of animals grazing these pastures.

Various other measurements of pasture quality (e.g., chemical composition, visual pasture score, dry matter digestibility, volatile fatty acid production and selectivity) have been utilized to determine the reasons for differences in the performance of animals grazing these two types of pasture. The more closely related these pasture quality measurements are to actual productive capacity of pastures in terms of body weight gain or some other economically important criterion of grazing animals, the more suitable these measures are in developing improved pasture species, formulating better pasture mixtures and improving pasture management systems.

Production of volatile fatty acids (VFA), both *in vivo* and *in vitro*, has been used to study pasture quality. Wang (1968) used various chemical and visual determinations in combination with 48-hour *in vitro* VFA production and was able to explain up to 76 and 73 percent of the variation in average daily gains (ADG) of cattle grazing orchardgrass and fescue pastures, respectively. It was suggested that VFA production from a shorter *in vitro* fermentation time might better predict ADG of grazing animals.

The objective of the experiment reported herein was to determine which pasture quality measurements would be most accurate and most practical in combination with VFA production after three specific *in vitro* fermentation time periods in predicting body weight gains of beef animals grazing orchardgrass-Ladino clover or fescue-lespedeza pastures.

CHAPTER II

REVIEW OF LITERATURE

I. PERFORMANCE OF CATTLE GRAZING TWO TYPES OF PASTURE

Several pasture experiments conducted at the Tennessee Agricultural Experiment Station during an eleven-year period from 1953-1963 provided data showing that yearling beef steers achieve different body weight gains depending on the pasture species being grazed. T. W. High et al. (1965a), Hobbs et al. (1965), J. W. High et al. (1965) and T. W. High et al. (1965b) all reported significantly higher gains ($P<.05$) (1.3-1.6 pounds per day) for steers grazing orchardgrass and legume pastures than for steers grazing fescue and legume pastures (0.9-1.2) for the spring and summer months (April to August). They also reported higher average daily gains (ADG) (0.5-1.3 pounds per day) for steers grazing fescue pastures in the winter over steers grazing orchardgrass pastures (0.3-1.2). However, only in the study by Hobbs et al. (1965) were the differences in winter gains significant ($P<.05$). The latter authors along with J. W. High et al. (1965) pointed out the importance of the legume content of pastures in increasing ADG of steers on orchardgrass-Ladino clover pastures and fescue-lespedeza pastures. Following each grazing study, a 70 to 100-day dry-lot feeding period was required for the cattle to reach the USDA good grade.

II. HISTORY OF *IN VITRO* FERMENTATION TECHNIQUES

The majority of the development of *in vitro* fermentation techniques has taken place in the last twenty years. Reviews of rumen microbial activity have been written by Baker and Harriss (1947) and Marston (1948). Early attempts at developing an *in vitro* fermentation system for studying rumen microbial activity consisted primarily of incubating strained rumen fluid with various substrates and measuring microbial activity in terms of end-products (Annison and Lewis, 1959). The primary difficulties in earlier systems were concerned with the techniques involved in handling anaerobic bacteria and protozoa in an articifial situation. Later attempts at a closed system were made by Pearson and Smith (1943) and Marston (1948). Marston (1948) designed an all-glass system for the analysis of volatile fatty acids (VFA) in addition to gaseous end-products of cellulose breakdown such as CH_4 and CO_2. Another artificial rumen technique was developed by Cheng *et al*. (1955) using washed-cell suspensions of rumen bacteria. Hall *et al*. (1953) employed this procedure to study the nutrient requirements of rumen microorganisms and Hall *et al*. (1962) used it to evaluate the quality of certain hays.

In more recent *in vitro* fermentation studies, attempts have been made to simulate the conditions in the rumen more exactly by using inoculum prepared in different manners, and by using semi-permeable membranes and removing certain soluble and gaseous end-products to simulate absorption from the rumen. One of the more important works in the development of *in vitro* fermentation systems was the publication of the mineral composition of sheep's saliva by McDougall (1948). In most of the recent "artificial rumen" methods, a mineral nutrient and buffer solution (artificial saliva), based on the composition of sheep's saliva, has been used.

In the early in vitro digestion trials, the digestion of cellulose was the primary criterion for measuring digestibility (Woodman and Evans, 1938; Pearson and Smith, 1943). In a series of experiments from 1874-1882, Wildt, Zuntz and Ducleaux (Marston, 1948) suggested that microorganisms are responsible for cellulose disappearance as feed passes through the digestive tracts of ruminants. However, the use of in vitro fermentation methods has not been limited to cellulose digestion. Protein synthesis has been studied in certain artificial rumens to determine the utilization of protein and non-protein nitrogen compounds by the ruminant (Pearson and Smith, 1943; Barth et al., 1961). In vitro production of VFA has been studied in connection with several aspects of rumen fermentation and was used as an important criterion for forage digestion studies by Barnett and Reid (1957a, b, c).

High coefficients of correlation between the results from in vivo and in vitro digestion trials (Pigden and Bell, 1955; Barnett, 1957; Baumgardt et al., 1958; Hershberger et al., 1959; Barth and Mohammed, 1967; and Barth et al., 1968) suggested that faster and more economical in vitro studies might be used as preliminary or screening techniques in the evaluation of the potential nutritive value of forages.

The use of in vitro digestible dry matter (DDM) in predicting *in vivo* DDM has been supported by more recent studies. One of the most promising newer *in vitro* fermentation methods for the estimation of the nutritive value of forages is the technique of Tilley and Terry (1963) describing an *in vitro* determination of dry matter digestibility. They used a wide range of forages (148) whose *in vivo* digestibilities previously had been measured in sheep trials and which had exhibited a high degree of association between *in vivo* and *in vitro* dry matter digestibility. Of particular note was the fact that the results were highly reproducible, the standard error between experiments being ±1.18 digestibility units.

The validity of the results from the Tilley and Terry (1963) method in estimating *in vivo* dry matter digestibility of forages and of mixed rations has been reported by Oh and Baumgardt (1966), Clark *et al*. (1968) and Clark and Barth (1970). The method is presently being evaluated on a large scale by the NC-64 and S-45 regional forage evaluation committees with the hope of adopting it as the standard *in vitro* fermentation method for the United States and Canada.

III. USE OF *IN VITRO* DIGESTIBILITY DATA FOR VOLUNTARY INTAKE ESTIMATES

Crampton (1957) has suggested that the level of intake (voluntary consumption) of a forage may be of primary importance in describing feeding value. Until 1957, digestibility of a forage had been accepted as the main criterion of its potential nutritive value. Crampton theorized that results from short-term fermentations were more related to intake and results from long-term fermentations were more related to digestibility. The hypothesis that a relationship exists between voluntary intake and short-term *in vitro* fermentation has been confirmed by Crampton *et al*. (1960) and Donefer *et al*. (1960). Reid *et al*. (1960), Van Soest (1965) and Chalupa and Lee (1966) using forage studies indicated also that short-term fermentation periods (up to 18 hours) are good predictors of voluntary intake. Barth and Mohammed (1967) indicated that short-term *in vitro* dry matter digestibility and cellulose digestibility were useful in predicting voluntary intake of mixed rations also.

Based on high correlations between *in vitro* cellulose digestion and *ad libitum* consumption (r = 0.91), Donefer *et al*. (1960) suggested that the Effective Nutritive Value Index for grass- and legume-hays could be predicted using 12-hour *in vitro* digestibility data. Crampton *et al*. (1960) proposed a Nutritive Value Index (NVI) which takes into account both intake level and availability (digestibility) of energy of a forage. They found simple correlations between NVI and ADG to be as high as 0.85. Davis *et al*. (1970) indicated that the relationship between ADG and NVI of cattle grazing orchardgrass-clover pastures was 0.90, while the same relationship for fescue-lespedeza pastures was only 0.50.

IV. VOLATILE FATTY ACID PRODUCTION IN THE RUMINANT AND ITS RELATION TO ANIMAL PERFORMANCE

In the monogastric animal, glucose is the major source of energy available for metabolism. The major source of energy for the ruminant is the volatile fatty acids which are found in the rumen and which arise primarily from the fermentation of dietary carbohydrates (Annison and Lewis, 1959). Dougherty et al. (1965) estimated that VFA accounted for 70 to 80 percent of the total energy utilized by the ruminant.

