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I am submitting herewith a thesis written by Robert R. Lyle entitled "Ruminal Parameters as Effected by Ration Change, Monensin, Protein Supplement Type and Level of Wheat in Steers Consuming Whole Shelled Corn Basal Rations." 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
and recommend its acceptance:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

RUMINAL PARAMETERS AS EFFECTED BY RATION CHANGE, MONENSIN, PROTEIN SUPPLEMENT TYPE AND LEVEL OF WHEAT IN STEERS CONSUMING WHOLE SHELLED CORN BASAL RATIONS

A Thesis

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ABSTRACT

This study was conducted initially to monitor changes occuring in ruminal pH, volatile fatty acid and ciliate protozoa concentrations as steers were adapted from all-forage rations to all-grain rations of whole shelled corn or mixtures of whole shelled corn and whole wheat. In addition, the effects of monensin additions and protein supplement type on these same rumen parameters in steers consuming basal rations of whole shelled corn were determined. Finally, the effects of varying levels of whole wheat, fed in combination with whole shelled corn, upon these same rumen parameters were determined.

In trial 1, four steers were adapted from all-forage rations to all-grain rations composed of whole shelled corn over a 15 day period (adaptation phase). The same steers were then fed whole shelled corn basal rations with and without monensin, supplemented with a commercial protein supplement or soybean meal for 112 days (switchover phase).

In trial 2, 47 steers were adapted from all-forage rations to all-grain rations composed of varying proportions of whole shelled corn and whole wheat over a 21 day period (adaptation phase). After adaptation to the all-grain rations, the same steers continued to receive the same corn - wheat rations for 50 days (finishing phase).

The effects of treatment on rumen variables observed within each trial are presented at a 5 percent level of significance. During the adaptation phase of trial 1, rumen fluid pH remained relatively high (6.70-6.85), but did decrease on day 15 (6.28). Total volatile fatty

acid concentration did not change during the adaptation phase (69.8-107.9 μm/ml), molar percent acetate decreased (73.6-49.7) and molar percent propionate tended to remain stable (12.6-15.0), but did increase (23.9) on day 12. Molar percents butyrate and valerate increased (9.4-29.7 and 0-2.7, respectively) during the adaptation phase. Molar percent isoacids did not change (.6-3.8) and protozoa concentrations, composed primarily of Entodinium, increased initially ($1.71-7.24 \times 10^5$/ml), and then decreased until all animals were defaunated after consuming all-grain, forage-free rations for 14 days.

During the adaptation phase of trial 2, rumen fluid pH (6.77-5.67), molar percent acetate (73.8-43.9) and molar percent butyrate (8.3-6.9) decreased, while total volatile fatty acid concentration (70.6-167.1 μm/ml) and molar percent propionate (15.4-45.4) increased. Molar percent valerate (.4-3.2) and isoacids (.8-1.3) did not change significantly. Total ruminal protozoa concentrations did not change between days 0 and 7 of the adaptation phase ($1.43-1.50 \times 10^5$/ml), however, by day 21, 85 percent of all steers were defaunated.

During the switchover phase of trial 1, monensin or protein supplement type did not effect ruminal pH (5.96-6.03), total volatile fatty acid concentration (121.0-130.0 μm/ml), molar percents acetate (43.7-46.7), propionate (39.0-44.0), butyrate (7.5-9.0), valerate (3.6-5.3) or isoacids (.4-.6).

During the finishing phase of trial 2, ruminal pH (5.94-6.22), total volatile fatty acid concentration (186.5-225.1 μm/ml), molar percents acetate (41.0-43.1), propionate (42.5-47.4), butyrate (7.3-

10.4), valerate (2.1-2.2) or isoacids (.9-1.4) were not effected by varying levels of wheat in the ration.

TABLE OF CONTENTS

LIST OF TABLES

TABLE (Continued) PAGE

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The availability of grain along with the current escalation in energy costs, the high costs of grain processing equipment and the need for an efficient system to rapidly finish backgrounded cattle with a minimum input of labor have provided incentives for the use of a feeding system which utilizes whole grain in the finishing phase. The feeding of whole grain, forage-free rations supplemented with a crude protein source to cattle has supported favorable average daily gains, feed conversions and carcass characteristics. An additional advantage of using whole rather than processed grain rations to finish cattle is the reduction in the incidence of digestive disturbances, which frequently occur when high-concentrate rations are used. This allows the feeder to utilize a ration of high energy density without using intensive management practices.

In the ruminant, ingested feed is extensively fermented by a diverse population of microorganisms in the rumino-reticulum, giving rise to volatile fatty acids, lactic acid and gases before passing into the lower digestive tract. A portion of these by-products produced in the rumen are utilized by the host. Previous research with cattle consuming forage or grain - forage mixed rations has indicated that monensin alters the fermentation processes in the rumen. However, little work reporting the effects of monensin upon the products of ruminal fermentation in cattle consuming whole shelled corn rations

has been published. Previous research has shown that quantitative changes occur in the production of volatile fatty acids and ammonia in the rumen as a result of feeding varying levels of protein to cattle consuming forage or grain - forage mixed rations. However, no data have been published regarding the effects of protein type upon the products of rumen fermentation in cattle consuming whole shelled corn rations. Reports have been published regarding changes in rumen metabolites as cattle are adapting from all-forage rations to various all-grain rations; however, none of these reports involved the use of whole shelled corn, whole wheat or combinations of the two.

The objectives of this study were to monitor the changes occuring in pH, volatile fatty acid and ciliate protozoa concentrations in the rumens of steers as they were gradually adapting from an all-forage ration to an all-grain ration of whole shelled corn or mixtures of whole shelled corn and whole wheat. In addition, the influence of protein supplement type and monensin on these same rumen parameters in steers consuming basal rations of whole shelled corn was determined. Finally, observations were made on the influence of varying levels of whole wheat fed in combination with whole shelled corn on pH, volatile fatty acid and ciliate protozoa concentrations in the rumen of feedlot cattle.

CHAPTER II

REVIEW OF LITERATURE

Use of All-Grain Rations

Gordon and Erwin (1960) and Wise et al. (1961, 1968) reported that the ruminant can perform normally for extended periods on rations devoid of roughage but otherwise balanced nutritionally. Data published by other researchers using all-grain finishing rations support these earlier findings (Hixon et al., 1969; Burkhardt et al., 1969; McLaren et al., 1971; Gerken et al., 1971; Vance et al., 1972; Gill et al., 1977).

Neumann and Snapp (1969) suggested that when all-grain rations are fed to cattle, grain should be ground or cracked for optimum performance, since some grain will escape chewing and digestion if the kernels are not broken. Wise et al. (1961) and Klay (1968) stressed the importance of including roughage or a buffer in cracked or ground grain rations fed to cattle as a means of maintaining a favorable rumen pH. This observation was supported by the findings of Goodrich and Meiske (1968), which showed that the performance of feedlot steers was improved slightly by the addition of small amounts of roughage to all-concentrate, ground corn rations. However, Beeson (1969) indicated that whole shelled grains may serve as a source of roughage in the rumen, and that the use of forage or dietary buffers in these rations was not essential. Vance et al. (1970) indicated that gross rumen wall changes such as papillae clumping and hair accumulation were less severe when whole shelled corn was fed in comparison to ground or steam-flaked corn.

Johnson et al. (1974) found that whole shelled corn rations supported higher ruminal pH levels in steers than did ground or heat processed corn rations.

When ruminants are fed all-grain rations, it is necessary to employ an ad libitum feeding system. Research conducted by Wise et al. (1961) and Mullen (1963) indicated that this type of feeding reduced the opportunity for the animal to overeat, thereby insuring a more uniform intake of feed with respect to time, which greatly reduced the incidence of bloat in feedlot animals.

Use of Corn in Finishing Rations

In previous feeding trials at Tennessee (Godfrey et al. 1977), whole shelled corn basal rations supplemented with protein have supported excellent average daily gains, feed conversions and carcass characteristics in heavy yearling finishing steers.

A study conducted by Hixon et al. (1969) showed that the feed efficiencies of steers receiving basal rations of either whole shelled corn or cracked corn were not significantly different. However, steers receiving the whole shelled corn ration had a significantly greater rate of gain (1.81 kg/day) than steers receiving the cracked corn ration (1.56 kg/day). They concluded that the costs involved in cracking corn were not justified. In contrast, Martin et al. (1971) and Weichenthal et al. (1972), in comparing the feedlot performance of steers receiving basal rations of either whole shelled corn, ground corn or rolled corn, indicated that the physical form of corn did not affect rate of gain or feed efficiency. The steers fed the whole shelled corn rations produced the most economical gains as a result of the absence of grain processing costs.

Data resulting from a digestion study by McCullough and Matsushima (1973) indicated that the percentage of total dietary starch digested in the rumen of steers was greater with whole shelled corn rations than with flaked corn rations (91 percent vs. 77 percent, respectively). This difference was attributed to an alteration in the rate of rumen turnover. In a similar study by Karr et al. (1966), data indicated that the quantity of dietary starch reaching the abomasum increased as the level of ground corn in the ration increased.

White et al. (1975), in studying the effects of physical form of corn on feedlot performance of cattle, demonstrated that dry matter intake and the incidence of liver abscesses were not effected by the physical form of grain; however, carcass weight and average daily gain tended to be higher in steers consuming whole shelled corn rations compared to steers consuming ground shelled corn rations. However, Wagner and Aimone (1976) indicated that feed intake, average daily gain, carcass characteristics and feed efficiency was not different in steers consuming whole shelled corn or rolled corn rations.

Use of Wheat in Finishing Rations

At times, wheat is competitively priced with other grains for use in livestock feeding. Except for being considerably higher in protein (12.8 percent vs. 8.8 percent) and containing much less carotene, wheat is quite similar to corn in composition and feeding value.

In a review of the literature, Brethour (1970) summarized that the relative feeding value of wheat to other grains is affected by processing methods, type and variety of wheat, and the conditions under which it is fed. He added that as a result of its potential to cause

rumen acidosis, wheat supports best results in the feedlot when it is mixed with another grain.

Generally, feedlot rations contain a maximum of 30 to 40 percent wheat. However, several reports have been published indicating that cattle can perform satisfactorily on higher levels of wheat. Wagner et al. (1971a, 1971b) reported favorable average daily gains and feed efficiencies in feedlot heifers consuming rations containing 70 and 84 percent wheat. In a later report, Martin and Wagner (1974) reported favorable average daily gains and feed efficiencies in feedlot cattle consuming rations containing up to 70 percent wheat. Oltjen et al. (1965) indicated that cracked wheat may make up 50 percent of the concentrate source in all-concentrate rations without adversely effecting rates of gain in cattle.

Oltjen et al. (1966) reported that the carbohydrate portion of wheat is readily degraded to volatile fatty acids in the rumen. In comparing wheat to corn in all-concentrate steer diets, Oltjen et al. (1967) found that total volatile fatty acid concentration was higher and ruminal pH was lower in steers fed all-wheat rations than in steers fed all-corn rations. He concluded that this difference resulted from the more rapid rate of fermentation of the endosperm of wheat compared to that of corn.

Protein Utilization by the Ruminant

Haskins et al. (1967) reported that with rations based on ground shelled corn supplemented with varying levels of soybean meal, steers receiving a 14 percent crude protein ration had higher average daily gains than steers consuming an 11 percent crude protein ration. Data

reported by Gill et al. (1977) indicated that in steers weighing up to 340 kg fed whole shelled corn rations supplemented with soybean meal to provide crude protein levels of 9.5, 10.3, 11.2 and 12.3 percent, rate of gain and feed efficiency increased as the protein level of the ration increased. They concluded that young steers fed whole shelled corn rations readily responded to increases in dietary protein levels up to 12 percent. However, in 390 kg steers which had been well fed during backgrounding, the 9.5 percent level of crude protein provided by the corn grain alone supplied an adequate amount of protein to meet the requirements of the animal.

Early studies indicated that dietary proteins, when introduced into the rumen, were actively hydrolyzed (Sym, 1938; Pearson and Smith, 1943). In several subsequent studies (El-Shazly, 1952; Annison, 1954; McDonald, 1954; Lewis, 1955; Lewis, 1961; Annison and Lewis, 1962; Blaxter, 1962; Lampila, 1964; Hungate, 1966; Leng, 1970), researchers demonstrated that microbial fermentation of proteins in the rumen gave rise to peptides, amino acids, ammonia and volatile fatty acids. In addition to these products, microbial protein was also synthesized provided an energy source was available.

The extent to which rumen microorganisms degrade dietary protein depends largely on the chemical properties of the protein. Blaxter (1962) noted that microbial proteolytic enzymes appeared to be relatively non-specific. Lewis (1961) and Annison and Lewis (1962) reported that the relative rate of proteolysis and synthetic reactions in the rumen exerted a controlling effect on the nutritive value of the protein fed. They reported that the two major factors which regulated this balance were the solubility of the ingested protein and the amount and type of

carbohydrate present in the rumen. A minimum amount of protein which was susceptible to microbial fermentation was essential for supplying the ammonia-nitrogen needed by the rumen microorganisms for amino acid synthesis. The rate of microbial protein synthesis increased as the level of readily soluble dietary protein increased, provided an adequate supply of energy was available to the microbes.

The effect of dietary protein on the concentration of volatile fatty acids in the rumen has been reported by El-Shazly (1952), Davis et al. (1957), Haskins et al. (1967) and Mahmoud (1977). Davis et al. (1957) found that as the level of dietary protein increased from 9 percent to 36 percent, the concentrations of acetate, propionate, butyrate and total volatile fatty acid in the rumen increased. Haskins et al. (1967), using ground shelled corn as a basal ration, reported that a 14 percent crude protein diet supported higher molar proportions of butyrate in the rumen of cattle than an 11 percent crude protein diet. A study conducted by Gill et al. (1977) showed that as the level of crude protein in the diet increased, the ratio of acetate to propionate in the rumen decreased. El-Shazly (1952) noted that dietary protein was the principle source of branched-chain volatile fatty acids found in the rumen.

Factors Effecting Ruminal pH

The pH of rumen ingesta appears to be related to the presence and concentration of several rumen metabolites and dietary constituents, each exerting its own influence on the acidity or alkalinity of rumen contents. In an early report, Phillipson and McAnally (1942) theorized that the pH of rumen contents is probably, to a large extent, a function

of volatile fatty acid concentration. However, Cason et al. (1954) reported that the ash content of the rumen ingesta exerts more influence on ruminal pH than does volatile fatty acid concentration. Briggs et al. (1957) hypothesized that the pH of rumen contents is determined more by the concentration of lactic acid in the rumen than by volatile fatty acid concentration. They noted that when high concentrations of lactic acid were present in the rumen, pH followed lactic acid closely and inversely, sometimes regardless of large changes in total volatile fatty acid concentration. However, they did indicate that the regression of pH on total volatile fatty acid concentration was significant and negative, with coefficients ranging from -.049 to -.213.

Reports have indicated that the pH - volatile fatty acid relationship is modified to some extent by the level of ammonia-nitrogen in the rumen (Clark and Weiss, 1952). Therefore, variations in protein solubility could affect the pH of rumen ingesta.

