To the Graduate Council:

I am submitting herewith a thesis written by Paul Kupen Su entitled "Cellulose Digestion Potential, Liquid Volume and Other Rumen Parameters of Market-Transit Stressed Steers." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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CELLULOSE DIGESTION POTENTIAL, LIQUID VOLUME AND OTHER RUMEN PARAMETERS OF MARKET-TRANSIT STRESSED STEERS

A Thesis

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ABSTRACT

The objectives of this study were to evaluate the error associated with estimating rumen fluid volume (V2) in field studies using the marker concentration in the rumen fluid sample collected 1 hr after dosing with either lithium sulfate or polyethylene glycol. A comparison of this estimate with the rumen volume (V20) calculated from predicted marker concentration at time of dosing using individual regression coefficients, and that (V20B) calculated from predicted marker concentration at the time of dosing using a common regression coefficient was also made. Rumen parameter (rumen fluid volume, in vitro gas producing potential and pH) changes of 172 weanling feeder-steer calves subjected to the market-transit chain were evaluated and factors affecting these changes were also examined.

Mean V2, V20 and V20B in shipment 7 were 34.6 ± 2.01ℓ, 31.6 ± 1.88ℓ and 31.9 ± 2.01ℓ respectively. In shipment 11, mean V2, V20 and V20B values were higher than those in shipment 7 but the mean difference between V2 and V20 were similar even though different markers were employed. Standard errors of mean V2, V20 and V20B were also similar in the 2 shipments though the coefficients of variation (CV) of the estimates of rumen fluid volume in shipment 7 were larger than the respective CV values in shipment 11. Mean estimates of V2, V20 and V20B in shipment 12 were slightly higher than those in shipment 7 but lower than the respective estimates in shipment 11. Difference between V2 and V20 in shipment 12 was only 1.01ℓ compared to 3.00ℓ and 2.92ℓ in the shipments 7 and 11, respectively. Comparison of the analysis of the three estimates V2, V20 and V20B in each of the shipments suggested that the method of calculation had no effect in the statistical interpretation of the results.

In vitro gas production (GP) of the rumen fluid and rumen fluid volume followed similar trends during the market-transit phase in the three trials. Increases in GP and slight increases in rumen fluid volume were observed on arrival at the auction barn, and GP and rumen fluid volume decreased after a 24 hour starvation period in the auction barn. The rumen parameters return to pre-shipment levels within 11 days after arrival at the feedlot.

The factors affecting GP during the market-transit chain include the farm of origin, pre-shipment treatments and the length of time the calves spent in the orderbuyer barn. Rumen fluid volume was significantly affected by the farm of origin differences when measured at the farms 30 days prior to marketing, and at most sampling points at the feedlot. The addition of sodium bicarbonate to the feedlot receiving diet did not have any significant effect on rumen fluid volume. Pre-shipment treatment had a significant effect on rumen fluid volume only at the arrival auction barn and departure orderbuyer barn phase of the market-transit chain in trial 3.

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CHAPTER I

INTRODUCTION

A majority of the calves produced in Tennessee and the Southeast are shipped at weaning to Southwest Great Plains or Northern Corn Belt feedlots for finishing and thus are subjected to market and transportation stresses. These stresses include weaning, crowding, starvation, dehydration, fatigue, anxiety and fright, temperature and weather changes and exposure to various bacterial and viral pathogens. The effects of morbidity and mortality on production costs are important economic considerations to both feeder calf producers and feedlot operators. Sinha and Abinanti (1962) and Herrick (1969) estimated the cost of treating a single calf at $10 to $20 and Flemming (1975) estimated the total losses in the U.S. due to Bovine-Shipping-Fever Complex at $500 million per year. Numerous hypotheses and research results concerned with factors influencing the incidence and prevention of Bovine-Shipping-Fever Complex have been reported (Sinha and Abinanti, 1962). However, there are inconsistencies in the literature on the type and time of treatment to alleviate these problems.

Purser and Moir (1959) and Esdale and Satter (1972) reported a reduction in rumen microbial activity during severe stress. Meiske et al. (1958) and Warner (1962) demonstrated that starvation markedly reduced the rumen microbial population. Since many stressful situations such as starvation are associated with shipping fever calves, part of the physiological changes in calves observed during and following marketing and shipping may result from nutritional stress or deficiencies.