The total concentration of VFA in the rumen and the amounts and proportions of individual VFA are dependent upon the diet and may have a substantial influence on the performance of ruminant animals (Annison and Lewis, 1959; Hungate, 1966). A relationship between animal performance and ruminal VFA concentration was reported by Balch (1960) and Shaw et al. (1960). In a study of lambs grazing fescue and orchardgrass pasture, Grimes et al. (1967) found that 48 percent of the variation in body weight gains could be accounted for by the variation in the molar percent of acetic and propionic acid in the rumen. Variation in concentration of propionic acid alone accounted for 44 percent of the variation. In contrast, Putnam et al. (1965) indicated that only 11 to 14 percent of the total variation in weight gains could be explained by variation in concentration of VFA in the rumen. The concentration of VFA in the rumen is affected by the absorption rate across the rumen wall. This rate is primarily dependent upon the pH of the rumen liquor, increasing with decreasing pH (Lewis, 1961).

V. VOLATILE FATTY ACID PRODUCTION IN RUMINANTS GRAZING PASTURES

Variation in pasture quality is the primary cause of variation in amounts and proportions of VFA present in the rumen. It has been pointed out that the proportions of acids present reflects both the relative concentration in and absorption from the rumen (Shaw et al., 1960). However, no one can say exactly what proportion of the propionic or butyric acids arise by biosynthesis from acetic acid or what proportion actually is produced by the breakdown of carbohydrates.

Diets consisting of younger, less mature plants tend to lower the pH in the rumen (Barnett and Reid, 1957a). Diets providing larger amounts of readily available soluble carbohydrates tend to decrease the rumen pH, inhibiting the activity of the microorganisms which break down cellulose (Donefer et al., 1963). This low rumen pH condition is conducive to the production of propionic acid by the microorganisms present (Davis et al., 1964). Elsden (1946) reiterated that the substrate which is more easily utilizable will result in the production of less acetic acid and more propionic acid, or decrease the acetic to propionic ratio.

Armstrong (1964) reported that a decrease in digestibility of a forage material was accompanied by an increase in acetic acid production. Bailey (1958) reported that legumes usually contained more soluble carbohydrates than did grasses, hence produced a greater proportion of propionic acid upon degradation.

The in vitro production of individual VFA tends to show somewhat different proportions compared to in vivo concentration. Under similar conditions, in vitro studies exhibit a greater production of propionic acid whereas in vivo studies show a greater acetic acid concentration (Elsden, 1946). It must be remembered that there is a continuous transport across the rumen epithelium which takes place in vivo, dispersing some of the VFA into the blood stream. This is not accomplished in the simpler in vitro systems, and is only approached in the more elaborate in vitro systems.

In an *in vivo* study, Wang (1968) showed a high relationship between ADG and total VFA when sampling from the dorsal area of the rumen. The coefficients of correlation between ADG and total VFA when sampling from the ventral area were lower and less consistent. The coefficients of simple correlation between ADG and molar percentages of the individual VFA or between ADG and acetic/propionic (A/P) ratio were reported to be generally low, inconsistent, nonsignificant and variable between pastures. Similar results have been reported by Balch (1960, Shaw *et al*. (1960) and Grimes *et al*. (1967). The latter authors indicated, however, that there was a positive correlation between ADG and propionic molar percent and a negative correlation between ADG and acetic acid molar percent. This negative correlation might be explained somewhat by the results of Armstrong *et al*. (1958) who indicated that for the growing animal, the efficiency of energy utilization is inversely proportional to the acetic acid molar percentage in the rumen.

VI. *IN VITRO* VOLATILE FATTY ACID PRODUCTION AND ITS RELATION TO THE PERFORMANCE OF ANIMALS GRAZING PASTURES

Wang (1968) indicated that there is a difference in *in vitro* VFA production of steers grazing either orchardgrass-clover (OC) or fescue-lespedeza (FL) pastures. The results of his analyses exhibited a slightly higher (nonsignificant) VFA production from OC as compared to FL samples. He suggested that the higher lignin content in FL pastures may account for the lower VFA production since his data indicate a highly significant ($P<.01$) negative correlation between lignin and VFA production. He reported no such relationship in OC pastures. He also indicated that, even though the coefficients of correlation between ADG and in vitro VFA production were generally low, there was a significant (P<.05) relationship between ADG and molar percent of propionic acid. This supported the findings of Grimes et al. (1967). In OC pastures, there was a positive correlation between ADG and both expressions (mmoles/l/g DM and molar percentage) of butyric acid production.

Davis (1969) noted a tendency for the available forage to decrease in VFA producing potential during the 28-day grazing period in July. This suggested a possible explanation for the decrease in forage quality reflected in loss of body weight during this period.

Davis (1969) obtained results similar to Wang (1968) in that the coefficients of correlation between ADG and the individual VFA on a percentage basis were not significant. However, his analysis did reveal significant (P<.05) simple correlations between ADG and VFA expressed in millimoles per liter, namely acetic, propionic and total VFA (0.49, 0.39 and 0.45, respectively). He noted also that regardless of pasture combinations, the orchardgrass portion of the pasture was more highly associated with ADG than the fescue portion, based on VFA analysis.

VII. IN VITRO VOLATILE FATTY ACID PRODUCTION AS A PREDICTOR OF ADG

Using a 48-hour fermentation process (Tilley and Terry, 1963), Davis et al. (1970) compared the usefulness of VFA production from samples obtained from three different pasture types in predicting ADG.

Using heifers to graze these pastures, they found that at most, 34 percent ($P<.01$) of the variation in ADG on fescue pastures, 67 percent ($P<.01$) of the variation in ADG on orchardgrass pastures and 72 percent ($P<.01$) of the variation in ADG on clover pastures could be accounted for simply by measuring the VFA production from samples of each pasture and fitting the proper prediction equation. They suggested that analysis of pure samples of the most utilized and preferred forage species (such as clover) is more useful for VFA analysis than a random sample (representative of the available forage) when predicting ADG. They noted also that no single VFA accounted for a large amount of the coefficient of multiple determination (R^2) and suggested that the relationship between ADG and VFA production is complex, involving interrelationships with other acids, and very possibly other measurements of pasture quality determined chemically or visually.

Davis (1969), in predicting ADG based solely on 48-hour _in vitro_ VFA production from pasture samples, noted a substantial difference between the predictability of ADG of heifers grazing fescue pastures ($R^2 = 0.40$, $P>.05$) and ADG of heifers grazing orchardgrass pastures ($R^2 = 0.69$, $P<.01$). He noted further that the accuracy of predicting ADG of heifers grazing fescue pastures increased significantly ($R^2 = 0.77$, $P<.01$) when certain other variables (_viz._, visual pasture score and chemical analyses of pasture samples) were included in a prediction equation. The R^2 for orchardgrass pasture also increased to 0.74 ($P<.01$) when these other variables were considered.

Wang (1968) derived prediction equations for estimating ADG of steers grazing orchardgrass-clover (OC) and fescue-lespedeza (FL) pastures using 48-hour *in vitro* VFA production and several variables obtained from pasture score measurements and chemical analyses. Using grazed and hand-clipped FL samples, he determined that 73 and 60 percent, respectively, of the variation in ADG of steers grazing FL pastures could be accounted for. These figures were not significant (P>.05). Using grazed and hand-clipped OC samples, he determined that 77 and 72 percent (P>.05), respectively, of the variation in ADG could be explained using the proper measurements of chemical analysis and visual pasture score. It was postulated that different time periods of *in vitro* fermentation of these hand-clipped OC and FL samples might yield VFA production data which could be used in combination with the chemical analyses and pasture score measurements to derive more accurate equations for the prediction of ADG of steers grazing these pastures.

CHAPTER III

EXPERIMENTAL PROCEDURE

Wang (1968) and Davis et al. (1969) conducted research to determine the usefulness of various measurements of pasture quality to predict performance of steers grazing orchardgrass-Ladino clover (OC) and fescue-lespedeza (FL) pastures. They determined differences in body weight gains of grazing beef steers and tried to relate these with in vitro production potential of the forages and other measurements of pasture quality such as: in vitro dry matter digestibility, and percent of crude protein, acid detergent fiber (ADF) and acid insoluble lignin (AIL). Using a 48-hour in vitro fermentation period, Wang (1968) also suggested that there was a correlation between VFA production and body weight gains.

It was postulated that VFA produced during shorter fermentation periods might improve the prediction of the performance of grazing animals. Therefore, fermentation periods of 12, 24 and 36 hours were chosen in the present study. Resulting VFA concentrations together with the other measures of pasture quality which had been obtained by Wang (1968) were used in order to predict daily gains of the grazing cattle by multiple regression equations.