Bailey (1961) indicated that the physical character of a ration indirectly influenced the pH of rumen contents. He reported that mastication, both during eating and ruminating, greatly increased salivary secretion. Earlier work by Gordon (1958) showed that mastication and rumination was considerably decreased on a high-concentrate diet, thereby reducing salivary flow to the rumen. A study by Clark and Weiss (1952) resulted in similar findings. They noticed that diets consisting largely of concentrates provide less mechanical stimulation of the cardiac region of the rumen, thereby reducing salivary flow to the rumen. McDougall (1948) indicated that a reduction in salivary flow to the rumen would reduce the buffering capacity of the rumen ingesta, thereby allowing ruminal pH to decrease substantially.

Several reports have been published regarding the pH of rumen contents in animals consuming all-grain rations. The pH of rumen ingesta taken from rumen-fistulated sheep consuming a cracked corn ration ranged from 5.3 to 6.2 in a study by Myburgh and Quinn (1943). However, Hungate et al. (1952) reported much lower pH values ranging from 4.1 to 4.7 in the rumen of sheep consuming all-grain diets.

Johnson et al. (1974) found that whole shelled corn rations resulted in less dramatic reductions in rumen pH of steers than did rations consisting of ground or heat processed corn. Steers consuming whole shelled corn rations had rumen pH values higher than 5.6.

Effect of Monensin on Rumen Metabolites

Monensin is the major factor in a group of closely related biologically active compounds produced by a strain of Streptomyces cinnamonensis. Monensin inhibits the growth of gram positive organisms, thereby altering the population of bacteria normally found in the rumen (Haney and Hoehn, 1967).

Extensive investigations conducted with monensin have unequivocally demonstrated that this compound alters the proportion of individual volatile fatty acids produced by the rumen microorganisms. Researchers (Dinius et al., 1976; Perry et al., 1976; Richardson et al., 1976; Raun et al., 1976; Boling et al., 1977; Mowat et al., 1977) have indicated that monensin supports an increase in the molar proportions of propionate and a decrease in the molar proportions of acetate and butyrate in the rumen of cattle consuming forage or grain and forage mixed rations. However, Gill et al. (1977) indicated that in steers consuming whole shelled corn rations, the addition of monensin to the

diet resulted in only subtle changes in the molar proportions of acetate, propionate and butyrate in the rumen.

Numerous data have been published regarding the effects of monensin on feed efficiency in cattle (Brown et al., 1974; Embry and Swan, 1974; Raun et al., 1974; Anthony et al., 1975; Farlin et al., 1975; Potter et al., 1976; Raun et al., 1976; Gill et al., 1977). These workers indicated that monensin improved feed efficiency in cattle by reducing feed intake without affecting average daily gain or carcass characteristics.

The mechanism by which monensin improves feed efficiency in the ruminant is not yet fully understood. Richardson et al. (1976) indicated that in changing the molar percentage of volatile fatty acids toward an increased proportion of propionate and a decreased proportion of acetate and butyrate, monensin theoretically increased the efficiency of converting feed energy to energy in the acid end products which are available for absorption from the rumen. They concluded that the animal would have more energy available for metabolic functions from the feed consumed. Raun et al. (1976) reported that monensin, by increasing propionate production, reduced the heat increment of rumen fermentation. An earlier study conducted by Armstrong et al. (1957) indicated that an increase in the proportion of propionate in the rumen reduced energy losses from the animal by reducing methane losses from the rumen.

Thornton et al. (1978) indicated that monensin improved the digestibility of whole shelled corn rations in feedlot steers, especially when dietary crude protein levels were low. Raun et al. (1976), in a study with monensin, indicated that an improved efficiency in rumen

fermentation is not the only benefit received by feeding monensin. Other potential energy savings include an increase in gluconeogenesis, a sparing of protein from ruminal fermentation and a stimulation of microbial synthesis in the rumen.

Factors Effecting Rumen Protozoa

Many genera and species of ciliate protozoa are normally found in the rumen. These obligate anaerobic organisms can be divided into two main orders based on morphological features and substrate specificity: (1) Holotrichs, which are ciliated over their entire body surface and utilize readily available carbohydrates, such as sugars; (2) Oligotrichs, which are generally ciliated in a single oral zone and utilize more complex polysaccharides such as starch and cellulose (Hungate, 1966; Barnett, 1961). Researchers have shown that the rumen protozoa not only aid in the digestion of carbohydrates, but they also digest proteins, both of dietary and microbial origin (Barnett, 1961; Annison and Lewis, 1962; Hungate, 1966).

The effects of diet on rumen protozoa populations have been extensively studied. Christiansen et al. (1964) found that when feeding frequency and particle size of the ration increased, protozoa numbers increased and when feed intake increased, protozoa numbers decreased, due to an increased rate of passage of ingesta through the gastro-intestinal tract. Data reported by Warner (1964a) indicated that when sheep were fed moderate amounts of starch, the concentration of protozoa in the rumen initially increased. However, as the starch level in the ration continued to increase, pH levels in the rumen decreased, thereby increasing rumen streptococci numbers. Finally, when the level of

starch in the diet peaked, rumen pH declined further and the number of rumen lactobacilli increased, accompanied by a sharp decline or complete absence of rumen protozoa.

Many researchers have indicated that low ruminal pH levels are detrimental to the ciliate protozoa (Oxford, 1955; Hungate, 1966; Purser and Moir, 1959; Warner, 1964a; Eadie and Mann, 1970; Abe et al., 1973). Purser and Moir (1959) indicated that the major factors controlling rumen protozoa concentration are the extent of reduction of ruminal pH and the length of time the pH remains depressed. However, they did not specify the minimum pH at which protozoa inhibition occurs. Their data indicated that the correlation of rumen pH with mean daily protozoa concentration was highly significant at a value of .90.

Of particular interest in this study were the Entodinium protozoa, which are grouped in the order Oligotricha. Numerous reports indicate that Entodinium protozoa flourish in the rumen of cattle consuming rations abundant in readily available forms of carbohydrates, such as starch (Oxford, 1955; Warner, 1956; Barnett, 1961; Annison and Lewis, 1962; Warner, 1964a; Abe et al., 1973; Hinkson et al., 1976). However, other researchers found that in ruminants consuming rations containing high levels of starch and little or no forage, the number of Entodinium may be sharply depressed or they may be completely eliminated from the rumen (Annison and Lewis, 1962; Slyter et al., 1965; Hungate, 1966; Mackie et al., 1978).

Ciliate protozoa can be both beneficial and detrimental to the overall nutrition of the ruminant. Some beneficial functions that protozoa serve in the rumen include: higher production of volatile

fatty acids, a reduction in the ratio of acetate to propionate (Abou Akkada and El-Shazly, 1964; Hungate, 1966; Eadie and Mann, 1970; Hinkson et al., 1976), storage of amylopectin starch within the ciliate's bodies, which is later conveyed to the host (Annison and Lewis, 1962; Eadie and Mann, 1970), and synthesis of microbial protein of high biological value, which can be utilized by the host (Oxford, 1955). Conversely, protozoa may reduce the efficiency of digestion in the rumen. Oxford (1955) cites specific examples such as wastage of ingesta and bacterial protein, destruction of B vitamins, and increased lactate concentrations in the rumen.

Conflicting reports have been published concerning the influence of protozoa on the rate of gain of ruminants. Christiansen et al. (1964) reported that a viable population of rumen ciliates seemed to improve live weight gains of sheep, possibly through a higher production of volatile fatty acids in the rumen. However, Eadie and Hobson (1962) and Williams and Dinusson (1973) indicated that no striking differences were observed in the performance of ruminants with or without viable populations of rumen protozoa.

Ruminal Fermentation

Researchers have long realized that in the ruminant, ingested feed is fermented extensively by a diverse population of anaerobic microorganisms in the rumeno-reticulum before passing into the lower digestive tract of the animal. Tappeiner (1883) demonstrated that the fermentation of cellulose in the rumen of cattle resulted in the formation of large amounts of volatile fatty acids. This observation was supported by the findings of Kellner (1900), which indicated that

the energy value of cellulose was equal to that of starch when fed to steers.

Much subsequent research unequivocally demonstrated that each of the carbohydrate constituents of the ruminant's diet was degraded to some extent in the rumen and that polysaccharides were fermented at rates which generally reflected their solubility and availability. These same reports indicated that starch was readily fermented in the rumen with the production of volatile and non-volatile fatty acids (Phillipson and McAnally, 1942; Reid et al., 1957; Annison and Lewis, 1962; Blaxter, 1962; Hungate, 1966). Phillipson and McAnally (1942) cited three ways by which starch can be digested by the ruminant: (1) some starch may bypass the rumen and go directly to the abomasum and small intestine, and be digested there, (2) some starch may be digested by the protozoa in the rumen and stored in their bodies as amylopectin, which is later conveyed to the host, (3) some starch may be fermented by the ruminal microorganisms, giving rise to volatile fatty acids.

For many years the volatile fatty acids produced in the rumen were considered to be of little nutritional significance to the animal. It is now well known that these short-chain organic acids constitute the major source of energy needed by the ruminant, since only a small proportion of the ingested carbohydrate escapes degradation in the rumen. Carroll and Hungate (1954) and Warner (1964b) reported that the volatile fatty acids produced in the rumen account for about 70 percent of the total energy requirement of the ruminant. Earlier work by Elsden (1946) led to the conclusion that volatile fatty acids are nearly

completely utilized by the ruminant, since only negligable amounts are excreted by the animal.

Carbohydrate metabolism in ruminants is markedly different from that in monogastric animals. In the ruminant, most of the dietary carbohydrates are fermented in the rumen, giving rise to short-chain organic acids. Gray et al. (1951, 1952) found that the mixture of ruminal volatile fatty acids included formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate and heptanoate. Acetate generally predominated in the rumen under all dietary conditions. They theorized that the longer-chain volatile fatty acids were synthesized in the rumen by the condensation of shorter-chain acids with a two-carbon compound in equilibrium with acetate. Edwards (1955) subsequently reported that acetate contributed to the formation of valerate and caproate and that propionate also contributed to the formation of valerate. In addition to this, he reported that amino acids hydrolyzed from dietary proteins in the rumen may be the primary source of branched-chain volatile fatty acids.

An investigation conducted by Leng (1970), partially supported by the discoveries of previous researchers, led to the formulation of several mechanisms by which the rumen microorganisms synthesize volatile fatty acids. He indicated that the phosphoroclastic reactions were the major reactions involved in the synthesis of acetate in the rumen. Requirements for these reactions included thiamine pyrophosphate, coenzyme-A and inorganic phosphate. Baldwin et al. (1962) and Leng (1970) indicated that there are two mechanisms by which lactate and pyruvate are converted to propionate in the rumen. The first pathway involves the formation of oxaloacetate and succinate and the second

involves the formation of acrylate. Under normal feeding conditions, the major pathway is the one involving succinate. However, the acrylate pathway may be more important in the rumen of cattle consuming grain rations. Leng (1970) reported that synthesis of butyrate in the rumen may involve acetate, or may occur from compounds giving rise to acetyl Co-A, such as pyruvate or glutamate. In addition to this, he added that two pathways may be involved in the synthesis of butyrate from acetate. The most likely pathway being the reversal of B-oxidation. A second possible pathway involved malonyl Co-A, but this pathway required twice as much energy as the other pathway.

Absorption of Volatile Fatty Acids From the Rumen

The cells lining the forestomach of the ruminant are classified histologically as keratinized, stratified, squamous epithelial cells, a type that does not produce mucus and is non-glandular. The epithelium of the reticulum is raised into folds forming small compartments with four, five or six sides. The epithelium of the rumen is raised into small papillae, being most dense in the ventral parts of the caudal and ventral sacs, which are the areas where, presumably, most absorption occurs (Church, 1976).

The earliest published reports indicating that volatile fatty acids are readily absorbed from the rumen are those by Phillipson and McAnally (1942), McAnally and Phillipson (1942) and Barcroft et al. (1944). Blaxter later indicated that the driving force which carries the acids across the rumen epithelial surface is probably attributed to a concentration gradient, which is maintained by the rapid removal of volatile fatty acids from the blood by the liver and other tissues as well.

Several factors affect the rate at which acids are absorbed across the rumen wall. The rate of disappearance of fatty acids from the rumen increases as the length of the carbon chain increases (Pfander and Phillipson, 1953; Sutton et al., 1963). Gray (1948) and Bloomfield et al. (1963) have presented data indicating that the rate at which volatile fatty acids were absorbed from the rumen was greater under acidic conditions than under alkaline conditions. Blaxter (1962) reported that the rumen wall was more permeable to the organic acids when they existed as the free acid, rather than as the anion. He noted that the pKa values of the volatile fatty acids ranged from 4.77 to 4.89. As ruminal pH was lowered, more of the acids existed as the undissociated molecule in contrast to the charged ion present at a higher ruminal pH.

Researchers have reported conflicting results regarding the effect of ruminal pH on the relative rates of absorption of the individual acids. Gray (1948) and Sutton et al. (1963) indicated that the order of absorption of the predominant volatile fatty acids from the rumen was butyrate > propionate > acetate under conditions of low pH. However, Barcroft et al. (1944) reported that when rumen contents were alkaline, the order of absorption of the acids from the rumen was acetate > propionate > butyrate. Gray (1948) could not detect any significant acid absorption when rumen conditions were basic, but he did confirm that propionate was absorbed more rapidly than acetate when rumen conditions were acidic.

Work by Pfander and Phillipson (1953) indicated that the concentration of an individual acid had a greater influence than did ruminal pH upon the quantity of acid absorbed across the rumen wall.

In the same report, they added that due to the metabolism of butyrate by the rumen epithelium, the proportion of butyrate reaching the venous blood stream was small in comparison to the proportion found in the rumen. Annison et al. (1957), in a similar study, noted that less butyrate, relative to acetate and propionate, was found in the portal blood and that there was a greater concentration of ketone bodies in portal than in peripheral blood, as a result of the active metabolism of butyrate by the rumen epithelium.

Metabolism of Volatile Fatty Acids

Many investigations into the utilization of the volatile fatty acids by the body tissues of the ruminant have been conducted. Acetate provides an immediate source of energy to the ruminant and is actively involved in the synthesis of milk and body fat (Stoddard et al., 1949; Kleiber et al., 1952; Jarrett et al., 1952). It appears that the function of propionate in the ruminant tissues is mainly glucogenic. Reid (1950) stated that propionate was the principle source of blood glucose for sheep and McClymont (1951) and Leng et al. (1967) indicated that all ruminants maintained their blood sugar level by gluconeogenesis from propionate. Pennington and Sutherland (1956) and Leng and Annison (1963) proposed two pathways by which glucose could be synthesized from propionate. Little precise information exists regarding the metabolic function of butyrate. Butyrate has been shown to have a high energy value (Kiddle et al., 1951; Pfander and Phillipson, 1953; Johnson, 1953). The highly ketogenic activity of butyrate in liver and rumen epithelial tissues has been demonstrated by Pennington (1952), but the effect of the acid on blood sugar is less well established.

Factors Effecting Volatile Fatty Acid Concentration in the Rumen

The concentration of a particular volatile fatty acid or of the total acids present in the rumen is not necessarily a good indicator of the rate of acid production. Annison and Lewis (1962) and Wheaton et al. (1970) both noted that the concentration of any acid in the rumen at any given time was influenced by several factors, such as: (1) the rate at which the acid is produced, (2) the rate at which the acid is absorbed from the rumen, (3) the rate or extent of passage of ingesta from the rumen to the omasum, (4) dilution of rumen contents with saliva, (5) the rate at which the acid is utilized by rumen microbes and (6) interconversion of acids or conversion of acids to other rumen metabolites.