The microbial population is responsible for the digestion of cellulose and subsequent production of volatile fatty acids (Marston, 1948). Caroll and Hungate (1954) reported that volatile fatty acid production in the rumen accounts for approximately 70% of the total energy requirement of the ruminant. Non-protein nitrogen is converted to digestible protein by the microbes and subsequently digested and absorbed in the gastrointestinal tract (Zuntz, 1891, cited by Johnson, 1963). During market-transit when the normal feeding schedule is disrupted and often the diet changed, a study of the rumen microbial activity could give a good indication of the nutritional status and possibly the stress conditions of the animal. Measurement of the capacity to ferment excess substrate in a closed in vitro system has been demonstrated by McAnally (1943) and Hungate (1956) as a rapid means of estimating the size and viability of microbial populations. The rate of the fermentation can be measured by the rate of gas production (El-Shazly and Hungate, 1965) and is directly correlated with rumen microbial activity.

The objectives of this study were: 1) to measure ruminal parameter changes in weaning feeder calves subjected to normal industry market-transit stresses and to subsequent feedlot adaptation, 2) to study the relationship among various pre-and post-transit ruminal parameters and performance traits and 3) to evaluate the influence of various pre-and post-transit nutritional-management practices on the ruminal microbial populations and other rumen parameters.

CHAPTER II

REVIEW OF LITERATURE

Reviews of earlier works on rumen microbial activities by Phillipson (1942) and Marston (1948) suggested that microorganisms were responsible for cellulose disappearance as feed passed through the gastrointestinal tract of the ruminant. Between 1880 and 1900 investigators using rumen bacteria to study fermentation of cellulose observed that large amounts of volatile fatty acids were produced. Many of these early investigators thought that cellulose digestion and the resultant fatty acids had little direct nutritional value to the animal. However, during this same period it was postulated that non-protein nitrogen was converted to protein by the rumen bacteria and that the subsequent digestion of these microorganisms contributed to the protein requirement of the animal.

Early attempts to measure microbial population size and activity were difficult and time consuming. However, more recently it has been shown that microbial population size can be determined by evaluating the microbes' ability to ferment substrate in a closed _in vitro_ system. The subsequent review will deal with methods of measuring changes in rumen microbial populations and with factors affecting these changes.

Results from numerous experiments have shown that ration composition and frequency of feeding can influence rumen microbial activity. Feeder cattle are frequently transported long distances by truck which means that they go without feed and water for a considerable period of time. Upon arrival at the feedlot, the observant feedlot operator tries

to manage the animals through an adjustment period by gradually bringing the animals to maximum daily intake of a concentrate ration. However, if this cautious and planned increase in daily intake does not occur, digestive disturbances are observed. Therefore, this review includes also studies of conditions observed in feeder calves during marketing and transporting.

A. IN VITRO GAS PRODUCTION POTENTIAL OF RUMEN FLUID

Importance of Rumen Microbes to Nutrition

The nutritional contribution to the ruminant by microorganisms has long been known by ruminologists. Warner (1966) stated that the nutritional economy of the ruminant animal depends on the absolute number of microorganisms present in the rumen and their flowing from the rumen into the omasum. Research conducted by McNaught et al (1954) and Oxford (1955) indicated that rumen protozoa may be a factor in the efficiency of nitrogen utilization and Pilgrim et al. (1970) and Weller and Pilgrim (1974) demonstrated that protozoa contributed to the dietary needs of the ruminant. Quin (1943) reported that ruminant nutrition is at least partially dependent upon the products from metabolism of bacteria which have been rendered available for absorption. The rumen absorbs and utilizes the fermentation products formed by the rumen microorganisms and also depends on the microbes for its protein supply (El-Shazly and Hungate, 1965). Klopfenstein et al. (1966) indicated that, in addition to protein and carbohydrates, lipid metabolism in ruminants is either directly or indirectly influenced by the rumen protozoa. Warner (1956) indicated that the predominant product of endogenous metabolism of the

protozoa was probably ammonia. Culture studies by Gutierrez (1955), Abou-Akkada and Howard (1962) and Williams et al. (1961) indicated that the major end products of several species of protozoa are large amounts of acetic and butyric acid with smaller amounts of propionic acid. However, Abou-Akkada and El-Shazly (1964) and Christiansen et al. (1964) reported that the presence of protozoa in the rumen of sheep resulted in greater amounts of propionic acid.