I. EXPERIMENTAL PASTURES

The experimental pastures were four three-acre plots of orchardgrass-Ladino clover and four three-acre plots of fescue-lespedeza found at the

University of Tennessee's Blount Farm. Steer and heifer grazing studies had been conducted for several years on these pastures. The pastures were grouped into four pasture pairs based on animal performance data collected previously and pasture quality. There were two pasture pairs of each pasture type.

Subjective visual pasture scoring was carried out at 28-day intervals throughout the experimental grazing period. Percent orchardgrass, percent fescue, percent legume and legume index (the product of percent legume and average height of the legume) were recorded.

Collection of Samples

The samples obtained from these pastures were hand-clipped according to the method described by High (1966) and considered characteristic of the forage available in the pasture. These forage samples were taken on the median day of each of six 28-day experimental periods beginning on May 5, 1967 and ending on September 22, 1967.

Chemical Analyses and In Vitro Dry Matter Digestibility

The samples were dried for 72 hours in a forced-air oven and ground in a Wiley mill through a 20-mesh screen. Acid detergent fiber and acid insoluble lignin determinations were conducted according to Van Soest (1963), and crude protein and moisture were determined according to the A.O.A.C. (1965) methods. *In vitro* digestible dry matter was obtained with the use of the Tilley and Terry (1963) method. These data were expressed on a dry matter basis.

II. EXPERIMENTAL ANIMALS

Sixteen Hereford steers averaging 243 kg. in body weight were divided into four groups of four each on the basis of weight and grade after having been dosed with thiabendazol. An additional animal was allowed to graze each pasture for a forage intake study. Each group was randomly assigned to a pasture pair and placed on its respective pasture from April 27, 1967 until the middle of October. The steers were rotated at one- and two-week intervals between the two pastures within each pasture pair. All animals were weighed at 28-day intervals beginning with the initial weigh day. A summary of the average daily gains for each period during the grazing season is presented in Table 1.

III. *IN VITRO* VOLATILE FATTY ACID PRODUCTION

A modified Tilley and Terry (1963) *in vitro* fermentation procedure was used for the production of *in vitro* volatile fatty acids. This procedure was originally designed to determine *in vitro* digestible dry matter. It employed a 48-hour fermentation period of the sample, followed by a 48-hour digestion period using a pepsin solution. In the present study the first stage of the Tilley and Terry (1963) procedure and three different periods of fermentation, 12, 24 and 36 hours, were utilized.

Collection of Rumen Liquor

Rumen liquor, hereafter denoted as inoculum, was collected from a 2-year old rumen-fistulated Hereford steer that had been grazing

TABLE 1

AVERAGE DAILY GAIN (KG.) OF STEERS GRAZING TWO TYPES OF PASTURES DURING THE GRAZING SEASON[a]

Period	Date (1967)	Pasture type: Orchardgrass-clover 2-6[b]	Orchardgrass-clover 3-8[b]	Fescue-lespedeza 1-5[b]	Fescue-lespedeza 4-7[b]
1	April 21-May 19	1.08	1.10	0.89	0.99
2	May 19-June 16	0.73	0.65	0.38	0.14
3	June 16-July 14	0.73	0.73	0.53	0.51
4	July 14-Aug. 11	0.49	0.38	0.28	0.43
5	Aug. 11-Sept. 8	0.69	0.63	0.75	0.60
6	Sept. 8-Oct. 6	0.02	0.57	0.25	0.38
Complete experiment[c]		0.72	0.68	0.51	0.51

[a]Averages based on four animals.

[b]2-6, 3-8, 1-5 and 4-7 represent pasture pairs.

[c]Differences between means of pasture types were statistically significant ($P<.05$).

an orchardgrass-fescue-Ladino clover pasture prior to each collection. On the collection day, the steer was removed from pasture at 7:00 a.m. and water and feed were withheld for two hours prior to collection. Ingesta were taken from the rumen, thoroughly mixed and then filtered through 8 layers of cheese cloth into a thermos bottle maintained at 39°C. Approximately 2 liters of the liquid portion of the ingesta (inoculum) were collected.

Collection of Supernatant Liquor

Following is a brief description of the first phase of the Tilley and Terry (1963) procedure:

Five-tenths gram of a forage sample was weighed and quantitatively transferred into a 75-ml centrifuge tube. Forty ml of McDougall's (1948) artificial "Saliva" and 10 ml of inoculum were added to each tube. Carbon dioxide gas was forced into each tube to displace the air. The tubes were incubated at 39°C in a water bath in the dark until the end of the fermentation periods of 12, 24 and 36 hours. At the end of each specific period, 1 ml of $HgCl_2$ was added in order to stop further microbial activity. Also two ml of Na_2CO_3 were added to aid in sedimentation of the sample. The tubes were then rotated to disperse the floating particles and centrifuged at 2,000 r.p.m. for 15 minutes. Five ml of the supernatant were transferred to a 15 ml centrifuge tube, one ml of 25 percent metaphosphoric acid was added and the tubes were mixed with a Vortex Genie shaker. After standing for 30 minutes, the tubes were centrifuged at 3,000 r.p.m. for 10 minutes.

IV. DETERMINATION OF VOLATILE FATTY ACID CONCENTRATION

Volatile fatty acid concentration in the supernatant liquor was determined using the method of Erwin *et al.* (1961). A 3-microliter quantity of each sample was injected into an Aerograph 600-C model HY-FI gas chromatograph. A stainless steel column packed with 15% Tween 80, 2 1/2% H_3PO_4 on chromosorb W A/W 60/80 was used. The injector block was maintained at 170°C. The oven temperature was 140°C. The range setting was 10 and the attenuator setting was 32. Nitrogen gas was used as the carrier gas at a rate of 20 ml/minute. Hydrogen gas was supplied at a rate of 20 ml/minute.

The peak heights were recorded for each sample. The concentrations of acetic acid, propionic acid and butyric acid in millimoles per liter per gram dry matter were obtained by comparing the peak height of the unknown sample to the peak height of a pure standard (Wang, 1968) after adjusting for the dissolution effect and the dry matter content.

V. STATISTICAL ANALYSIS

Statistical analysis was accomplished with a 7040 IBM computer following a program designated "BMDO2R-Stepwise Regression-Version of December 17, 1965 Health Science Computing Facility, UCLA." The data were analyzed by simple correlation procedures (Steel and Torrie, 1960) and stepwise regression procedures as described by Draper and Smith (1966) to determine the relationship between ADG and *in vitro* VFA production.

Trial Design

Three Tilley and Terry (1963) artificial rumen trials were conducted during three consecutive weeks. The design format was:

2 pasture types

2 pasture pairs

6 grazing periods

3 fermentation times

3 replicate runs

2 replicate tubes

3 VFA's + total VFA + A/P ratio

2 ways of expressing VFA production

The value used for analysis as an independent variable was an average of the six values obtained for each VFA from three replicate runs and two replicate tubes, expressed either as millimoles per liter per gram dry matter or molar percent.

Correlation Coefficients

Concentrations of the three individual VFA and their sum (total VFA) for each specific fermentation period were correlated with the respective body weight gains within periods of animals grazing both types of pasture. Correlations between gains and several estimates of pasture quality, such as percent grass, percent legume, legume index, *in vitro* digestible dry matter, and percent of crude protein (DMB), AIL (DMB) and ADF (DMB), were also computed.

Multiple Regression Equations

Multiple regression equations which predict body weight gains were computed in a stepwise fashion. The general form for the prediction equation for predicting body weight gains was:

$$\hat{Y}_i = a + \sum_j b_j x_j$$

Where:

$$i = 1,2,\ldots,6$$

$$j = 1,2,3,\ldots,k$$

$$a = \bar{Y} + \sum_j b_j(0-\bar{x}_j).$$

The b_j's are the coefficients of partial regression of the dependent variable on the independent variables.

The x_j's are the independent variables measuring the in vitro VFA production at different fermentation periods, the pasture characteristics determined from clipped samples and values from the chemical analyses of the clipped samples.

The $\bar{Y}_i$'s are the means of the average daily gains.

The $\hat{Y}_i$'s are the predicted values of average daily gain for the specified values of the x_j's.

The $\bar{x}_j$'s are the means of the k-th independent variable.

The calculations were made within each pasture type and within each fermentation period.