Despite the active mixing of rumen ingesta by contractions of the rumeno-reticulum wall, rumen contents are non-homogenous (Pearson and Smith, 1943; Balch, 1950; Wheaton et al., 1970; Church, 1976). By visually examining the rumen contents of rumen-fistulated steers consuming all-grain rations, Wheaton et al. (1970) noted that rumen ingesta were principally localized in the ventral sac of the rumen, where a layer of grain was observed covering the ventral floor. Their analyses indicated that pH was lowest in this area and volatile fatty acid concentration was highest, probably because the formation of acids occurs primarily in the solid fraction of the rumen ingesta. However, molar percent volatile fatty acids did not differ between sampling sites in the rumen. In the same study, when obtaining rumen samples from fistulated animals both via stomach tube and fistula, they reported that pH values were higher in the samples taken via stomach tube

compared to that in the samples taken via fistula. They attributed this difference to salivary and mucus contamination resulting from passing the stomach tube down the esophagus. A similar study conducted by Raun and Burroughs (1962b) indicated no observable differences in molar percent volatile fatty acids between rumen samples taken via fistula or stomach tube. However, total volatile fatty acid concentration was lower and rumen fluid pH was higher in the samples taken by tube. They attributed these differences to salivary dilution in the anterior area of the rumeno-reticulum, which was the site of withdrawal of the tube samples. They concluded that the differences between molar percent volatile fatty acid in the samples taken via stomach tube or fistula were consistent, such that they did not lend any error to comparative measurements between different rations.

In the determination of volatile fatty acid concentration and pH of rumen contents taken via stomach tube, one must realize that pH is likely to be higher and acid concentration lower than that of samples taken via fistula. Despite this discrepancy, one may still accurately make comparative measurements of the molar proportions of volatile fatty acids between ration treatments using rumen samples taken from animals via stomach tube.

Effect of Ration on Volatile Fatty Acid Concentration

Much research has indicated that the total concentration of volatile fatty acids in the rumen is significantly affected by the diet. High-energy finishing rations usually support higher total acid concentrations and higher proportions of propionate relative to acetate and butyrate than do low-energy, forage rations (Reid *et al.*,

1957; Annison and Lewis, 1962; Raun et al., 1962a; Blaxter, 1962; Hungate, 1966; Hinkson et al., 1976). This alteration in acid production was accompanied by a reduction in the pH of rumen contents. Reid et al. (1957) attributed the increased levels of propionate to a qualitative change in the rumen microorganisms in response to the low pH levels associated with the continued feeding of a high-starch diet. However, they noted that the possibility existed that a change in the proportion of individual volatile fatty acids at varying pH levels may reflect changes in relative rates of absorption of acids from the rumen.

CHAPTER III

EXPERIMENTAL PROCEDURE

Description of Trials

Trial 1. Four Hereford steers (average initial weight 200 kg) were taken off pasture and placed in separate free-standing stalls. Stalls were equipped with automatic waterers and individual feed tubs. Steers were assigned to the same stall for the duration of the trial. Trial 1 consisted of two phases: the first 15 days of the trial served as the adaptation phase, in which all steers were adapted from an all-forage ration of grass hay to an all-concentrate ration consisting of whole shelled corn supplemented with protein and minerals. The remainder of the trial (112 days) served as the switchover phase, in which the same four steers were used in two successive, balanced four by four Latin square design trials.

The 15 day adaptation phase was designed to allow the changes in rumen fluid pH, volatile fatty acid and protozoa concentrations to be monitored as steers were gradually adapting from an all-forage ration to an all-concentrate ration. On the first day of the adaptation phase, each steer was fed 2.0 kg of whole shelled corn, topdressed with .5 kg of a protein supplement (36 percent crude protein) and hay was fed ad libitum. As the adaptation phase progressed, the amount of whole shelled corn given each steer was increased by .45 kg per day, the amount of protein supplement given each steer remained constant at .5 kg per day

and the amount of hay fed each steer was reduced by .5 kg per day. By the last day of the adaptation phase, each steer was consuming a forage-free ration of whole shelled corn ad libitum, supplemented with protein. A trace mineralized salt, dicalcium phosphate, ground limestone mixture (1:2:1 ratio, respectively) was available free-choice to each steer throughout the adaptation phase.

To observe any ruminal changes resulting from the ration change, rumen fluid samples were taken from each steer on the day immediately preceding the first day of the adaptation phase and subsequently on days 3, 6, 9, 12 and 15 of the adaptation phase. The method by which rumen samples were taken was similar to that described by Raun and Burroughs (1962b). A stainless steel strainer was secured to one end of a small flexible tygon tube (.5 cm i.d.). A speculum was held in the animal's mouth and the tube was passed down the esophagus until the strainer entered the rumeno-reticulum. A 100-120 ml sample of rumen fluid was aspirated with a 60 ml catheter tip syringe.

Upon completion of the adaptation phase, the same steers were immediately used in the second phase of trial 1, the 112 day switchover phase. This phase was designed to evaluate the effects of protein supplement type and monensin on the pH, volatile fatty acid and protozoa concentrations in the rumens of steers consuming forage-free basal rations of whole shelled corn. In the switchover phase, two successive, balanced four by four Latin square design trials were used. There were no differences in the design of the two trials, one merely followed the other. Within each Latin square design trial, each of the four steers was fed each of the four rations shown in table 1 for a 14 day period.

TABLE 1

GRAIN RATIONS FED DURING SWITCHOVER PHASE-- TRIAL 1

Ration	Daily Allotment[a]			
	Whole Shelled Corn[b]	Commercial Protein[c]	Soybean Meal[d]	Monensin[e]
1	Ad libitum	.5 kg		
2	Ad libitum	.5 kg		300 mg
3	Ad libitum		.5 kg	
4	Ad libitum		.5 kg	300 mg

[a]All rations were topdressed with 70 g of a trace mineralized salt-dicalcium phosphate- ground limestone mixture (1:2:1, respectively).

[b]Corn, dent yellow, grain, ref. no. 4-02-935.

[c]Tend-R-Leen[R] 36% crude protein.

[d]Soybean, seed, solv-extd., toasted ground, ref. no. 5-04-607 44% crude protein.

[e]Monensin was fed via .22 kg rice meal pellets.

Within each 14 day period, rumen fluid samples were taken via stomach tube from each steer in the morning (9:00 a.m.) and afternoon (3:00 p.m.) of days 5, 11 and 14.

Trial 2. This trial consisted of two phases: an adaptation phase, in which steers were adapted from all-forage rations to all-grain rations over a 21 day period and a finishing phase, in which steers were fed all-grain rations for 50 days.

Forty-seven heavy yearling steers were taken off pasture and assigned to four pens, twelve steers in each of three pens and eleven steers in the fourth pen. All steers in each pen were gradually adapted to all-grain basal rations containing varying proportions of whole shelled corn and whole wheat (table 2) over a 14 day period by the same process as that described in the adaptation phase of trial 1. Rumen fluid samples were taken via stomach tube from each steer on the day immediately preceding the adaptation phase and subsequently on days 7 and 21 of the adaptation phase to observe changes occuring in ruminal pH, volatile fatty acid and protozoa concentrations. Although adaptation from an all-forage ration to an all-grain ration occured over a 14 day period, observations were made on rumen samples taken through day 21 of the trial. Therefore, the "adaptation phase" of this trial actually involved ruminal changes occuring over a 21 day period.

After the adaptation phase was completed, the same steers continued to receive the same whole shelled corn - whole wheat mixture during the 50 day finishing phase of the trial. Finally, on day 50 of the finishing phase, rumen fluid samples were taken from each steer to

TABLE 2

GRAIN RATIONS FED DURING ADAPTATION AND FINISHING PHASES-- TRIAL 2

Ration	Daily Allotment[ab]		
	Basal Ration	Protein Supplement[c]	Monensin[d]
1	100% Whole Shelled Corn (WSC)[e]	.5 kg	300 mg
2	80% WSC 20% Whole Wheat (WW)[f]	.5 kg	300 mg
3	60% WSC 40% WW	.5 kg	300 mg
4	40% WSC 60% WW	.5 kg	300 mg

[a]All steers had access to a trace mineralized salt block.

[b]Upon completion of the adaptation phase, basal rations were fed ab libitum.

[c]Tend-R-Leen[R], 36% crude protein.

[d]Monensin was added to the protein supplement during formulation (600 ppm).

[e]Corn, yellow dent, grain, ref. no. 4-02-935.

[f]Wheat, soft red winter, grain, variety Arthur, ref. no. 4-05-294.

determine if varying levels of wheat in the rations effected ruminal pH, volatile fatty acid or protozoa concentrations.

Sample Processing Procedures

All rumen fluid samples taken from steers during both phases of trials 1 and 2 were processed in the same manner. As each rumen sample was taken from each steer, the pH of the fluid was determined immediately. A portable pH meter with a glass electrode, standardized with pH 7.0 and 4.0 solutions, was used. For protozoa counts, a 10 ml aliquot of each rumen sample was preserved by diluting with 10 ml of 40 percent formalin, sealed in a test tube, agitated slightly and stored at room temperature until protozoa counts were made. Finally, a 70 ml aliquot of rumen fluid was sealed in a plastic bag, immediately cooled by immersion in cold water and then frozen until volatile fatty acid analyses could be performed.

Volatile Fatty Acid Analysis

Volatile fatty acid analyses on all rumen samples were performed in duplicate. The procedure used was similar to that described by Erwin et al. (1961). Each frozen rumen sample was thawed at room temperature and 40 ml were transferred into a 45 ml tube and centrifuged under refrigeration at 10,000 g for 5 minutes. From the centrifuged sample, two 5 ml aliquots of the supernate were taken and each was acidified with 1 ml of a 25 percent meta-phosphoric acid solution which contained 2-ethyl butyric acid as an internal standard. The acidified sample was refrigerated for 30 minutes, after which, the sample was centrifuged again under refrigeration at 10,000 g for 10 minutes. The supernate

was then decanted and frozen to further coagulate any protein not precipitated by the acidifying process. This frozen sample was then thawed and centrifuged at 2000 g for 5 minutes to remove any coagulated protein. Volatile fatty acids were quantitated by gas-liquid chromatography.[1] A microliter syringe[2] was used to inject a 1 µl aliquot of the supernate into a 1.8 meter glass column (2.0 mm i.d.) packed with 10 percent SP-1200/1 percent H_3PO_4 on 80/100 mesh Chromosorb W AW[3] for separation of volatile fatty acids. Peak areas of individual volatile fatty acids were compared to similar areas from standard acid solutions and quantitated to volume using the 2-ethyl butyric acid factor.

Protozoa Counts

All protozoa counts were performed in duplicate on each rumen sample. The methods used to stain, dilute and count protozoa were similar to those described by Boyne et al. (1957) and Purser and Moir (1959). A 1 ml aliquot of the formalin-preserved rumen fluid (1:2 dilution) was pipetted into a 16 x 150 mm tube. Two drops of brilliant green dye were added, the contents were mixed and allowed to stand at room temperature for at least four hours. Nine ml of 30 percent glycerol were then added to the stained sample, giving a 1:20 dilution of the original rumen contents. A 1 ml aliquot of the final dilution was then

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[1]A Bendix 2500 gas-liquid chromatograph equipped with an Infotronics CRS-309 digital integrator was used.

[2]Hamilton Company, Reno, Nevada.

[3]Supelco Incorporated, Bellefonte, Pennsylvania.

pipetted into a Sedgwick-Rafter counting chamber. A square micrometer eyepiece scale which projected an area of .25 mm^2 onto the base of the counting chamber was used. Using a calibrated microscope stage, the protozoa in 50 fields were counted. The 50 fields were evenly spaced over the 20 x 50 x 1 mm counting chamber. The counting chamber was then rotated 180° and a second 50-field count was made. The average of these two counts was used for calculating the number of protozoa per ml of rumen fluid.

Statistical Analysis

Trial 1. Data obtained during the adaptation and switchover phases were analyzed separately. Analysis of variance (Barr et al., 1976) was used on data obtained from the adaptation phase to determine if any rumen variables were significantly affected by the independent variables. The model used was:

$$Y_{ij} = \mu + a_i + d_j + e_{ij}.$$

where:

Y_{ij} = dependent variables.

μ = theoretical population mean.

a_i = effect of animal, i = 1-4.

d_j = effect of day, j = 1-6.

e_{ij} = random error.

If differences were determined by F tests, means were separated using the Student-Newman-Keuls test (Sokal and Rohlf, 1969). In addition, volatile fatty acid and pH data obtained during the adaptation phase were fitted to a binomial regression on day. The regression equation used was:

$$\hat{Y} = a + b_1(day) + b_2(day^2).$$

where: $\hat{Y}$ = dependent variables.

a = intercept.

day = day of adaptation phase.

b_1, b_2 = the regression coefficients for each dependent variable on the independent variable = day, day^2.

Protozoa counts made during the adaptation phase were fitted to a linear regression on day. The regression equation used was:

$$\hat{Y} = a + b_1(day).$$

where: $\hat{Y}$ = dependent variables.

a = intercept.

day = day of adaptation.

b_1 = the regression coefficient for each dependent variable on the independent variable = day.

Data obtained from the switchover phase of trial 1 were statistically analyzed as a split plot in time (Harvey, 1964), due to the successive observations made on the same whole unit over a period of time. Programs prepared by Barr et al. (1976) were used to conduct analyses of variance to determine if the rumen variables were significantly affected by the independent variables. The two models used were:

(1) $Y_{ijklm} = \mu + t_i + p_j + a_k + r_l + A_{ijkl} + (d_m p_j) + e_{ijklm}.$

(2) $Y_{ijklm} = \mu + t_i + p_j + a_k + r_l + (d_m p_j) + e_{ijklm}.$

where in models 1 and 2:

Y_{ijklm} = dependent variables.

μ = theoretical population mean.

t_i = effect of treatment, i = 1-4.

p_j = effect of row of Latin square, j = 1-4.

a_k = effect of animal, k = 1-4.

r_l = effect of replication of Latin square design trial, l = 1, 2.

$(d_m p_j)$ = effect of sampling day within row of Latin square design trial, m = 1-3.

e_{ijklm} = random error.

and in model 1:

A_{ijkl} = error A $(t_i * p_j * a_k * r_l)$.

To conduct the split plot analysis of variance, the mean squares for treatment, row of Latin square, animal and replication of Latin square were taken from model 2 and tested against the mean squares of error A from model 1. The mean squares of sampling day within row of Latin square were taken from model 1 and tested against the mean squares of the residual error of model 1. If differences were determined by F test, means were separated using the Student-Newman-Keuls test.

Trial 2. Data obtained during the adaptation and finishing phases of this trial were analyzed separately. Analysis of variance (Barr et al., 1976) was used on data obtained within each phase of the trial to determine if any rumen variables were significantly affected by the independent variables. Data obtained during the 21 day adaptation phase were analyzed statistically as a split plot in time, due to the successive observations made on the same whole unit over a period of time. The two models used were:

(1) $Y_{ijk} = \mu + t_i + (a_j t_i) + d_k + t_i * d_k + e_{ijk}$.

$$(2)\quad Y_{ij} = \mu + t_i + d_j + t_i * d_j + e_{ij}.$$

where in model 1:

Y_{ijk} = dependent variables.