In an effort to measure microbial contribution to the rumen nutritional status, researchers have developed numerous methods of estimating the microbial population size and microbial activity (McAnally, 1943; Elsden, 1945; Marston, 1948; Louw et al., 1949; Burroughs et al., 1951; Johns, 1951; McBee, 1953; Doetsch et al., 1953; and Carroll and Hungate, 1954). Procedures using diaminopimelic acid to measure the bacterial content and mechanical separation of the protozoa populations proved to be time consuming and makes the analysis of large numbers of samples difficult, according to Weller et al. (1958). Johnson (1963) enumerated problems associated with in vitro fermentation systems and suggested areas in which the technique is critical to the study of nutritional problems. These areas were: 1) cellulose digestion and factors affecting cellulose digestion, 2) utilization of non-protein nitrogen, 3) use as a screening technique for drugs, 4) in vitro forage evaluation and 5) several others.

Fermentation contributes one of the most characteristic feature of ruminal activity (Quin, 1943). When food is in excess and conditions constantly favorable, fermentation is maximum and is a linear function of population size, according to El-Shazly and Hungate (1965). Change in the in vitro fermentation rate of rumen fluid over time (1hour) has been

shown by El-Shazly and Hungate (1965, 1966) and El-Din and El-Shazly (1969b) to be a true measure of change in viable microorganisms in the rumen. El-Din and El-Shazly (1969a) reported high coefficients of correlation between changes in *in vitro* fermentation capacity and changes in percentage of viable bacteria (r = .99) and total ciliated protozoa count (r = .98). The major products of rumen fermenration are either gases CO_2 and methane, or acids which release gases from the rumen bicarbonate (El-Shazly and Hungate, 1965). Quin (1943), McAnally (1943), Hungate (1956) and El-Shazly and Hungate (1965, 1966) demonstrated that the rate of gas production is a useful index of the rate of fermentation. Trei *et al.* (1970) reported that gas production per gram of dry matter incubated was highly correlated with dry matter disappearance from the incubation flask. Hungate (1965) and El-Din and El-Shazly (1969a) found the rate of gas production was related to the rate of volatile fatty acid production.

Methods of Measuring Microbial Activity

Many methods of measuring gas production have been used by various investigators. Upon review of the literature, all methods were found to be very similar. The methods used or the modification of a method depended on the investigator and the needs of the experiment. Quin *et al.* (1938) described a method for the continous recording of the amount of gas generated within the rumen by connecting the rumen to a large graduated manometer by way of a tube through a fistula.

After reviewing the various methods proposed for studying microbial activity Hungate *et al.* (1955) developed a modified Warburg manometric method for *in vitro* measurement of gas production. The flasks attached to the manometers were placed in a water bath at rumen temperature and

and carbon dioxide was employed to maintain anaerobic conditions. A period of 40 minutes to one hour between collection and first measurement is included in this method. They found this manometric method permitted quantitative determinations of the acids, carbon dioxide and methane produced during the fermentation and that it was useful in assaying microbial activity in the bovine rumen. Later Hungate et al. (1961) developed a method for measuring the gas production with a hypodermic syringe which had the advantage that measurements can be made almost immediately. They reported that continuous shaking of the incubation flask was not necessary although occasional mixing was necessary to redisperse the solids which may rise to the top of the liquid. Use of this method was reported by Shibata et al. (1961) and El-Shazly and Hungate (1965), who suggested that measuring only the amounts of gas with the hypodermic syringe permitted the use of larger samples and increased precision. They used approximately 200 g of rumen contents in a prewarmed incubator jar that was tightly closed with a rubber stopper. The needle of a 10 ml water lubricated syringe was inserted through the rubber stopper and measurements were started within 15 minutes of removing the sample from the animal. El-Din and El-Shazly (1969b) modified the apparatus by putting the syringe in a horizontal position thereby reducing the resistance of the plunger. They also employed a T-tube with a 3-way stop-clock to allow the syringe to be emptied or to allow the collection of gas for analysis with greater ease. They found this method to be a reliable means of measuring net growth of rumen microorganisms over time. Trei et al. (1970) incubated 75 ml of innoculum which represented the fluid collected from 35 g of pressed rumen ingesta, with substrate

and incorporated a calibrated water manometer. Throughout the literature, carbon dioxide was employed to facilitate anaerobic conditions and incubation temperatures employed varied from 37^{o}c to 40^{o}c.