For each fermentation period, the VFA variable which had the highest correlation with ADG was forced into the regression equation. Various other pasture measurements were inserted into the equation in a stepwise fashion, the variable being added in a given step being the one among those not yet included which would increase the R^2 value most. The variable inserted would have the highest partial correlation with ADG while having the least partial correlation with the other variables already in the equation. Slight variations in the selection of independent variables will be discussed fully in Chapter IV.

CHAPTER IV

RESULTS AND DISCUSSION

I. *IN VITRO* VFA PRODUCTION FOLLOWING 12, 24 AND 36 HOURS OF FERMENTATION

The *in vitro* production of VFA is expressed in two different ways: production in terms of concentration (millimoles/liter/gram of forage dry matter) and production in relation to other acids (molar percentage). A summary of the *in vitro* VFA production from OC pastures is presented in Tables 2 and 3. Each datum represents the mean of six observations on one pasture pair for a given 28-day grazing period.

VFA Production From OC Pasture Samples

The *in vitro* VFA production from OC samples is shown in Table 2. Generally, VFA production from OC samples was higher than from FL samples. In the case of each VFA, approximately 40 percent of the VFA which was produced in 36 hours of fermentation is present after 12 hours, and in most cases, over 80 percent was produced after 24 hours. Total VFA produced tended to decrease in the middle of the grazing season and then increase toward the end of the grazing season.

The molar percentages of VFA produced *in vitro* from OC forage samples are presented in Table 3. For the first two fermentation periods, the molar percentage of acetic acid tended to increase until the middle of the grazing season and then decrease until the end of the grazing season. The molar percentages of propionic and butyric acids tended to follow the opposite trend.

TABLE 2

IN VITRO VOLATILE FATTY ACID PRODUCTION (IN MMOLES/L/G DM) FROM ORCHARD-GRASS-LADINO CLOVER SAMPLES COLLECTED DURING DIFFERENT TIMES OF THE GROWING SEASON[a]

Grazing period	Fermentation time, hr. 12		24		36	
	2-6[b]	3-8[b]	2-6[b]	3-8[b]	2-6[b]	3-8[b]
			Acetic acid			
4/21-5/19	26.2	28.5	50.1	52.6	66.9	69.6
5/19-6/16	26.8	32.5	47.5	49.7	59.7	61.5
6/16-7/14	27.1	32.4	48.0	48.6	61.3	63.1
7/14-8/11	25.1	29.7	50.4	48.6	62.1	69.5
8/11-9/8	29.5	28.5	49.9	46.9	64.0	64.2
9/8-10/6	33.3	31.8	55.5	50.5	64.5	65.6
			Propionic acid			
4/21-5/19	8.4	8.7	16.1	17.7	20.6	21.8
5/19-6/16	7.9	9.4	15.0	14.9	18.1	18.2
6/16-7/14	8.3	9.0	14.9	14.8	18.9	19.2
7/14-8/11	6.8	8.4	15.3	14.6	19.4	20.2
8/11-9/8	8.2	8.1	14.7	15.6	19.5	21.1
9/8-10/6	9.8	9.5	16.7	14.9	19.3	19.8
			Butyric acid			
4/21-5/19	2.31	2.08	5.76	5.58	7.74	7.58
5/19-6/16	2.25	2.54	5.04	4.73	6.28	5.97
6/16-7/14	2.04	2.39	4.44	5.37	5.84	6.85
7/14-8/11	1.70	2.25	5.17	4.88	6.56	7.59
8/11-9/8	2.10	2.37	4.88	4.86	6.86	6.57
9/8-10/6	3.50	2.95	4.56	5.55	8.28	7.65
			Total VFA			
4/21-5/19	36.8	39.2	71.9	75.8	95.3	99.0
5/19-6/16	36.8	44.5	67.6	69.3	84.2	85.7
6/16-7/14	37.5	43.8	67.5	68.8	86.1	89.1
7/14-8/11	33.6	40.3	70.8	68.0	88.1	97.2
8/11-9/8	39.9	39.0	69.4	67.4	90.3	91.8
9/8-10/6	46.7	44.5	76.8	71.0	91.8	93.1

[a]Based on 6 observations.

[b]2-6 and 3-8 designate pasture pairs.

TABLE 3

IN VITRO VOLATILE FATTY ACID PRODUCTION (IN MOLAR PERCENT) FROM ORCHARDGRASS-LADINO CLOVER SAMPLES COLLECTED DURING DIFFERENT TIMES OF THE GROWING SEASON[a]

Grazing period	Fermentation time, hr. 12		24		36	
	2-6[b]	3-8[b]	2-6[b]	3-8[b]	2-6[b]	3-8[b]
			Acetic acid			
4/21-5/19	71.0	72.6	69.7	69.4	70.2	70.3
5/19-6/16	72.8	73.0	70.4	71.7	71.0	71.8
6/16-7/14	72.2	74.0	71.0	70.7	71.1	70.8
7/14-8/11	74.7	73.5	71.1	71.5	70.6	71.5
8/11-9/8	73.9	73.0	71.9	69.5	70.9	69.9
9/8-10/6	71.4	71.5	72.3	71.2	70.3	70.4
			Propionic acid			
4/21-5/19	22.8	22.2	22.3	23.4	21.6	22.0
5/19-6/16	21.4	21.1	22.2	21.5	21.5	21.2
6/16-7/14	22.1	20.5	22.1	21.5	21.9	21.6
7/14-8/11	20.1	20.8	21.5	21.4	22.0	20.8
8/11-9/8	20.7	20.7	21.2	23.2	21.6	23.0
9/8-10/6	20.9	21.4	21.8	21.0	21.0	21.3
			Butyric acid			
4/21-5/19	6.27	5.31	9.01	7.36	8.12	7.66
5/19-6/16	6.11	5.78	9.85	6.82	7.46	6.97
6/16-7/14	5.44	5.46	7.58	7.81	6.78	7.68
7/14-8/11	5.06	5.58	7.30	7.17	7.45	7.81
8/11-9/8	5.26	6.09	7.02	7.20	7.60	7.16
9/8-10/6	7.50	6.63	5.94	7.82	9.02	8.21
			Acetic/propionic ratio			
4/21-5/19	3.12	3.27	3.12	2.97	3.25	3.20
5/19-6/16	3.40	3.46	3.17	3.33	3.30	3.38
6/16-7/14	3.26	3.61	3.22	3.29	3.25	3.57
7/14-8/11	3.71	3.53	3.30	3.33	3.20	3.44
8/11-9/8	3.58	3.53	3.39	3.00	3.29	3.04
9/8-10/6	3.41	3.34	3.31	3.39	3.34	3.31

[a]Based on 6 observations.

[b]2-6 and 3-8 designate pasture pairs.

VFA Production From FL Samples

The *in vitro* production of VFA from FL pastures is shown in Tables 4 and 5. In Table 4, VFA tended to decrease sharply in the second period and then to increase slightly for the remainder of the grazing season. Wang (1968) noted that the extreme decrease in the second period reflected both in ADG (Table 1, page 17) and in VFA production may be due to the advanced stage of maturity of the grasses or lower rainfall observed during this period. The fact that the pastures had been clipped four days prior to weighing also may have had some effect.

The molar percentages of VFA from FL samples are shown in Table 5. Although there is some indication of an increasing and then decreasing trend in acetic acid, the other acids tended not to follow any pattern.

II. COEFFICIENTS OF SIMPLE CORRELATION BETWEEN ADG AND OTHER MEASUREMENTS OF PASTURE QUALITY FROM TWO TYPES OF PASTURE

Coefficients of simple correlation between ADG and other measurements of pasture quality taken from OC and FL pastures are shown in Table 6. The measurements of pasture quality were determined by Wang (1968) and Davis (1969) and appear in Appendix Table 19.