μ = theoretical population mean.

t_i = effect of treatment, i = 1-4.

$(a_j t_i)$ = effect of animal within treatment, j = 1-12.

d_k = effect of day, k = 1, 2.

e_{ijk} = random error.

where in model 2:

Y_{ij} = dependent variables.

μ = theoretical population mean.

t_i = effect of treatment, i = 1-4.

d_j = effect of day, j = 1, 2.

e_{ij} = random error.

To conduct the split plot analysis of variance, the mean squares of treatment were taken from model 2 and tested against the mean squares of animal within treatment from model 1. The mean squares of sampling day and treatment*sampling day were taken from model 1 and tested against the residual mean squares from model 1. If differences were determined by F test, means were separated using the Student-Newman-Keul test.

Analysis of variance (Barr et al., 1976) was conducted on data collected during the second phase of trial 2 by the model:

$$Y_{ij} = \mu + t_i + e_{ij}.$$

where: Y_{ij} = dependent variables.

μ = theoretical population mean.

t_i = effect of treatment, i = 1-4.

e_{ij} = random error.

If differences were determined by F test, means were separated using the Student-Newman-Keuls test.

Data obtained from rumen samples taken on days 7, 21 and 71 of trial 2 were used to graphically illustrate the changes occuring in rumen fluid pH, volatile fatty acid and ciliate protozoa concentrations between rations and between days of the trial. Means were calculated and used to construct three-dimensional histograms.

CHAPTER IV

RESULTS AND DISCUSSION

Trial 1

Adaptation phase. Rumen fluid pH and volatile fatty acid concentrations measured during the adaptation phase are presented in table 3 and mean squares from the analysis of variance of these data are presented in table A1. As shown in table 3, rumen fluid pH, initially at 6.85, did not change significantly until day 15 of the adaptation phase, when a value of 6.28 was noted. The binomial regression of rumen fluid pH on day of adaptation is presented in figure 1 and regression coefficients from which the curve was plotted are listed in table A3. Other researchers (Hungate et al., 1952; Reid et al., 1957; Krogh, 1961; Raun et al., 1962a) have reported that ruminal pH decreased as the level of starch in the diet increased. In this study, it is likely that the presence of hay in the diet through day 14 of the adaptation phase supported high ruminal pH levels through the period. This hypothesis is supported by the findings of other researchers (McDougall, 1948; Clark and Weiss, 1952; Bailey, 1961), who noted that ruminal pH was influenced by the physical character of the ration, where rations requiring much mastication and rumination supported a high ruminal pH by maintaining a copious flow of saliva to the rumen.

Total ruminal volatile fatty acid (VFA) concentration (table 3) did not change significantly during the adaptation phase ($P > .05$),

TABLE 3

MEANS BY DAY FOR RUMINAL pH AND VFA LEVELS--
ADAPTATION PHASE, TRIAL 1

Variable	Day of Adaptation Phase					
	0	3	6	9	12	15
pH	6.85[a]	6.83[a]	6.75[a]	6.70[a]	6.78[a]	6.28[b]
Total VFA (μm/ml)	85.7	69.8	107.9	70.7	83.7	81.9
Acetate, %	73.6[a]	61.9[ab]	64.0[ab]	54.8[ab]	49.7[b]	59.9[ab]
Propionate, %	14.7[a]	15.0[a]	12.6[a]	12.9[a]	23.9[b]	12.7[a]
Isobutyrate, %	.8	.6	.8	.8	1.1	1.1
Butyrate, %	9.4[a]	19.9[ab]	20.3[ab]	29.7[b]	18.8[ab]	21.2[ab]
Isovalerate, %	1.4	2.5	1.8	1.5	3.8	1.8
Valerate, %	.2[ab]	.0[ab]	.5[a]	.4[ab]	2.7	1.4[ab]
AC:PR Ratio	5.02	4.14	5.15	4.24	2.13	5.47

[a], [b]Values within a row not sharing a common superscript are significantly different, $P < .05$.

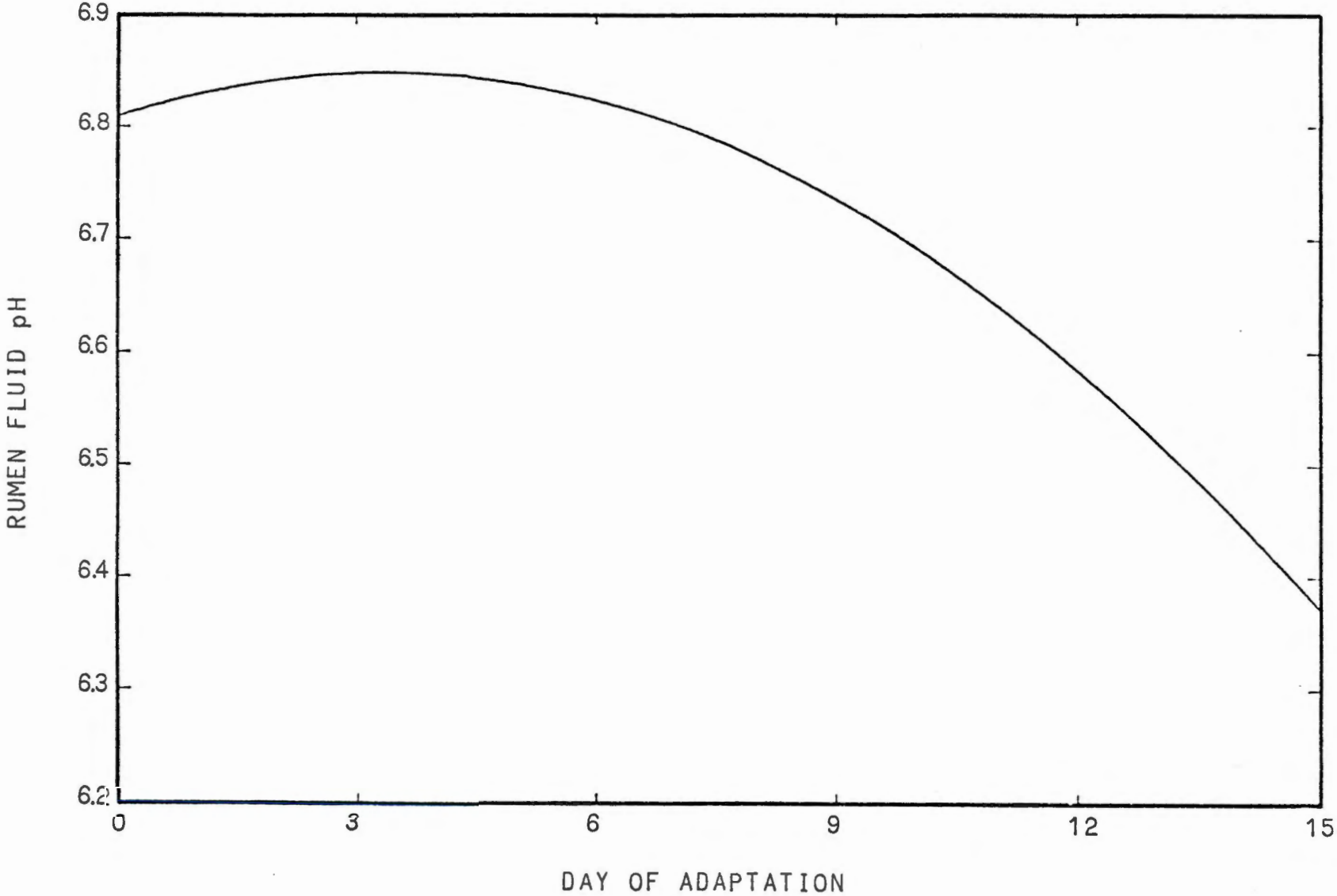

Figure 1. Binomial regression curve for rumen fluid pH -- Adaptation phase, Trial 1.

which is in contrast to the reports of Raun et al. (1962a), Wise et al. (1968) and Mackie et al. (1978), which indicated that total volatile fatty acid concentration increased as levels of dietary starch increased.

Molar proportions of individual volatile fatty acids changed ($P < .05$) during the adaptation phase. Molar percent acetate, initially at 73.6, tended to decrease throughout the period, but no significant change occurred until day 12, when acetate proportions decreased ($P < .05$) to 49.7 percent of the total volatile acids. Raun et al. (1962a), Wise et al. (1968) and Mackie et al. (1978) also reported that ruminal acetate levels decreased as levels of dietary starch increased.

In the rumens of steers consuming forage (day 0), propionate comprised 14.7 percent of the total volatile fatty acid. As steers were adapting to the whole shelled corn ration, no significant changes ($P > .05$) in propionate proportions were immediately noted, however, on day 12 of the adaptation phase, molar percent propionate increased ($P < .05$) to 23.9. This propionate increase is in agreement with reports of Annison and Lewis (1962), Raun et al. (1962a), Blaxter (1962) and Hungate (1966), which indicated that ruminal propionate levels were higher in animals consuming grain rations than in animals consuming forage rations. In this study, the molar proportion of propionate unexplainably decreased ($P < .05$) to 12.7 percent on day 15 of the adaptation phase.

Observations made during the adaptation phase revealed that, beginning on day 3, butyrate proportions increased, however, a significant increase did not occur until day 9, when butyrate reached a level of 29.7 molar percent. Butyrate levels remained abnormally high

through the remainder of the adaptation phase. Allison et al. (1964) reported that in sheep consuming large quantities of grain, the molar proportions of butyrate reached 40-45 percent of the total ruminal volatile fatty acids. They attributed these abnormally high levels of butyrate to the proliferation of rumen microorganisms similar to or identical with Peptostreptococci elsdenii, which had previously been shown to produce butyrate in large quantities (Elsden et al., 1956). Eadie and Mann (1970) observed high levels of butyrate in the rumen of sheep fed barley ad libitum. They suggested that high concentrations of protozoa in the rumen of these animals were directly responsible for the increased butyrate levels. Reid et al. (1957) reported that levels of butyrate in the rumen of sheep decreased as levels of dietary starch increased; however, this observation was made after ruminal conditions had stablized.

Binomial regressions of total volatile fatty acid, acetate, propionate and butyrate concentrations on day of adaptation are illustrated in figure 2. The regression coefficients for these variables are listed in table A3. As shown in figure 2, a concomitant decrease in acetate and increase in butyrate concentrations allowed the total volatile fatty acid concentration to remain stable during the adaptation phase.

Molar percent valerate (table 3) fluctuated only slightly during the adaptation phase, with a maximum value of 2.7 occuring on day 12. Isobutyrate and isovalerate levels did not change ($P > .05$) during the adaptation phase, nor did the ratio of acetate to propionate (AC:PR).

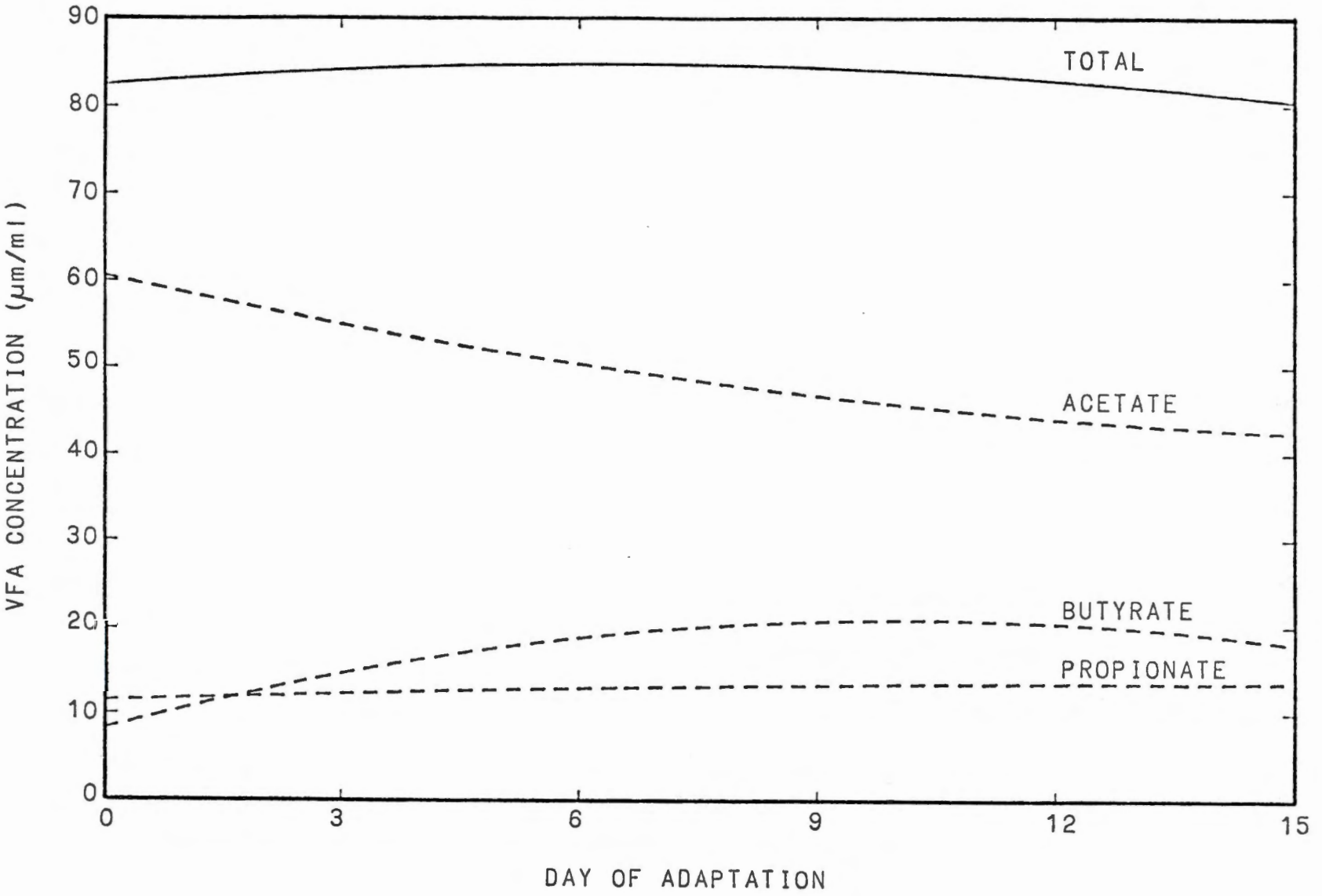

Figure 2. Binomial regression curves for ruminal VFA concentrations-- Adaptation phase, Trial 1.

Davis et al. (1957) indicated that in cattle, the adjustment of the rumen to varying levels of dietary protein was not complete at 13 days and that a period of 20 days was required for ruminal conditions to stabilize. In this study, it appears that ruminal conditions had not stabilized by day 15 of the adaptation phase.

Ruminal pH and protozoa concentrations measured on rumen samples taken during the adaptation phase and the first 14 days of the switchover phase are presented in table 4. Mean squares from the analysis of variance performed on these data are presented in table A2. As shown in table 4, total protozoa concentrations averaged 1.71×10^5/ml of rumen fluid as steers were consuming all-forage rations (day 0). By day 6 of the adaptation phase, protozoa numbers had increased ($P < .05$) to 7.24×10^5/ml and remained significantly higher on day 9, after which concentrations decreased until all steers were defaunated by day 14 of the switchover phase and remained so throughout the remainder of the trial.

Data reported by Warner (1964a) indicated that when sheep were fed increasing amounts of starch, ruminal protozoa concentrations initially increased as a result of the abundant supply of readily fermentable carbohydrates in the rumen. However, he noted that the numbers of protozoa decreased as the amount of dietary starch was further increased, presumably as a result of the low ruminal pH associated with high levels of dietary starch.

In this study, it should be noted that protozoa numbers decreased as rumen fluid pH decreased (table 4), and these steers were not defaunated until ruminal pH reached 5.93 on day 14 of the switchover phase.

TABLE 4

MEANS BY DAY FOR RUMINAL pH AND PROTOZOA CONCENTRATIONS--
ADAPTATION AND SWITCHOVER PHASE, TRIAL 1

Variable[a]	Day of Adaptation Phase						Day of Switchover Phase		
	0	3	6	9	12	15	5	11	14
pH	6.85^{b}	6.83^{b}	6.75^{b}	6.70^{b}	6.78^{b}	6.28^{c}	6.27^{c}	6.20^{c}	5.93^{c}
Total Protozoa	1.71^{b}	1.73^{b}	7.24^{c}	6.02^{c}	1.84^{b}	1.92^{b}	1.68^{b}	1.50^{b}	$.00^{b}$
Epidinium	.11	.03	.14	.13	.03	.02	.03	.26	.00
Entodinium	1.18^{bce}	1.53^{bce}	6.32^{d}	5.46^{ad}	1.79^{be}	1.89^{be}	1.65^{be}	1.24^{bce}	$.00^{e}$
Diplodinium	$.23^{bcd}$	$.14^{bcd}$	$.56^{b}$	$.36^{bcd}$	$.02^{cd}$	$.01^{cd}$	$.00^{cd}$	.00	.00
Dasytrich	$.12^{b}$	$.04^{cd}$	$.10^{b}$	$.07^{bde}$	$.00^{cd}$	$.00^{c}$	.00	.00	.00
Isotrich	$.02^{b}$	$.00^{c}$	$.12^{d}$	$.00^{c}$	.00	.00	.00	.00	.00
Charon	$.06^{b}$	$.00^{c}$	.00	.00	.00	.00	.00	.00	.00

[a]All protozoa values expressed as number x 10^{5}/ml rumen fluid.

b, c, d, e Values within a row not sharing a common superscript are significantly different, $P < .05$.

Krogh (1959), Eadie and Mann (1970) and Abe et al. (1973) reported that when ruminal pH dropped below 5.5, a marked decrease in protozoa numbers occured. Oxford (1955) and Purser and Moir (1959) indicated that ciliate protozoa could not survive when ruminal pH was below 6.0.

Differential protozoa counts performed on rumen samples taken during the adaptation phase and first 14 days of the switchover phase (table 4) revealed that concentrations of Epidinium, Dasytrich, Isotrich and Charon genera were initially low and remained so throughout the period. However, the numbers of Entodinium were initially high (1.18×10^5/ml) and increased further ($P < .05$) to 6.32×10^5/ml on day 6 of the adaptation phase and remained significantly higher on day 9, at a concentration of 5.46×10^5/ml rumen fluid. Throughout the remainder of the adaptation phase and during the first 11 days of the switchover phase, the numbers of Entodinium gradually decreased, until none were found on day 14 of the switchover phase. Oxford (1955) found that in animals consuming rations high in starch, Entodinium were virtually the only genus of protozoa present in the rumen. Observations made during this study support his findings.

Diplodinium numbers did not increase ($P > .05$) during the adaptation phase. However, a significant decrease was noted on day 12 of the adaptation phase, after which numbers decreased to zero.

Linear regressions of ruminal protozoa concentrations on day of adaptation are illustrated in figures 3 and 4 and regression coefficients from which these lines were plotted are listed in table A3.

Mackie et al. (1978) reported that numbers of rumen protozoa decreased by 50 to 80 percent within 7 days as sheep were adapted from

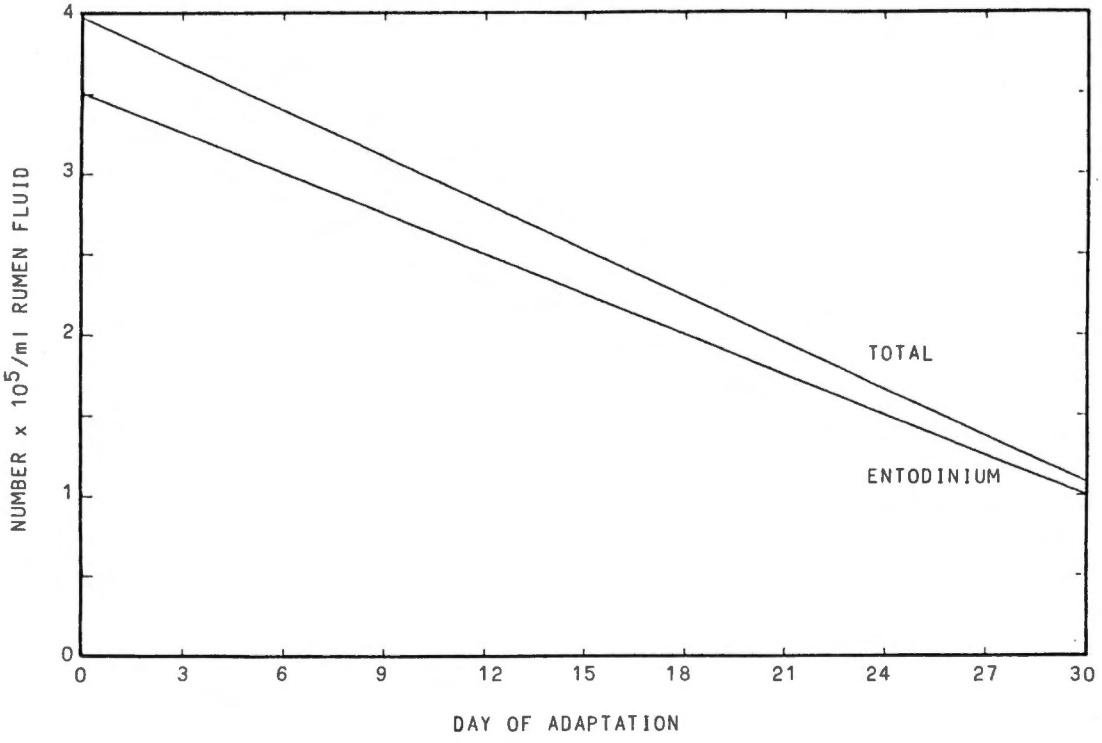

Figure 3. Linear regression curves for total and Entodinium protozoa concentrations-- Adaptation phase, Trial 1.

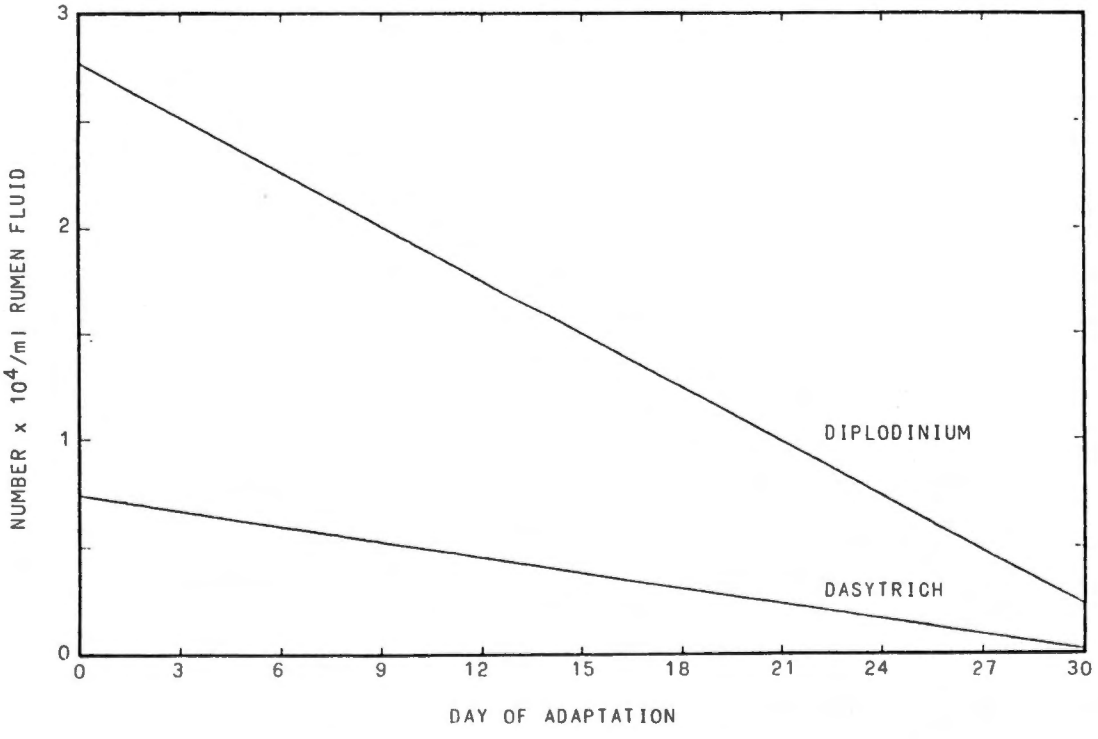

Figure 4. Linear regression curves for Diplodinium and Dasytrich protozoa concentrations-- Adaptation phase, Trial 1.

a forage ration to a ground corn ration. However, in this study, 29 days were required for protozoa numbers to decrease by more than 50 percent. It is likely that the relatively high ruminal pH levels supported through day 11 of the switchover phase were responsible for the delayed reduction in protozoa numbers.

Switchover phase. Means by ration for rumen fluid pH and volatile fatty acid concentrations pooled across both Latin square design trials of the switchover phase are listed in table 5. Mean squares from the split plot analysis of variance of these data are listed in table A4. As shown in table 5, monensin had no effect ($P > .05$) on ruminal pH, total volatile fatty acid concentration or molar proportions of volatile fatty acids during the switchover phase. Previous reports by Richardson et al. (1976), Dinius et al. (1976), Raun et al. (1976), Mowat et al. (1977) and Boling et al. (1977) indicated that monensin feeding resulted in a decreased concentration of acetate and an increased concentration of propionate in the rumen of cattle. Although in this study, monensin did not support a significant shift in ruminal acetate or propionate levels, there was a trend toward a lower molar percent acetate (43.8 vs. 46.5) and a higher molar percent propionate (42.8 vs. 39.4) in the rumen of monensin-fed steers compared to those not fed monensin, respectively. It is likely that the shift in ruminal acetate and propionate levels normally supported by monensin may have been eclipsed by the drastic shift in acetate and propionate levels already supported by the whole shelled corn rations used in this study. Gill et al. (1977) found

TABLE 5

MEANS BY RATION FOR RUMINAL pH AND VFA LEVELS--
SWITCHOVER PHASE, TRIAL 1

Variable[b]	Ration[a] Supplements			
	Commercial Protein[c]	Commercial Protein + Monensin	Soybean Meal	Soybean Meal + Monensin
pH	5.96	5.99	5.99	6.03
Total VFA (μm/ml)	130.0	130.0	125.0	121.0
Acetate, %	46.7	43.7	46.2	43.8
Propionate, %	39.8	44.0	39.0	41.5
Isobutyrate, %	.6	.5	.5	.5
Butyrate, %	8.2	7.5	9.0	8.6
Isovalerate, %	.5	.6	.4	.4
Valerate, %	4.1	3.6	4.4	5.3
AC:PR Ratio	1.36	1.12	1.55	1.13

[a]Basal rations consisted of whole shelled corn, fed ad libitum.

[b]No significant differences were noted among variables between rations, $P > .05$.

[c]Tend-R-Leen[R].

that the addition of monensin to whole shelled corn rations resulted in only subtle changes in the molar proportions of acetate and propionate in the rumen of cattle.

Reports have indicated that varying levels of dietary protein influenced ruminal concentrations of acetate, butyrate, isobutyrate, valerate and isovalerate in cattle (El-Shazly, 1952; Davis et al., 1957; Haskins et al., 1967; Mahmoud, 1977). However, no reports have been published regarding the influence of protein supplement type on ruminal volatile fatty acid concentrations. An objective of this study was to determine if differences existed in ruminal volatile fatty acid concentrations in steers consuming whole shelled corn rations supplemented with soybean meal or a commercial protein supplement[4]. Data presented in table 5 indicate that protein supplement type did not affect ($P > .05$) ruminal pH, total volatile fatty acid concentration or molar proportions of volatile fatty acids.

Shown in table 6 are ration means for rumen fluid pH and volatile fatty acid levels measured within each Latin square design trial of the switchover phase. The only variable affected by ration was molar percent valerate, which, during the first trial of the switchover phase, was higher ($P < .05$) in rumen samples taken from the steer consuming the soybean meal + monensin-supplemented ration (7.8) than

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[4]The commercial protein supplement used was Tend-R-Leen^{R}, hereafter referred to as TRL, a 36 percent crude protein pelleted supplement franchised by Domain Industries, New Richmond, Wisconsin and manufactured by the Tennessee Farmers Co-op, Lavergne, Tennessee. See table A5 for composition.

TABLE 6

MEANS BY RATION FOR RUMINAL pH AND VFA LEVELS MEASURED WITHIN LATIN SQUARE DESIGN TRIAL--SWITCHOVER PHASE, TRIAL 1

	First Latin Square Design Trial				Second Latin Square Design Trial			
	Ration[a] Supplements				Ration[a] Supplements			
Variable	Commercial Protein	Commercial Protein + Monensin	Soybean Meal	Soybean Meal + Monensin	Commercial Protein	Commercial Protein + Monensin	Soybean Meal	Soybean Meal + Monensin
pH	6.02	5.97	5.94	6.05	5.90	6.02	6.03	6.02
Total VFA (μm/ml)	125.0	139.0	132.0	127.0	134.0	120.0	117.0	116.0
Acetate, %	49.0	43.3	46.8	43.1	44.7	44.2	45.6	44.4
Propionate, %	30.6[b]	41.5	32.9[b]	34.9[b]	48.2[c]	46.6	45.1[c]	47.6[c]
Isobutyrate, %	.5	.6	.5	.4	.6	.5	.4	.5
Butyrate, %	13.2[b]	9.9	13.1[b]	13.4[b]	3.6[c]	5.1	4.9[c]	4.2[c]
Isovalerate, %	1.0	.8	.5	.4	.2	.5	.4	.4
Valerate, %	5.8[bg]	4.0[g]	5.2[g]	7.8[bh]	2.6[c]	3.1	3.7	3.0[c]
AC:PR Ratio	1.82	1.27	2.06[b]	1.31	.94	.96	1.04[c]	.97

[a]Basal rations consisted of whole shelled corn, fed ab libitum.

[b, c]Ration means between trials not sharing a common superscript are significantly different, $P < .05$.

[g, h]Ration means within each trial not sharing a common superscript are significantly different, $P < .05$.

in the steers consuming the other rations (5.8, 5.2, 4.0).