In OC pastures, the coefficients of correlation between ADG and measures of pasture quality tended to be low, with the exception of AIL and lignin:fiber ratio, which were both significantly ($P<.05$) negatively correlated with ADG. These expressions tended to show

TABLE 4

IN VITRO VOLATILE FATTY ACID PRODUCTION (IN MMOLES/L/G DM) FROM FESCUE-LESPEDEZA SAMPLES COLLECTED DURING DIFFERENT TIMES OF THE GROWING SEASON[a]

Grazing period	Fermentation time, hr. 12		24		36	
	1-5[b]	4-7[b]	1-5[b]	4-7[b]	1-5[b]	4-7[b]
			Acetic acid			
4/21-5/19	27.8	26.6	43.0	46.9	56.4	60.2
5/19-6/16	15.0	18.3	35.8	37.1	48.2	50.8
6/16-7/14	28.0	21.9	39.5	39.0	52.4	46.9
7/14-8/11	25.7	21.7	39.2	39.9	53.8	52.5
8/11-9/8	23.0	26.5	38.6	39.3	46.2	46.2
9/8-10/6	28.0	30.9	41.4	42.1	47.5	55.7
			Propionic acid			
4/21-5/19	10.1	9.9	14.1	14.6	25.2	24.0
5/19-6/16	5.1	6.6	12.1	11.0	24.1	24.6
6/16-7/14	8.7	7.9	12.2	12.7	23.9	26.2
7/14-8/11	9.3	8.0	13.3	12.7	25.8	25.2
8/11-9/8	8.0	9.2	11.8	11.8	26.1	25.4
9/8-10/6	10.2	11.0	13.2	14.5	27.3	25.1
			Butyric acid			
4/21-5/19	2.10	2.27	2.63	3.27	7.35	6.97
5/19-6/16	1.13	1.31	2.83	2.18	6.51	7.11
6/16-7/14	2.18	1.58	3.40	2.98	7.56	7.20
7/14-8/11	2.11	1.95	3.55	3.52	6.92	7.11
8/11-9/8	1.87	2.32	3.39	2.99	7.37	6.64
9/8-10/6	2.50	2.57	3.52	3.39	6.80	6.47
			Total VFA			
4/21-5/19	40.0	38.8	59.9	64.8	83.5	87.0
5/19-6/16	21.4	26.2	50.6	50.1	69.5	74.6
6/16-7/14	38.6	31.3	55.1	54.8	76.4	70.5
7/14-8/11	37.1	31.7	56.0	56.0	80.0	77.5
8/11-9/8	33.0	38.0	53.8	54.0	69.4	67.9
9/8-10/6	40.8	44.5	58.2	60.0	72.0	81.4

[a]Based on 6 observations.

[b]1-5 and 4-7 designate pasture pairs.

TABLE 5

IN VITRO VOLATILE FATTY ACID PRODUCTION (IN MOLAR PERCENT) FROM FESCUE-LESPEDEZA SAMPLES COLLECTED DURING DIFFERENT TIMES OF THE GROWING SEASON[a]

Grazing period	Fermentation time, hr.					
	12		24		36	
	1-5[b]	4-7[b]	1-5[b]	4-7[b]	1-5[b]	4-7[b]
			Acetic acid			
4/21-5/19	69.6	68.6	71.8	72.4	67.5	69.2
5/19-6/16	70.4	70.0	70.7	73.9	69.3	68.1
6/16-7/14	72.4	69.9	71.8	71.2	68.6	66.5
7/14-8/11	69.4	68.5	70.0	71.3	67.3	67.8
8/11-9/8	69.7	69.7	71.8	72.7	66.6	68.0
9/8-10/6	68.6	69.5	71.3	70.2	66.0	68.4
			Propionic acid			
4/21-5/19	25.3	25.5	23.5	22.6	25.2	24.0
5/19-6/16	23.8	25.1	23.9	21.9	24.1	24.6
6/16-7/14	22.4	25.3	22.2	23.2	23.9	26.2
7/14-8/11	25.0	25.2	23.7	22.7	25.8	25.2
8/11-9/8	24.3	24.2	22.0	21.8	26.1	25.4
9/8-10/6	25.0	24.7	22.8	24.1	27.3	25.1
			Butyric acid			
4/21-5/19	5.26	5.85	2.63	3.27	7.35	6.97
5/19-6/16	5.29	5.01	2.83	2.18	6.51	7.11
6/16-7/14	5.64	5.04	3.40	2.98	7.56	7.20
7/14-8/11	5.69	6.16	3.55	3.52	6.92	7.11
8/11-9/8	5.67	8.29	3.39	2.99	7.37	6.64
9/8-10/6	6.13	5.78	3.52	3.39	6.80	6.47
			Acetic/propionic ratio			
4/21-5/19	2.75	2.69	3.05	3.21	2.68	2.88
5/19-6/16	2.96	2.79	2.96	3.37	2.88	2.77
6/16-7/14	3.23	2.77	3.24	3.07	2.87	2.53
7/14-8/11	2.78	2.72	2.95	3.15	2.61	2.69
8/11-9/8	2.87	2.88	3.26	3.34	2.55	2.68
9/8-10/6	3.05	2.81	3.13	2.91	2.41	2.73

[a]Based on 6 observations.

[b]1-5 and 4-7 designate pasture pairs.

TABLE 6

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN ADG AND OTHER MEASUREMENTS OF PASTURE QUALITY TAKEN FROM TWO PASTURE TYPES

Pasture quality measurement	Pasture type	
	OC	FL
Percent fescue	0.15	0.12
Percent orchardgrass	0.36	----
Percent legume	0.57	-.07
Legume index	0.36	0.07
In vitro digestible dry matter	0.36	0.36
Crude protein percent, DMB	-.21	-.20
Acid-detergent fiber percent, DMB	-.24	-.59*
Acid-insoluble lignin percent, DMB	-.69*	-.33
Cellulose percent, DMB	0.13	-.60*
Lignin/fiber ratio	-.65*	-.21

*(.01<P<.05).

similar relationships with ADG, since the lignin:fiber ratio is calculated from AIL and ADF. The correlation between ADG and percent legume approached significance.

There were two significant relationships in the FL data. The correlations between ADG and ADF, and between ADG and percent cellulose were statistically significant ($P<.05$). The coefficients of simple correlation between ADG and chemical composition in FL pastures tended to be negative.

III. COEFFICIENTS OF SIMPLE CORRELATION BETWEEN ADG AND *IN VITRO* VFA PRODUCTION

Orchardgrass-Ladino Clover Forage

The coefficients of simple correlation between ADG and *in vitro* VFA production after the three fermentation times from OC pasture samples are presented in Table 7. Most coefficients of simple correlation between ADG and both ways of expressing *in vitro* VFA production tended to be low and variable. When production of acids was considered, butyric acid after 24 hours of *in vitro* fermentation showed the only significant ($P<.05$) relationship with ADG.

There were two notable relationships between ADG and molar percentages of acids formed. Molar percentage of acetic acid following 24-hour fermentation showed a highly significant ($P<.01$) relationship with ADG and propionic acid molar percentage following 12-hour fermentation was significantly ($P<.05$) correlated with ADG of animals grazing OC pastures. This supports the findings of Grimes *et al.*

TABLE 7

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN ADG AND IN VITRO VFA PRODUCTION FROM SAMPLES OF ORCHARDGRASS-LADINO CLOVER PASTURES

VFA variable	Fermentation time		
	12-hour	24-hour	36-hour
	mmoles/liter/gram dry matter		
Acetic acid	-.46	-.32	0.15
Propionic acid	-.21	0.21	0.34
Butyric acid	-.55	0.61*	-.21
Total acids	-.44	-.10	0.16
	molar %		
Acetic acid	-.08	-.74**	-.10
Propionic acid	0.64*	0.48	0.39
Butyric acid	-.47	0.33	-.41
Acetic/propionic ratio	-.48	-.57	-.22

*(.01<P<.05).

**P<.01.

(1967) and Wang (1968) who found that the single factor most correlated with ADG was propionic acid concentration.

Fescue-Lespedeza Forage

The coefficients of simple correlation between ADG and *in vitro* VFA production from FL pasture samples are presented in Table 8. Production of acetic acid following 24-hour *in vitro* fermentation shows the only significant (P<.05) relationship with ADG. There tended to be a consistent, moderate, positive correlation between ADG and VFA production from FL pasture samples. No such trend was found in the correlation between ADG and molar percentage of acids formed. These relationships tended to coincide with data presented by both Wang (1968) and Davis (1969) in that the coefficients of simple correlation between ADG and *in vitro* VFA production are low and variable for FL pastures.