In the first trial of the switchover phase, the rations containing monensin tended to support a lower molar percent acetate (44.3, 43.1) than did the rations not containing monensin (49.0, 46.8); however, these differences were not significant ($P > .05$). In the second trial of the switchover phase, differences in acetate levels between rations were much less pronounced, with monensin-supplemented rations supporting acetate levels of 44.2 percent and 44.4 percent and non-supplemented rations supporting levels of 44.7 percent and 45.6 percent.

In the first trial of the switchover phase, the ration containing TRL + monensin tended to support a higher propionate level (41.5 molar percent) than the other rations (30.6, 32.9, 34.9 molar percent), however, this difference was not significant ($P > .05$). During the second trial of the switchover phase, all rations supported similar levels of propionate (48.2, 46.6, 45.1, 47.6 molar percent). In comparing ration means between trials, the only ration which did not support higher ($P < .05$) ruminal propionate levels in the second trial than in the first was the one supplemented with TRL + monensin (41.5 vs. 46.6 molar percent).

Molar percent butyrate in the rumens of these steers was not effected ($P > .05$) by ration within either trial of the switchover phase; however, differences in butyrate levels did occur between trials. The only ration which did not support higher ($P < .05$) molar proportions of butyrate in the first trial than in the second trial was the TRL + monensin-supplemented ration (9.9 vs. 5.1).

During the first trial of the switchover phase, the whole shelled corn ration supplemented with soybean meal + monensin supported a higher ($P < .05$) molar percent valerate than the other rations (7.8 vs. 5.8, 5.2, 4.0). In comparing ration means across both trials of the switchover phase, the whole shelled corn rations supplemented with TRL or soybean meal + monensin supported higher ($P < .05$) molar proportions of valerate during the first trial of the switchover phase (5.8, 7.8, respectively) than during the second trial (2.6, 3.0, respectively).

During the first Latin square design trial, rations containing monensin tended to support lower ratios of acetate to propionate (AC:PR) than rations not containing monensin (1.27, 1.31 vs. 1.82, 2.06, respectively), however, these differences were not significant ($P > .05$). During the second Latin square design trial, all rations supported similar acetate to propionate ratios (.94, .96, 1.04, .97). In comparing ration means between trials of the switchover phase, the rations not containing monensin tended to support higher acetate to propionate ratios in the first trial (1.82, 2.06) than in the second trial (.94, 1.04), while the difference in acetate to propionate ratios supported by rations containing monensin was less pronounced (1.27, 1.31 vs. .96, .97). However, the only ration which supported a significantly different acetate to propionate ratio between trials was the one which contained soybean meal (2.06 vs. 1.04).

Presented in table 7 are means by Latin square design trial for rumen variables measured during the switchover phase. Ruminal pH, total volatile fatty acid concentration and molar percents acetate, isobutyrate and isovalerate did not change ($P > .05$) during the switchover phase.

TABLE 7

MEANS BY LATIN SQUARE DESIGN TRIAL FOR RUMINAL pH AND VFA LEVELS--SWITCHOVER PHASE, TRIAL 1

Variable	Latin Square Design Trial 1	Latin Square Design Trial 2
pH	5.99	5.99
Total VFA (μm/ml)	131.1	121.7
Acetate, %	45.5	44.7
Propionate, %	35.1[a]	46.9[b]
Isobutyrate, %	.5	.5
Butyrate, %	12.4[a]	4.4[b]
Isovalerate, %	.7	.3
Valerate, %	5.6[a]	3.1[b]
AC:PR Ratio	1.62[c]	.98[d]

[a], [b]Values within a row not sharing a common superscript are significantly different, $P < .01$.

[c], [d]Values within a row not sharing a common superscript are significantly different, $P < .05$.

However, molar percent propionate was higher ($P < .05$) during the second trial (46.9) than during the first trial (35.1), which resulted in a reduction ($P < .05$) in the ratio of acetate to propionate during the second trial. In the first trial of the switchover phase, molar percents butyrate and valerate were significantly higher (12.4, 5.6, respectively) than during the second trial (4.4, 3.1, respectively).

Data presented in table 7 reveal that, between the two trials of the switchover phase, differences in molar proportions of ruminal volatile fatty acids centered around propionate, butyrate and valerate, with acetate and isoacid levels remaining stable during the entire switchover phase. This alteration in volatile fatty acid levels indicates that ruminal conditions were still changing during the first trial of the switchover phase as a result of the previous ration change which occurred during the adaptation phase.

Trial 2

Adaptation phase. Means by day for rumen fluid pH, total volatile fatty acid concentration and molar percent volatile fatty acids measured during the adaptation phase are presented in table 8 and mean squares from the split plot analysis of variance of these data are listed in table A5. On day 0 of the adaptation phase, steers were grazing pasture, on day 7, steers were consuming an approximately 50 percent forage - 50 percent grain ration, and by day 21, steers had been consuming their respective corn - wheat, forage-free rations for 7 days.

TABLE 8

MEANS BY DAY FOR RUMINAL pH AND VFA LEVELS--
ADAPTATION PHASE, TRIAL 2

Variable	Day of Adaptation Phase		
	0[a]	7	21
pH	6.77[b]	5.90[c]	5.67[d]
Total VFA (μm/ml)	70.6[b]	115.5[c]	167.1[d]
Acetate, %	73.8[b]	46.1[c]	43.9[d]
Propionate, %	15.4[b]	38.7[c]	45.4[d]
Isobutyrate, %	1.0[b]	1.3[c]	1.0[b]
Butyrate, %	8.3[bc]	9.2[b]	6.9[c]
Isovalerate, %	1.2	1.3	.8
Valerate, %	.4	3.2	2.7
AC:PR Ratio	4.83[b]	1.29[c]	.99[d]

[a]Observations made on day 0 were not included in the analysis of variance.

[b, c, d]Values within a row not sharing a common superscript are significantly different, $P < .05$.

Rumen fluid samples taken from steers consuming their respective corn - wheat rations (table 8) indicate that ruminal pH, initially at 6.77, decreased ($P < .05$) by day 7 to 5.90. By day 21, rumen fluid pH had decreased further ($P < .05$) to 5.67. It should also be noted that the decrease in ruminal pH during the adaptation phase of this trial was more abrupt than that during the adaptation phase of trial 1. This could be attributed to the dissimilar conditions under which the animals were confined.

Total volatile fatty acid concentration, initially at 70.6 μm/ml in animals grazing pasture, increased ($P < .05$) on day 7 to 115.5 μm/ml and increased further ($P < .05$) on day 21 to 167.1 μm/ml. Wise et al. (1968), Raun et al. (1962a) and Mackie et al. (1978) found that total volatile fatty acid concentration increased as the level of starch in the diets of cattle and sheep increased.

As shown in table 8, molar percent acetate decreased ($P < .05$) from 73.8 on day 0 to 46.1 on day 7 and then decreased further ($P < .05$) to 43.9 on day 21 of the adaptation phase. This is in agreement with data presented in the adaptation phase of trial 1. Raun et al. (1962), Wise et al. (1968) and Mackie et al. (1978) indicated that molar percent acetate decreased in the rumen of cattle and sheep as they were adapted to rations containing high levels of grain.

Ruminal propionate levels increased ($P < .05$) from 15.4 molar percent on day 0 to 38.7 molar percent on day 7. After the steers had been consuming their respective all-grain, forage-free rations for 7 days (day 21), propionate levels increased ($P < .05$) to 45.4 molar percent. However, this is not in agreement with data presented in the

adaptation phase of trial 1, where molar percent propionate increased only slightly. Wise et al. (1968), Raun et al. (1962a), Reid et al. (1957) and Hungate (1966) indicated that ruminal propionate levels were higher in steers consuming rations high in starch, compared with those in steers consuming rations high in forage. The ratio of acetate to propionate (AC:PR), initially at 4.83 on day 0, decreased ($P < .05$) to 1.29 by day 7 and then decreased further ($P < .05$) by day 21 to .99.

Molar percent butyrate averaged 8.3 on day 0 of the adaptation phase and did not increase ($P > .05$) during the period; however, butyrate levels decreased ($P < .05$) between day 7 (9.2 molar percent) and day 21 (6.9 molar percent). This is in contrast to data reported by Raun et al. (1962a), Allison et al. (1964) and Eadie and Mann (1970), who reported that high levels of dietary starch initially supported relatively high concentrations of butyrate in the rumen. Likewise, data presented in the adaptation phase of trial 1 indicated that butyrate levels increased significantly as steers were fed increasing quantities of grain.

Molar proportions of isobutyrate in the rumens of these steers increased ($P < .05$) on day 7 (1.3 percent) and then decreased ($P < .05$) by day 21 (1.0 percent). Molar percent isovalerate did not change ($P > .05$) during the adaptation phase, with values remaining near 1.0. Molar percent valerate did not change ($P > .05$), however, values tended to increase between day 0 (.4 percent) and day 7 (3.2 percent).

Means by day for ruminal pH and protozoa concentrations measured during the adaptation phase are presented in table 9 and mean squares from the split plot analysis of variance of these data are listed in

TABLE 9

MEANS BY DAY FOR RUMINAL pH AND PROTOZOA CONCENTRATIONS--
ADAPTATION PHASE, TRIAL 2

Variable[ab]	Day of Adaptation Phase		
	0[c]	7	21
pH	6.77[d]	5.90[e]	5.67[f]
Total Protozoa	1.43[d]	1.50[d]	.64[e]
Epidinium	.02	.00	.00
Entodinium	1.16	1.48	.63
Diplodinium	.05	.01	.01
Dasytrich	.20[d]	.01[e]	.00[f]

[a]All values expressed as number x 10^5/ml rumen fluid.

[b]Isotrich and Charon were absent from all samples.

[c]Observations made on day 0 were not included in the analysis of variance.

[d, e, f]Values within a row not sharing a common superscript are significantly different, $P < .05$.

table A6. Differential protozoa counts revealed that concentrations of Epidinium and Diplodinium genera were initially low (.02 and .05 x 10^5/ml rumen fluid, respectively) and remained so throughout the adaptation phase.

Concentrations of Entodinium protozoa averaged 1.16 x 10^5/ml rumen fluid on day 0 and increased ($P > .05$) by day 7 to 1.48 x 10^5/ml. By day 21 of the adaptation phase, Entodinium concentrations averaged .63 x 10^5/ml rumen fluid. It should be noted that the Entodinium genus predominated in the rumens of these steers during the adaptation phase. Oxford (1955) and Hungate (1966) reported that the Entodinium genus predominated in the rumens of animals consuming high-starch rations.

Total protozoa concentrations in these steers did not change ($P > .05$) between days 0 and 7 (1.43-1.50 x 10^5/ml, respectively), however, 12 steers were defaunated during this period. These observations are partially in contrast to the report of Warner (1964a), who noted that as sheep consumed moderate levels of starch, ruminal protozoa concentrations initially increased. It should also be noted that a significant increase occured in total protozoa concentrations during the adaptation phase of trial 1, which is in contrast to data presented in this trial. By day 21 of the adaptation phase of this trial, 40 of the 47 steers were defaunated, but protozoa concentrations in the remaining seven steers were relatively high (4.3 x 10^5/ml). Warner (1964a) noted that the concentration of rumen protozoa decreased when sheep consumed rations composed solely of starch. Data published by Oxford (1955) and Purser and Moir (1959) indicated that ciliate protozoa could not survive when ruminal pH was at 6.0 for a considerable length

of time. In this trial, when rumen fluid pH averaged 5.67, 85 percent of all steers were defaunated.

Numbers of Dasytrich protozoa, initially at .20 x 10^5/ml rumen fluid, decreased ($P < .05$) by day 7 to .01 x 10^5/ml. By day 21 of the adaptation phase, no Dasytrichs were found in the rumen fluid of these steers.

Finishing phase. Means by ration for rumen fluid pH, total volatile fatty acid concentration and molar proportions of volatile fatty acids measured on day 50 of the finishing phase are presented in table 10 and mean squares from the analysis of variance of these data are listed in table A7.

As shown in table 10, the level of wheat in these rations did not effect ($P > .05$) rumen fluid pH (6.00, 6.13, 5.94, 6.22). This is in contrast to reports of Oltjen et al. (1966, 1967) and Slyter et al. (1970), who indicated that ruminal pH in cattle decreased as wheat replaced corn in finishing rations.

Total volatile fatty acid (VFA) concentration, although varying markedly, was not effected ($P > .05$) by the level of wheat in these rations (225.1, 188.1, 224.8, 186.5 μm/ml), nor were any consistent trends observable in an altered total acid concentration between rations. Oltjen et al. (1966, 1967) reported that total ruminal volatile fatty acid concentrations were higher in steers consuming rations predominantly composed of wheat compared to those in steers consuming rations predominantly composed of corn.

No data have been published regarding the effects of varying levels of whole wheat fed in combination with whole shelled corn upon

TABLE 10

MEANS BY RATION FOR RUMINAL pH AND VFA LEVELS--
FINISHING PHASE, TRIAL 2

Variable[a]	Basal Ration[b]			
	100% WSC	80% WSC 20% WW	60% WSC 40% WW	40% WSC 60% WW
pH	6.00	6.13	5.94	6.22
Total VFA (μm/ml)	225.1	188.1	224.8	186.5
Acetate, %	41.0	43.1	42.4	42.9
Propionate, %	47.4	43.5	44.7	42.5
Isobutyrate, %	1.3	1.3	1.2	1.4
Butyrate, %	7.3	8.9	10.4	10.4
Isovalerate, %	.9	1.1	1.0	1.2
Valerate, %	2.2	2.1	2.1	2.2
AC:PR Ratio	.87	1.01	.99	1.03

[a]No differences were noted among variables between rations, $P > .05$.

[b]All rations were topdressed daily with .5 kg Tend-R-Leen[R] protein supplement, containing monensin at 600 ppm, WSC = whole shelled corn, WW = whole wheat.

molar proportions of individual ruminal volatile fatty acids. In this study, varying levels of wheat in the rations did not effect ($P > .05$) the molar proportions of acetate, propionate, butyrate or isoacids in the rumen of these cattle.

Observations were made on protozoa concentrations in rumen fluid samples taken on day 50 of the finishing phase. Although ruminal pH averaged 6.07 on this day, 40 of the 47 steers were defaunated. Oxford (1955) and Eadie and Mann (1970) found that ciliate protozoa could survive in the rumen at a pH above 5.5. Therefore, it appears that in this study, protozoa populations were effected by some factor other than ruminal pH. Slyter et al. (1965) found that all-grain rations predominantly composed of wheat supported viable populations of ruminal protozoa, whereas all-grain rations composed predominantly of corn did not. However, Warner (1964a), Annison and Lewis (1962), Slyter at al. (1970), Hungate (1966) and Mackie et al. (1978) indicated that ruminal protozoa populations were low or absent in animals consuming rations high in starch, which is in agreement with data presented in this study.

Changes occuring in rumen fluid pH, total volatile fatty acid concentration and molar proportions of acetate, propionate and butyrate between the four rations and between days 7, 21 and 71 of this trial are graphically illustrated in figures 5, 6, 7, 8 and 9, respectively. Least-squares means from which these histograms were constructed are listed in table A9.

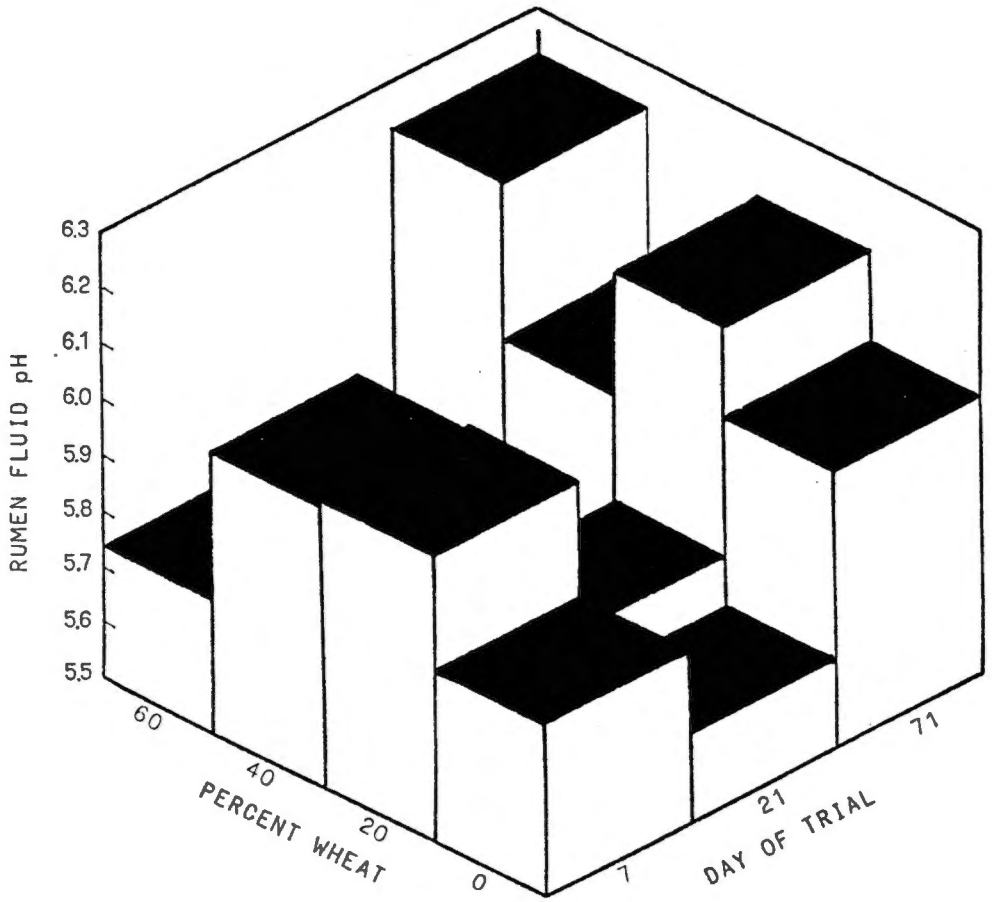

Figure 5. Histogram of rumen fluid pH vs. percent wheat vs. day of Trial 2.

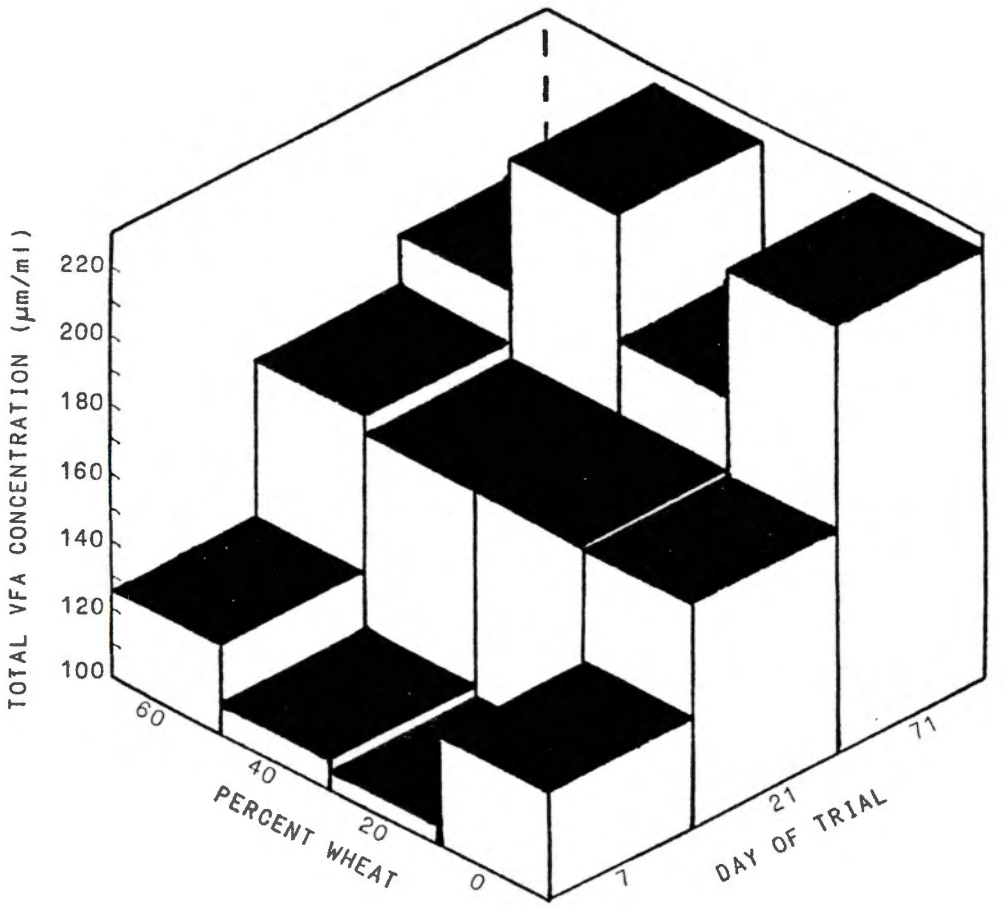

Figure 6. Histogram of total ruminal VFA concentration vs. percent wheat vs. day of Trial 2.

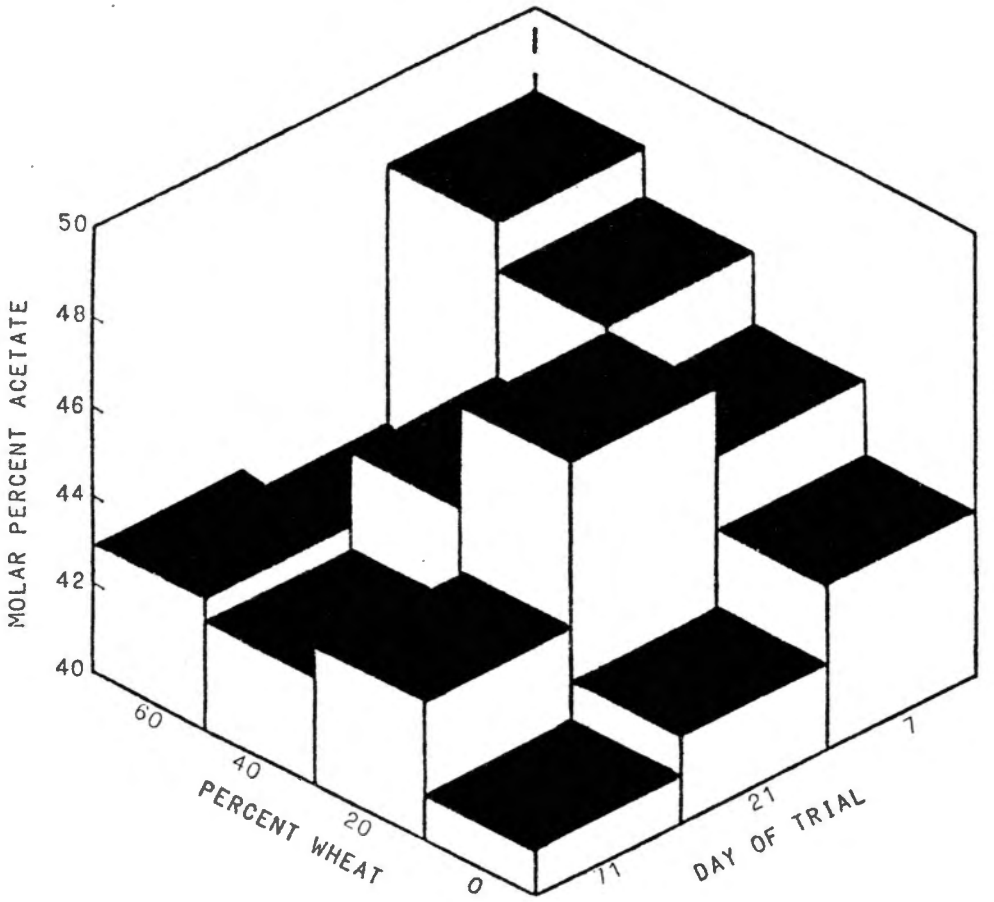

Figure 7. Histogram of molar percent ruminal acetate vs. percent wheat vs. day of Trial 2.

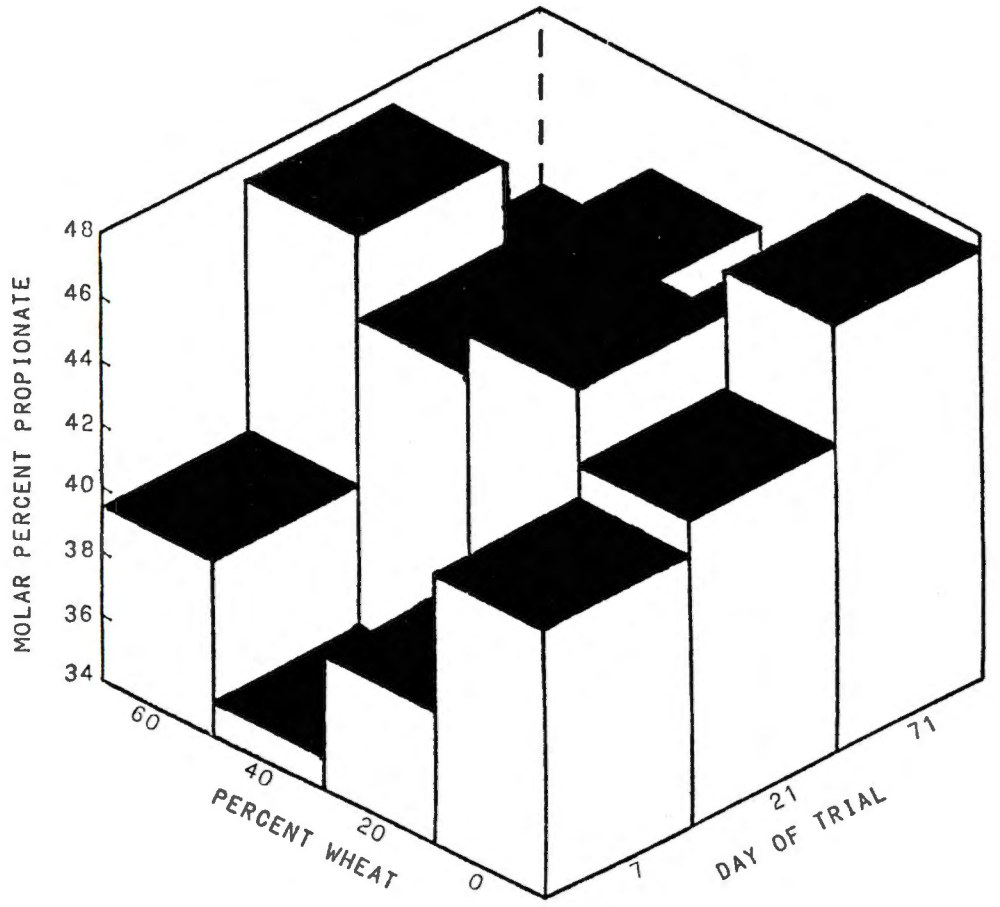

Figure 8. Histogram of molar percent ruminal propionate vs. percent wheat vs. day of Trial 2.

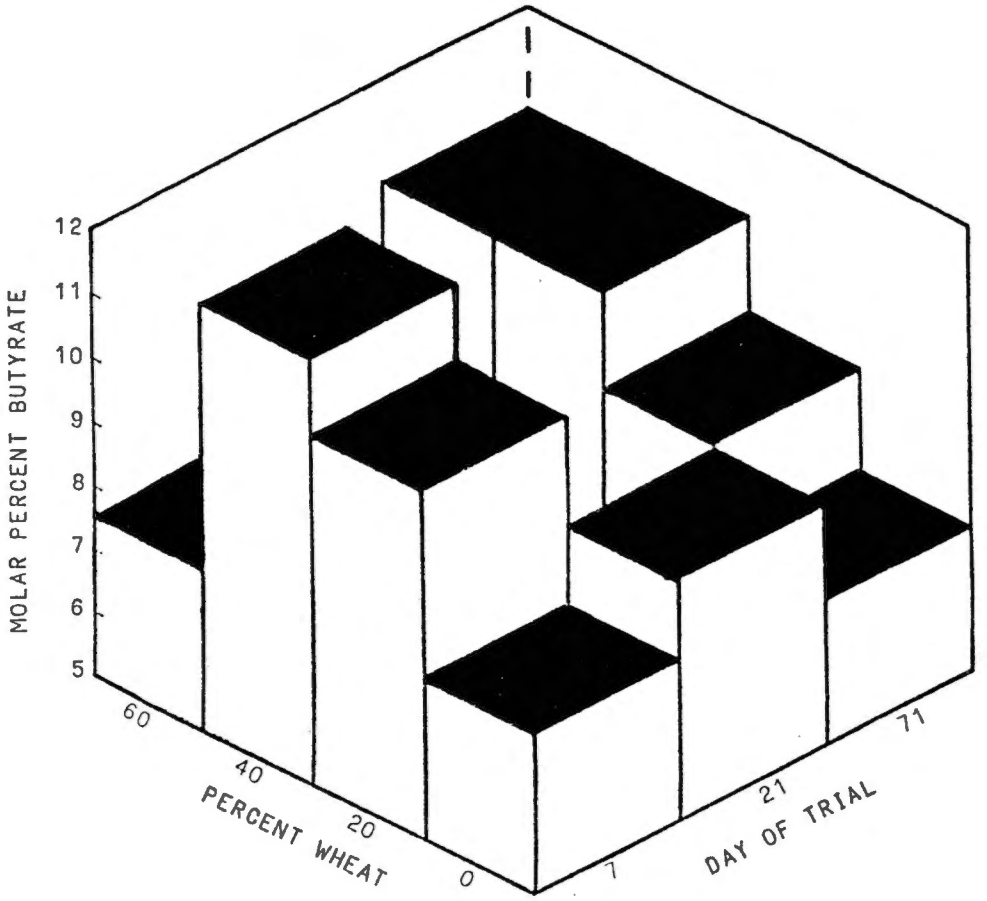

Figure 9. Histogram of molar percent ruminal butyrate vs. percent wheat vs. day of Trial 2.

CHAPTER V

SUMMARY AND CONCLUSIONS

The objectives of this study were to observe changes occuring in ruminal pH, volatile fatty acid and protozoa concentrations as steers in two separate trials were adapting from all-forage rations to all-grain rations and to observe the effects of monensin, protein supplement type and varying levels of whole wheat fed in combination with whole shelled corn upon these same rumen parameters in steers consuming all-grain rations. Trial 1 consisted of a 15 day adaptation phase and a 112 day switchover phase. Trial 2 consisted of a 21 day adaptation phase and a 50 day finishing phase.