IV. MULTIPLE REGRESSION ANALYSES

In the multiple regression analyses, equations to predict ADG were developed using various combinations of independent variables and were compared in order to evaluate their accuracy in predicting ADG. These independent variables included visual pasture score (percent grass, percent legume and legume index), chemical composition (crude protein, ADF, AIL and cellulose content, and lignin:fiber ratio), *in vitro* digestible dry matter and VFA production (acetic acid, propionic acid, butyric acid, total VFA and acetic/propionic ratio). The objective was to compare the accuracy of predicting ADG using

TABLE 8

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN ADG AND IN VITRO VFA PRODUCTION FROM SAMPLES OF FESCUE-LESPEDEZA PASTURES

VFA variable	Fermentation time 12-hour	24-hour	36-hour
		mmoles/liter/gram dry matter	
Acetic acid	0.30	0.63*	0.37
Propionic acid	0.31	0.42	0.25
Butyric acid	0.24	0.05	0.48
Total acids	0.30	0.57	0.37
		molar %	
Acetic acid	-.08	0.16	0.16
Propionic acid	0.08	-.13	-.24
Butyric acid	0.11	-.22	0.34
Acetic/propionic ratio	-.25	0.14	0.21

*(.01<P<.05).

various combinations of independent variables at three different lengths of fermentation, _viz_., 12-hour, 24-hour and 36-hour time periods. The prediction equations for ADG were calculated on a within-type-of-pasture basis.

The general form for the equations developed for the prediction of ADG was:

$$\hat{Y} = a + b_1\bar{x}_1 + b_2\bar{x}_2 + \ldots + b_i\bar{x}_i$$

Where: _a_ is a constant which can be calculated in the following manner:

$$a = \bar{Y} - b_1\bar{x}_1 - b_2\bar{x}_2 \ldots - b_i\bar{x}_i.$$

The _b_'s are partial regression coefficients.

For example, one equation was developed to estimate ADG of beef cattle grazing OC pastures from acetic acid production, percent legume and ADF: _i.e._,

$$\hat{Y} = 4.7453 - 0.0590\bar{x}_1 + 0.0216\bar{x}_2 - 0.0684\bar{x}_3$$

Where: $\hat{Y}$ = predicted value of ADG

$\bar{x}_1$ = average acetic acid production in mmoles/1/g DM

$\bar{x}_2$ = average percentage legume in OC pastures

$\bar{x}_3$ = average percentage acid detergent fiber in samples from OC pastures.

Coefficients of Multiple Determination

For each pasture type two sets of multiple determination (R^2) values are presented. The R^2 value represents the amount of the variation in ADG which can be accounted for using certain independent variables in a multiple regression system. Tables 9 and 10 (for OC

TABLE 9

COEFFICIENTS OF MULTIPLE DETERMINATION (R^2) ASSOCIATED WITH EQUATIONS FOR PREDICTING ADG FROM INDIVIDUAL VFA VALUES AND FIVE SELECTED OC PASTURE VARIABLES[a]

VFA variable	Fermentation time 12-hour	24-hour	36-hour
	mmoles/liter/gram dry matter		
Acetic acid	0.72	0.88*	0.79
Propionic acid	0.64	0.54	0.70
Butyric acid	0.71	0.66	0.73
Total VFA	0.76	0.77	0.70
	molar %		
Acetic acid	0.50	0.86	0.77
Propionic acid	0.58	0.62	0.92**
Butyric acid	0.58	0.75	0.74
Acetic/propionic ratio	0.53	0.69	0.62

[a]Selected pasture variables: percent orchardgrass, legume index, and crude protein (DMB), AIL (DMB), and cellulose (DMB) content.

*(.01<P<.05).

**P<.01.

TABLE 10

COEFFICIENTS OF MULTIPLE DETERMINATION (R^2) ASSOCIATED WITH EQUATIONS FOR PREDICTING ADG FROM INDIVIDUAL VFA VALUES AND FIVE SELECTED FL PASTURE VARIABLES[a]

VFA variable	Fermentation time 12-hour	24-hour	36-hour
		mmoles/liter/gram dry matter	
Acetic acid	0.76	0.83*	0.80
Propionic acid	0.77	0.80	0.80
Butyric acid	0.77	0.77	0.76
Total VFA	0.76	0.82	0.81
		molar %	
Acetic acid	0.83	0.77	0.76
Propionic acid	0.80	0.77	0.77
Butyric acid	0.76	0.76	0.79
Acetic/propionic ratio	0.76	0.77	0.77

[a]Selected pasture variables: percent fescue, legume index, and crude protein (DMB), DDM and cellulose (DMB) content.

*(.01<P<.05).

and FL, respectively) show the amount of variation which can be explained using each individual VFA and five selected independent variables, which are different depending on pasture type. The reason for the use of selected variables was an attempt to decrease the number of pasture quality measurements which must be obtained for a given set of samples in order to predict ADG most easily without significant loss in accuracy. It was ultimately decided that determining the five pasture quality variables which were selected necessitated almost as much laboratory work as determining all ten pasture quality variables and allowing the computer to analyze the data and arrive at a more accurate result. Therefore, the concept of selected variables is not discussed further.

Tables 11 and 12 (for OC and FL, respectively) depict the R^2 value for each prediction equation using each VFA individually and any five of the ten independent variables (chosen on the basis of their partial correlations with ADG) to predict ADG. As shown in Table 11, three combinations of variables at the 24-hour fermentation period resulted in R^2 values which were significant at the .05 level of probability, and one combination of variables at the 24-hour fermentation period resulted in a significant R^2 value at the .01 level of probability. A similar situation occurred at the 36-hour fermentation period when all ten independent variables were considered for use in the prediction equation.

As shown in Table 12 only one equation based on 36-hour fermentation data produced a significant reduction in residual sum of squares in the final step. However, one equation from the 24-hour fermentation and one equation from the 36-hour fermentation separate from the one discussed previously yielded a significant reduction at some step other than the final step.

TABLE 11

COEFFICIENTS OF MULTIPLE DETERMINATION (R^2) ASSOCIATED WITH EQUATIONS FOR PREDICTING ADG FROM INDIVIDUAL VFA VALUES AND ANY FIVE OC PASTURE VARIABLES

VFA variable	Fermentation time 12-hour	 24-hour	 36-hour
		mmoles/liter/gram dry matter	
Acetic acid	0.81	0.91*	0.82
Propionic acid	0.77	0.73	0.66
Butyric acid	0.77	0.91*	0.88*
Total VFA	0.76	0.72	0.76
		molar %	
Acetic acid	0.61	0.88*	0.87*
Propionic acid	0.65	0.64	0.93**
Butyric acid	0.66	0.94**	0.75
Acetic/propionic ratio	0.61	0.85	0.66

*(.01<P<.05).

**P<.01.

TABLE 12

COEFFICIENTS OF MULTIPLE DETERMINATION (R^2) ASSOCIATED WITH EQUATIONS FOR PREDICTING ADG FROM INDIVIDUAL VFA VALUES AND ANY FIVE FL PASTURE VARIABLES

VFA variable	Fermentation time 12-hour	24-hour	36-hour
		mmoles/liter/gram dry matter	
Acetic acid	0.76	0.74	0.74
Propionic acid	0.73	0.80	0.75
Butyric acid	0.77	0.77	0.82
Total VFA	0.76	0.70	0.71
		molar %	
Acetic acid	0.83	0.77	0.76
Propionic acid	0.77	0.78	0.77
Butyric acid	0.76	0.76	0.92*
Acetic/propionic ratio	0.76	0.77	0.77

*(.01<P<.05).

Since the goal set by the investigator was to determine the best combination of variables for predicting ADG from the three fermentation periods, the discussion has been limited to the combination of variables which yielded the highest significant R^2 from the 24- and 36-hour fermentation periods, using in vitro VFA production expressed both in mmoles/l/g DM and molar percentage.

Multiple Regression Equations to Estimate ADG From In Vitro VFA Production and Several Other Variables Obtained from Two Types of Pastures

The results of the regression analyses for both OC and FL data pertaining to 12-hour fermentation were not as promising as the results from other fermentation times, as seen in Tables 11 and 12. The coefficients of multiple determination were in the 0.60-0.80 range, and not statistically significant, and therefore, not large enough to make valid predictions.

OC pasture samples--24-hour fermentation. Results from the 24-hour fermentation period together with other independent variables appear to be more promising in the prediction of ADG. As shown in Table 13, butyric acid production accounted for a significant ($P<.05$) 37 percent of the variation in ADG. The addition of percent legume increased the R^2 value to 0.67, a figure significant at the .01 level of probability. Further additions of percent fescue, percent cellulose and legume index accounted for additional variation in ADG of 10, 9 and 3 percent, respectively. The R^2 values (.78, .86 and .89, respectively) are highly significant ($P<.01$). Approximately 90 percent of the variation in ADG of the animals grazing OC pastures in this experiment can be explained using this combination of variables.