During the adaptation phase of trial 1, rumen fluid samples were taken from four yearling steers on the day preceding the trial and at three day intervals during the trial as steers were gradually adapting from all-forage rations to all-grain rations composed of whole shelled corn supplemented with protein. During this phase, rumen fluid pH decreased ($P < .05$) by day 15. Total volatile fatty acid concentration did not change ($P > .05$) during the 15 day period, however, alterations in the molar proportions of the individual volatile fatty acids did occur. Molar percent acetate tended to decrease throughout the period, but this reduction was not significant until day 12. Molar percent propionate did not change until day 12 of the period, when an increase ($P < .05$) occurred. During the adaptation phase, molar percent butyrate increased ($P < .05$), molar percent

isoacids did not change ($P > .05$) and protozoa populations, composed primarily of Entodinium, initially increased ($P < .05$) and then decreased to zero after steers had consumed all-grain, forage-free rations for 14 days.

During the switchover phase of trial 1, the same steers were used in two successive, balanced four by four Latin square design trials. Within each Latin square design trial, each steer was fed each of the following rations for 14 days: (1) whole shelled corn (WSC) ad libitum plus .5 kg TRL/day, (2) WSC ad libitum plus .5 kg TRL/day plus 300 mg monensin/day, (3) WSC ad libitum plus .5 kg SBM/day, (4) WSC ad libitum plus .5 kg SBM/day plus 300 mg monensin/day. Rumen fluid samples were taken from each steer on days 5, 11 and 14 of each ration period. Monensin and protein supplement type did not support any significant ($P > .05$) changes in ruminal pH, volatile fatty acid or protozoa concentrations.

In trial 2 of this study, forty-seven yearling steers were taken off pasture and used in both phases of the trial. During the adaptation phase of the trial, steers were assigned to four pens and all steers in each pen were adapted to one of the following all-grain basal rations over a 21 day period: (1) 100 percent whole shelled corn (WSC), (2) 80 percent WSC-20 percent whole wheat (WW), (3) 60 percent WSC-40 percent WW, (4) 40 percent WSC-60 percent WW. All rations were topdressed with a commercial protein supplement (TRL) containing monensin. Rumen fluid samples were taken from each steer on the day preceding the trial and subsequently on days 7 and 21 of the adaptation phase. During the adaptation phase, rumen fluid pH, molar percent

acetate and molar percent butyrate decreased ($P < .05$), total volatile fatty acid concentration and molar percent propionate increased ($P < .05$), molar percents valerate and isoacids did not change ($P > .05$) and total protozoa concentrations, composed primarily of Entodidium, decreased ($P < .05$). No ration effects ($P > .05$) were observed during the adaptation phase.

During the finishing phase of trial 2, steers in each pen were fed the same respective proportions of whole shelled corn and whole wheat as they had received during the adaptation phase of the trial. No forage was fed during the finishing phase. On day 50 of the finishing phase, rumen fluid samples were taken from each steer. Rumen fluid pH, volatile fatty acid and protozoa concentrations were not effected ($P > .05$) by the level of wheat in these rations.

These data indicate that ruminal pH decreases, total volatile fatty acid concentration increases, molar percent acetate decreases, molar percent propionate increases and protozoa concentrations decrease as cattle are adapting from all-forage rations to all-grain rations of whole shelled corn or whole shelled corn and whole wheat. It appears that 14 to 21 days are required for ruminal conditions to stabilize when such a ration change occurs. The results of this study also indicate that the effects of monensin on ruminal acetate and propionate levels in steers consuming all-grain rations of whole shelled corn is not as marked as that reported in steers consuming forage or grain and forage mixed rations. It is likely that the shift in ruminal acetate and propionate levels normally supported by monensin may have been eclipsed by the drastic shift in acetate and propionate levels already

supported by the whole shelled corn rations used in this study. Finally, data indicate that the level of whole wheat fed in combination with whole shelled corn does not effect ruminal pH, volatile fatty acid or protozoa concentrations in steers.

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APPENDIX

APPENDIX

LIST OF SYMBOLS

TVFA = Total VFA Concentration (μm/ml)

AC = Acetate

PR = Propionate

IB = Isobutyrate

BU = Butyrate

IV = Isovalerate

VA = Valerate

AC:PR = Ratio of Acetate to Propionate

TABLE A1

MEAN SQUARES FOR MOLAR PERCENT VFA AND pH
DATA-- ADAPTATION PHASE, TRIAL 1

Source of Variation	Degrees of Freedom	Mean Squares								
		Total VFA, Molar Percent VFA, pH and AC:PR Ratio								
		Total VFA	AC%	PR%	IB%	BU%	IV%	VA%	pH	AC:PR
Animal	3	1258.0	2.7	.9	.2	13.7	.1	4.5	.1	.6
Day	5	761.7	266.6*	75.0**	.1	166.8*	3.3	4.3*	.2*	5.9
Error	15	809.8	87.6	7.7	.1	54.8	2.4	1.5	.04	2.3
Total	23									

*Indicates that resulting F value is significant, $P < .05$.

**Indicates that resulting F value is significant, $P < .01$.

TABLE A2

MEAN SQUARES FOR PROTOZOA CONCENTRATION DATA-- ADAPTATION AND SWITCHOVER PHASE, TRIAL 1

Source of Variation	Degrees of Freedom	Mean Squares						
		Total Protozoa and Differential Counts						
		Total	Isotrich	Charon	Epidinium	Entodinium	Diplodinium	Dasytrich
Animal	3	8.4×10^{10}	7.3×10^{5}	4.2×10^{6}	9.1×10^{8}	8.1×10^{10}	1.2×10^{9}	2.5×10^{7}
Day	11	1.9×10^{11}**	1.6×10^{6}**	1.0×10^{7}*	2.6×10^{8}	1.5×10^{11}**	1.2×10^{9}*	6.8×10^{7}***
Error	24	4.4×10^{10}	5.0×10^{5}	3.5×10^{6}	3.6×10^{8}	4.5×10^{10}	4.8×10^{8}	9.9×10^{6}
Total	38							

*Indicates that resulting F value is significant, $P < .05$.

**Indicates that resulting F value is significant, $P < .01$.

***Indicates that resulting F value is significant, $P < .001$.

TABLE A3

INTERCEPTS AND REGRESSION COEFFICIENTS FOR RUMINAL pH, MOLAR PERCENT VFA AND PROTOZOA CONCENTRATIONS[a] ON DAY OF ADAPTATION PHASE -- TRIAL 1

Variable	Intercept	B_1	B_2
pH	6.809821	.023541	-.003522
Total VFA	82.558285	.749571	-.059285
Acetate	60.625831	-2.028968	.054375
Propionate	11.476500	.308564	-.011512
Isobutyrate	.557143	.021929	-.000600
Butyrate	8.276250	2.481775	-.122292
Isovalerate	1.336607	.146839	-.006400
Valerate	.058393	.034361	.006065
AC:PR Ratio	5.203632	-.300228	-.017030
Total Protozoa	397228.630	-9625.757	
Isotrich	776.818	-26.958	
Charon	1564.666	-57.045	
Epidinium	9626.088	-122.369	
Entodinium	350422.521	-8339.104	
Diplodinium	27713.960	-846.910	
Dasytrich	7407.777	-240.529	

[a]As a result of the random variation in protozoa concentrations, quadratic regressions were not performed on these data.

B_1 = Linear regression coefficient for day.

B_2 = Quadratic regression coefficient for day.

TABLE A4

MEAN SQUARES FOR MOLAR PERCENT VFA AND pH
DATA-- SWITCHOVER PHASE, TRIAL 1

Source of Variation	Degrees of Freedom	Mean Squares								
		Total VFA, Molar Percent VFA, pH and AC:PR Ratio								
		TVFA	AC%	PR%	IB%	BU%	IV%	VA%	pH	AC:PR
Treatment	3	461.4	55.0	123.3	.05	9.9	.2	11.0	.02	1.0
Row of Latin Sq.	3	964.8	99.1	224.0	.14	44.9	2.7	9.3	.14	3.1
Animal	3	525.7	7.8	56.7	.05	12.7	.6	6.4	.26	.6
Replication of Trial	1	1931.0	16.7	3329.2***	.00	1478.7***	2.5	148.2***	.00	10.0*
Error A	21	1222.5	68.8	220.4	.13	46.1	1.3	11.7	.21	1.3
Day(Row)	8	975.5**	11.3	56.1	.03	16.1**	.4*	5.6***	.20	.2
Error B	54	361.3	16.4	28.2	.04	6.1	.2	1.2	.06	.2
Total	93									

*Indicates that resulting F value is significant, $P < .05$.

**Indicates that resulting F value is significant, $P < .01$.

***Indicates that resulting F value is significant, $P < .001$.

TABLE A5

COMPOSITION OF TEND-R-LEEN^{R} BEEF FINISHER
CONCENTRATE PROTEIN SUPPLEMENT

Active Drug Ingredient	
Chlortetracycline	133.4 g/Ton
Guaranteed Analysis	
Crude Protein (min.)	36.00 %
This includes not more than 12 percent equivalent crude protein from non-protein nitrogen.	
Crude Fat (min.)	1.25 %
Crude Fiber (max.)	12.00 %
Calcium (Ca) (min.)	2.75 %
(Ca) (max.)	3.50 %
Phosphorous (P) (min.)	1.00 %
Iodine (I) (min.)	.00006 %
Salt (NaCl) (min.)	2.00 %
(max.)	3.00 %
Vitamin A (min.)	30,000 USP units/lb.
Vitamin D-3 (min.)	6,000 USP units/lb.

Ingredients

Linseed Meal, Sunflower Seed Meal, Soybean Meal, Cottonseed Meal, Meat and Bone Meal, Dehydrated Alfalfa Meal, Cane Molasses, Wheat Middlings, Brewers Dried Grains, Urea, DiCalcium Phosphate, Salt, Defluorinated Phosphate, Calcium Carbonate, Manganous Sulfate, Iron Sulfate, Copper Oxide, Calcium Iodate, Cobalt Carbonate, Zinc Oxide, Vitamin A Palmitate, D-Activated Animal Sterol, Vitamin E Supplement, Vitamin B-12 Supplement, Riboflavin Supplement, Choline Chloride, Calcium Pantothenate, Folic Acid, Menadione Sodium Bisulfite, Biotin, Pyrodoxine, Thiamine, Niacin, Dried Extracted Streptomyces Meal, Dried Fermentation Solubles, Dried Whey Products, Dried Fermented Corn Extractive, Ethoxyquin (a preservative).

TABLE A6

MEAN SQUARES FOR MOLAR PERCENT VFA AND pH
DATA-- ADAPTATION PHASE, TRIAL 2

Source of Variation	Degrees of Freedom	Mean Squares								
		Total VFA, Molar Percent VFA, pH and AC:PR Ratio								
		Total VFA	AC%	PR%	IB%	BU%	IV%	VA%	pH	AC:PR
Treatment	3	741.7	51.7	58.0	.2	11.9	.4	7.1**	.3	.3
Error A	43	1260.4	25.9	52.0	.2	17.4	1.6	1.7	.1	.2
Day	1	57526.6***	8781.0*	1040.0***	1.7**	144.9**	4.0	6.3	1.3***	1.9***
Treatment*Day	3	522.5	453.0	78.7	.1	58.9*	.1	2.5	.0	.2
Error B	41	1532.2	370.6	40.5	.2	15.7	1.1	2.3	.1	.1
Total	91									

*Indicates that resulting F value is significant, $P < .05$.

**Indicates that resulting F value is significant, $P < .01$.

***Indicates that resulting F value is significant, $P < .001$.

TABLE A7

MEAN SQUARES FOR PROTOZOA CONCENTRATION
DATA-- ADAPTATION PHASE, TRIAL 2

Source of Variation	Degrees of Freedom	Mean Squares						
		Total Protozoa and Differential Counts						
		Total	Isotrich[a]	Charon[a]	Epidinium[a]	Entodinium	Diplodinium	Dasytrich
Treatment	3	3.5×10^{10}	0	0	0	3.7×10^{10}	2.9×10^{7}	1.6×10^{6}
Error A	43	4.9×10^{10}	0	0	0	4.8×10^{11}	2.3×10^{7}	1.2×10^{6}
Day	1	$1.7 \times 10^{11*}$	0	0	0	$1.7 \times 10^{11*}$	1.0×10^{6}	$1.8 \times 10^{7***}$
Treatment*Day	3	$1.1 \times 10^{11*}$	0	0	0	$1.1 \times 10^{11*}$	1.3×10^{6}	2.1×10^{7}
Error B	38	3.1×10^{10}	0	0	0	3.1×10^{10}	1.2×10^{7}	1.7×10^{6}
Total	88							

[a]These genera were absent from all rumen samples.

*Indicates that resulting F value is significant, $P < .05$.

***Indicates that resulting F value is significant, $P < .001$.

TABLE A8

MEAN SQUARES FOR MOLAR PERCENT VFA AND pH
DATA-- FINISHING PHASE, TRIAL 2

Source of Variation	Degrees of Freedom	Mean Squares								
		Total VFA, Molar Percent VFA, pH and AC:PR Ratio								
		Total VFA	AC%	PR%	IB%	BU%	IV%	VA%	pH	AC:PR
Treatment	3	5544.3	10.5	54.5	.2	26.8	.3	.0	.2	.1
Error	43	2035.0	20.2	26.7	.3	19.3	1.0	.2	.2	.1
Total	46									

TABLE A9

LEAST-SQUARES MEANS FOR RUMINAL pH AND MOLAR PERCENT VFA DATA--
ADAPTATION AND FINISHING PHASES, TRIAL 2, HISTOGRAM POINTS

Percent Wheat	Day of Trial	AC:PR	pH	TVFA	AC%	PR%	IB%	BU%	IV%	VA%
60	7	1.26	5.74	125.6	48.1	39.4	1.1	7.5	1.2	2.6
60	21	.90	5.56	171.3	42.3	47.3	1.0	6.8	.7	1.9
60	71	1.03	6.22	186.5	42.9	42.5	1.4	10.4	1.2	2.2
40	7	1.48	6.01	108.6	47.0	35.0	1.3	11.7	1.4	3.6
40	21	1.02	5.69	166.0	44.6	44.7	1.1	5.7	.8	2.6
40	71	.99	5.94	224.8	42.4	44.7	1.2	10.4	1.0	2.1
20	7	1.34	6.02	105.3	45.4	38.2	1.5	10.5	1.4	3.2
20	21	1.07	5.75	166.1	47.0	46.0	1.0	6.3	1.0	2.4
20	71	1.01	6.16	188.1	43.1	43.5	1.3	8.9	1.1	2.1
0	7	1.04	5.81	131.7	43.7	42.5	1.3	7.5	1.0	3.4
0	21	.99	5.66	165.1	41.9	43.6	1.0	8.8	.7	3.8
0	71	.87	6.00	225.1	41.0	47.4	1.3	7.3	.9	2.2

VITA

Robert R. Lyle was born in Nashville, Tennessee on May 18, 1955. He attended elementary school in that city and was graduated from McGavock Senior High School in 1973. He then entered the University of Tennessee at Martin and in May 1977, he received the Bachelor of Science degree in Agriculture. In the Summer of 1977, he began graduate studies in the Animal Science Department of the University of Tennessee at Knoxville as a graduate research assistant. In June 1979, he received the Master of Science degree in Animal Science.

He is married to Patricia Sneed of Nashville, Tennessee.