TABLE 13

MULTIPLE REGRESSION EQUATIONS FOR ADG ESTIMATED FROM 24-HR IN VITRO VFA PRODUCTION[a] (IN MMOLES/L/G DM) AND SEVERAL OTHER VARIABLES OBTAINED FROM ORCHARDGRASS-LADINO CLOVER PASTURES

Equation components	Equation number					
	1	2	3	4	5	6
Constant	-1.4542	-1.6648	-2.0822	-4.5586	-4.3548	-3.8726
Butyric acid	0.4152	0.4017	0.4600	0.5714	0.5351	0.5949
Percent legume		0.0193	0.0195	0.0137	0.0291	0.0310
Percent fescue			0.0098	0.0148	0.0135	0.0124
Cellulose percent				0.0572	0.0558	0.0654
Legume index					-.0034	-.0037
In vitro digestible dry matter						-.0161
R^2	.37*	.67**	.78**	.86**	.89**	.91*

[a]Measured in supernatant liquor after 24 hours of fermentation *in vitro* using the Tilley and Terry (1963) fermentation technique.

*(.01<P<.05). **P<.01.

As shown in Table 14, a number of independent variables in combination with 24-hour molar percentage of butyric acid yielded a high and statistically significant R^2. Although the butyric acid molar percentage after 24 hours of fermentation did not explain much of the variation in ADG by itself ($R^2 = 0.11$), in combination with AIL and percent fescue in the OC pastures, it resulted in a significant ($P<.01$) coefficient which accounted for 78 percent of the variation in ADG of the steers grazing these pastures. The combination of variables which had the highest R^2 value, and therefore explains the largest part of the variation which occurred in ADG of the animals grazing OC pastures, is the combination of: molar percentage of butyric acid after 24 hours of *in vitro* fermentation, AIL content, percent fescue in the OC pastures, lignin:fiber ratio, percent cellulose and the percent crude protein present in the samples from the OC pastures. This combination of variables explains approximately 94 percent of the variation in ADG of the steers grazing OC pastures.

OC pasture samples--36-hour fermentation. Butyric acid production *in vitro* after 36 hours of fermentation and several other pasture variables were used in developing the regression equations for predicting ADG found in Table 15.

Although the predictive value of equation one is negligible, the addition of AIL increased the R^2 value to 0.53 ($P<.05$). The addition of four other variables, percent cellulose, lignin:fiber ratio, percent orchardgrass and ADF increased the predictive value of the equations by 10, 12, 8 and 5 percent, respectively.

The best combination of variables after 36 hours of *in vitro*

TABLE 14

MULTIPLE REGRESSION EQUATIONS FOR ADG ESTIMATED FROM 24-HR IN VITRO VFA PRODUCTION[a] (IN MOLAR PERCENT) AND SEVERAL OTHER VARIABLES OBTAINED FROM ORCHARDGRASS-LADINO CLOVER PASTURES

Equation components	Equation number					
	1	2	3	4	5	6
Constant	0.1610	1.2010	0.8547	0.6527	-5.4556	-12.2310
Butyric acid	0.0646	0.0799	0.1127	0.1371	0.1567	0.1795
Acid insoluble lignin[b]		-.2171	-.2258	-.6311	-1.9312	-3.3555
Percent fescue			0.0118	0.0096	0.0085	0.0096
Lignin:fiber ratio				0.1489	0.6648	1.2451
Cellulose percent					0.1558	0.3457
Crude protein percent						-.0463
R^2	.11	.64*	.78**	.84**	.88*	.94**

[a]Measured in supernatant liquor after 24 hours of fermentation *in vitro* using the Tilley and Terry (1963) fermentation technique.

[b]Van Soest (1963) method.

*(.01<P<.05). **P<.01.

TABLE 15

MULTIPLE REGRESSION EQUATIONS FOR ADG ESTIMATED FROM 36-HR IN VITRO VFA PRODUCTION[a] (IN MMOLES/L/G DM) AND SEVERAL OTHER VARIABLES OBTAINED FROM ORCHARDGRASS-LADINO CLOVER PASTURES

	Equation number					
Equation components	1	2	3	4	5	6
Constant	1.2051	2.3811	5.2829	20.9690	25.9790	33.4590
Butyric acid	-.0795	-.0899	-.1811	-.3405	-.4175	-.5542
Acid insoluble lignin[b]		-.2071	-.2474	2.4885	3.4815	5.1218
Cellulose percent			-.0607	-.4406	-.5784	-.0573
Lignin:fiber ratio				-1.1017	-1.4857	-1.8522
Percent orchardgrass					0.0103	0.0113
Acid detergent fiber						-.7019
R^2	.05	.53*	.63*	.75*	.83*	.88*

[a]Measured in supernatant liquor after 36 hours of fermentation in vitro using the Tilley and Terry (1963) fermentation technique.

[b]Van Soest (1963) method.

*(.01<P<.05).

fermentation included the molar percentage of propionic acid in combination with other pasture variables, as shown in Table 16. Once again, the variation in VFA alone did not account for a great deal of the variation which occurred in ADG, but in combination with AIL, the R^2 increased to 0.61 ($P<.05$). These variables in combination with percent orchardgrass ($R^2 = 0.73$, $P<.05$), percent crude protein ($R^2 = 0.79$, $P<.05$), and lignin:fiber ratio yielded a notable R^2 of 0.93, which was significant at the .01 level of probability. If, for some reason, a study necessitated 36 hours of fermentation *in vitro* of OC pasture samples, and the other pasture variables had been determined, then the investigator should use the above combination of variables in order to most accurately predict ADG of beef animals grazing these OC pastures.

FL pasture samples--24-hour fermentation. There were no equations based on 24-hour *in vitro* VFA production expressed in either mmoles/1/g DM or molar percent that yielded statistically significant coefficients of multiple determination. The R^2 values, as shown in Table 12, page 39, ranged from 0.76 to 0.78 ($P>.05$).

FL pasture samples--36-hour fermentation. The best combination of variables for predicting ADG based on mmoles/1/g DM production of VFA from FL samples, presented in Table 17, yielded a significant ($P<.05$) 81 percent of the variation in ADG explainable using butyric acid production, percent cellulose, *in vitro* DDM, percent legume and percent crude protein as predictors.

Using molar percentage of VFA produced *in vitro* after 36 hours of fermentation (Table 18), a combination of butyric acid, percent

TABLE 16

MULTIPLE REGRESSION EQUATIONS FOR ADG ESTIMATED FROM 36-HR IN VITRO VFA PRODUCTION[a] (IN MOLAR PERCENT) AND SEVERAL OTHER VARIABLES OBTAINED FROM ORCHARDGRASS-LADINO CLOVER PASTURES

Equation components	Equation number 1	2	3	4	5	6
Constant	-3.6115	-2.3582	-5.9974	-7.0485	-8.5240	-10.4080
Propionic acid	0.1971	0.1888	0.3078	0.3601	0.4193	0.5010
Acid insoluble lignin[b]		-.2016	-.1430	-.0923	-.0080	-.3775
Percent orchardgrass			0.0135	0.0196	0.0224	0.0273
Crude protein percent				-.0383	-.0699	-.0851
Legume index					0.0029	0.0029
Lignin:fiber ratio						-.1310
R^2	.15	.61*	.73*	.79*	.88*	.93**

[a]Measured in supernatant liquor after 36 hours of fermentation *in vitro* using the Tilley and Terry (1963) fermentation technique.

[b]Van Soest (1963) method.

*(.01<P<.05). **P<.01.

TABLE 17

MULTIPLE REGRESSION EQUATIONS FOR ADG ESTIMATED FROM 36-HR IN VITRO VFA PRODUCTION[a] (IN MMOLES/L/G DM) AND SEVERAL OTHER VARIABLES OBTAINED FROM FESCUE-LESPEDEZA PASTURES

	Equation number					
Equation components	1	2	3	4	5	6
Constant	-.7288	2.8857	11.7890	10.7010	8.4526	8.0835
Butyric acid	0.2334	0.1693	0.2200	0.3255	0.3485	0.3148
Cellulose percent		-.0935	-.2406	-.2448	-.1992	-.1972
In vitro digestible dry matter			-.0613	-.0529	-.0325	-.0289
Legume percent				0.0205	0.0287	0.0303
Crude protein percent					-.0537	-.0711
Percent fescue						0.0057
R^2	.23	.47	.66*	.79*	.81*	.82

[a]Measured in supernatant liquor after 36 hours of fermentation *in vitro* using the Tilley and Terry (1963) fermentation technique.

*(.01<P<.05).

TABLE 18

MULTIPLE REGRESSION EQUATIONS FOR ADG ESTIMATED FROM 36-HR IN VITRO VFA PRODUCTION[a] (IN MOLAR PERCENT) AND SEVERAL OTHER VARIABLES OBTAINED FROM FESCUE-LESPEDEZA PASTURES

Equation components	Equation number					
	1	2	3	4	5	6
Constant	0.9625	4.5311	5.5417	4.3260	11.5500	10.5460
Butyric acid	-.0807	-.0533	-.1364	-.0850	-.0380	-.0286
Cellulose percent		-.1062	-.1250	-.1473	-.2780	-.2586
Legume index			0.0070	0.0096	0.0084	0.0090
Percent fescue				0.0185	0.0242	0.0270
In vitro digestible dry matter					-.0519	-.0041
Crude protein percent						0.0314
R^2	.12	.64**	.78**	.83**	.91**	.92*

[a]Measured in supernatant liquor after 36 hours of fermentation *in vitro* using the Tilley and Terry (1963) fermentation technique.

*(.01<P<.05). **P<.01.

cellulose, legume index, percent fescue and *in vitro* DDM yielded a highly significant R^2, 0.91 ($P<.01$). This combination would be most accurate if it were necessary to use VFA production after 36 hours of *in vitro* VFA production for estimating the ADG of beef animals grazing FL pastures.

CHAPTER V

SUMMARY

The objective of this experiment was to determine which pasture quality measurements would be most accurate and most practical in combination with _in vitro_ volatile fatty acid production after three specific periods of fermentation time in predicting body weight gains of beef animals grazing two types of pasture.

A grazing trial had been conducted previously with steers on orchardgrass-clover and fescue-lespedeza pastures to determine changes in body weight gains throughout the growing season. Chemical composition (AIL, ADF, cellulose and crude protein content, and lignin:fiber ratio), visual estimates of forage species composition (percent grass, percent legume and legume index) and _in vitro_ digestible dry matter of pasture samples had also been determined at 28-day intervals during the spring-summer grazing season.

In the present study, an _in vitro_ fermentation trial to determine VFA production was conducted with forage samples from these pastures, using 12, 24 and 36 hours of fermentation.

Multiple regression equations were developed using measurements of pasture quality and _in vitro_ VFA production.

It was concluded that:

1. There is no combination of variables which can be used with 12-hour VFA production data which will accurately predict ADG of beef animals grazing either pasture species.

2. The combination of variables which explains the most variation in ADG of animals grazing OC pastures includes 24-hour molar percentage of butyric acid, acid insoluble lignin, percentage of fescue in the OC pasture, lignin:fiber ratio, percent cellulose and percent crude protein. The significant R^2 ($P<.01$) indicates that this combination of variables accounts for 94 percent of the variation in ADG. For animals grazing FL pastures, the 24-hour equation using the combination of variables which accounted for the most variation in ADG yielded an R^2 of .80 ($P>.05$), which was not as good as results following 36 hours of fermentation.

3. The combination of variables which most accurately estimates variation in ADG of animals grazing FL pastures includes 36-hour molar percentage butyric acid in combination with percent cellulose, legume index, percent fescue, in vitro digestible dry matter and percent crude protein. The significant R^2 ($P<.01$) associated with this equation indicates that 91 percent of the variation in ADG of the cattle grazing FL pastures is accounted for by this combination of variables. For animals grazing OC pastures the 36-hour equation using the combination of variables which accounted for the greatest amount of variation in ADG yielded an R^2 of .93 ($P<.01$) which was not as accurate as results from 24 hours of fermentation.

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APPENDIX

TABLE 19

MEASUREMENTS OF PASTURE QUALITY COMPILED FROM ORCHARDGRASS-CLOVER AND FESCUE-LESPEDEZA PASTURES DURING DIFFERENT TIMES OF THE GROWING SEASON

Grazing period	Pasture type: Orchardgrass-clover 2-6	Orchardgrass-clover 3-8	Fescue-lespedeza 1-5	Fescue-lespedeza 4-7
		Percent fescue		
4/21-5/19	10	15	96	97
5/19-6/16	00	2	96	95
6/16-7/14	30	15	90	92
7/14-8/11	00	10	95	87
8/11-9/8	15	30	84	80
9/8-10/6	10	10	82	89
		Percent orchardgrass		
4/21-5/19	60	62	--	--
5/19-6/16	55	64	--	--
6/16-7/14	50	55	--	--
7/14-8/11	43	73	--	--
8/11-9/8	60	45	--	--
9/8-10/6	40	63	--	--
		Percent legume		
4/21-5/19	25	15	1	1
5/19-6/16	15	30	20	3
6/16-7/14	18	20	8	5
7/14-8/11	5	12	2	11
8/11-9/8	15	10	10	10
9/8-10/6	5	3	1	1
		Legume index		
4/21-5/19	73	45	3	2
5/19-6/16	45	150	4	6
6/16-7/14	72	100	40	15
7/14-8/11	20	48	6	50
8/11-9/8	30	30	30	30
9/8-10/6	10	15	3	2

TABLE 19 (continued)

Grazing period	Pasture type: Orchardgrass-clover 2-6	Orchardgrass-clover 3-8	Fescue-lespedeza 1-5	Fescue-lespedeza 4-7
	In vitro digestible dry matter			
4/21-5/19	67.7	65.6	63.2	66.6
5/19-6/16	61.5	62.6	57.1	56.8
6/16-7/14	60.5	62.2	53.8	55.6
7/14-8/11	65.5	66.5	60.9	61.3
8/11-9/8	62.4	58.6	60.1	60.5
9/8-10/6	60.9	62.0	61.3	65.4
	Percent crude protein			
4/21-5/19	13.7	14.2	14.9	14.3
5/19-6/16	16.6	18.2	16.4	14.8
6/16-7/14	17.0	18.0	13.7	13.8
7/14-8/11	15.9	19.8	15.7	16.2
8/11-9/8	14.7	18.5	14.9	14.0
9/8-10/6	14.0	16.8	13.6	14.2
	Percent acid detergent fiber			
4/21-5/19	34.8	33.9	34.1	33.6
5/19-6/16	39.0	36.5	37.8	37.5
6/16-7/14	37.9	35.5	37.8	36.9
7/14-8/11	37.0	36.4	36.1	35.8
8/11-9/8	35.2	34.4	36.6	35.8
9/8-10/6	35.9	35.2	35.3	34.7
	Percent acid insoluble lignin			
4/21-5/19	3.67	3.08	2.91	3.47
5/19-6/16	5.47	4.31	4.64	3.53
6/16-7/14	4.62	5.13	4.01	4.21
7/14-8/11	4.91	4.61	3.81	3.85
8/11-9/8	5.17	5.71	4.23	4.10
9/8-10/6	5.92	6.07	3.89	3.61
	Percent cellulose			
4/21-5/19	31.1	30.8	31.2	30.2
5/19-6/16	33.6	32.2	33.2	34.0
6/16-7/14	33.3	30.4	33.8	32.1
7/14-8/11	32.1	31.8	32.3	31.9
8/11-9/8	30.0	28.7	32.4	31.6
9/8-10/6	30.0	29.2	31.4	31.1

TABLE 19 (continued)

Grazing period	Pasture type: Orchardgrass-clover 2-6	Orchardgrass-clover 3-8	Fescue-lespedeza 1-5	Fescue-lespedeza 4-7
	Lignin/fiber ratio			
4/21-5/19	10.6	9.1	8.5	10.3
5/19-6/16	14.0	11.8	12.3	9.4
6/16-7/14	12.2	14.4	10.6	11.4
7/14-8/11	13.3	12.6	10.6	10.8
8/11-9/8	14.7	16.6	11.6	11.4
9/8-10/6	16.5	17.2	11.0	10.4

VITA

Peter Eldon Shumway was born in Bronxsville, New York, on October 14, 1944.

Upon completion of high school at Skaneateles Central School, in Skaneateles, New York, in June, 1962, he entered Clemson University, in Clemson, South Carolina. He earned a B.S. degree in Animal Science. While attending the University, he was a member of the Block and Bridle Club and the Pershing Rifles Crack Drill Platoon. After graduation, he taught junior high school until he entered the Graduate School of the University of Tennessee to attain a Master's degree, majoring in Animal Husbandry. Having been awarded an Assistantship in Animal Husbandry, he received his M.S. during the March, 1970, commencement.

He is married to the former Pamela W. Nichols, and they have three children, Pete, Jud and Anne.