El-Shazly and Hungate (1965) collected their samples with a stainless steel sampler inserted deep into the various regions of a fistulated rumen. Hungate et al. (1955) reported that duplicates of the samples agreed in results and El-Din and El-Shazly (1969b) reported a variation of less than 2 percent. Carroll and Hungate (1954) suggested that because of the large samples (50 to 200 ml rumen fluid) error due to sampling is less than in other techniques. Trei et al. (1970) indicated that replication is not necessary since sampling error is considerably smaller than the error between trials. In addition, variation between samples collected through fistula and stomach tube were found to be no greater than 3.5 percent (El-Din and El-Shazly, 1969a, 1969b).

Numerous reports in the literature support the practice of sampling bovine or ovine rumen contents by orally inserting a stomach tube fitted with some vacuum device and a stainless steel strainer. The accepted procedure for estimating bacterial population size is in vitro measurement of the volume of gases produced when a sample of the rumen fluid is incubated with a substrate in the anaerobic environment of a simple closed in vitro system.

Factors Affecting In Vitro Gas Production

Time of withdrawal of ingesta after feeding, type of substrate used and length of starvation of the animal has been shown by numerous investigators to affect the in vitro gas production by rumen fluid. Warner (1966) reported that the concentration of microorganisms in the rumen

changed with time and according to patterns characteristic of the specie. Time of sampling ingesta was found to be an important factor influencing fermentation rates by McAnally (1943), Quin (1943), Hungate et al. (1960), El-Shazly and Hungate (1965) and El-Din and El-Shazly (1969a, 1969b).

The cellulose fermentative rate of rumen microorganisms obtained from sheep fed hay was highest immediately after feeding (15 to 20 minutes) and the lowest rate was always observed before feeding (El-Din and El-Shazly, 1969a). Quin (1943) found that no gas was produced by ruminal fluid drawn before feeding in the early morning. He suggested that this early morning pre-feeding absence of active gas production affords evidence of the intermittency of the fermentative process and its dependence on fresh food in the forestomachs. El-Shazly and Hungate (1965) reported that maximal rates of gas production from rumen samples of hay-concentrate fed animals occured immediately after feeding and that the rate dropped to pre-feeding levels after 3 hours. The above gas production rates were measured over an incubation period of 1 hour. However, when incubation takes place for more than 1 hour the net microbial growth will decline unless ideal conditions of dilution, feed concentration and carbon-nitrogen (C/N) are maintained during the entire period and El-Din and El-Shazly (1969b) and Putnam et al. (1961) observed an increase in protozoa numbers when cattle were fed more than twice daily and Purser (1961) found that rumen protozoa numbers dropped when lambs were fed once instead of twice daily.

Rumen samples concentration and substrate concentration influenced the fermentation rate in studies conducted by El-Shazly and Hungate (1965) and El-Din and El-Shazly (1969a). They suggested that 15 g of feed for

each 50 g of rumen sample plus 150 ml of mineral solution gave the highest growth rate of rumen microorganisms. Trei et al. (1970) reported that when the same volume of rumen fluid (75 ml) was used, gas production per gram of dry matter significantly decreased as substrate sample weight increased above 2 g. Therefore they suggested that the most efficient rumen fluid to substrate concentration was 75 ml to 2 g. When concentrates plus straw were used as the substrate, they supported maximum fermentation rate when compared to glucose, sucrose or starch alone or in combination with urea, sodium citrate or succinic acid (El-Din and El-Shazly, 1969a). They further demonstrated that maximum in vitro gas production resulted when the feed being consumed by the animal was used as the substrate and that the highest net microbial growth rate was obtained when (C/N) ratios (16.5 and 17.5) were closest to the (C/N) of the feed consumed by the donor animal. These results tend to establish the fact that time is required for the microbes to adapt to changes in feed ingested by the host animal.

McAnally (1943) reported that glucose is the most readily fermented substrate followed closely by sucrose. In the same year, Quin, (1943) demonstrated that glucose and fructose undergo an extremely rapid and identical type of fermentation and that sucrose has a somewhat slower fermentation rate although it exhibits a definite peak rate within the first 10 minutes. Maltose and mannite were fermented at much slower rates and lactose, galactose, arabinose, xylose and rhamnose showed almost no evidence of fermentation. They found that starch, either baked or raw, failed to undergo in vitro fermentative disintegretion within 30 minutes, however, after the first hour of relatively low production, soluble starch produced a total of 2.54 ml of gas within 4 hours compared to 8.5 ml for the raw starch.

Starvation has long been known to influence the rumen microbial population and various experiments have been conducted to study the effect of periods of starvation on the rate of return to normal metabolic activity by the microorganisms. Moir and Somers (1957) suggested that rumen protozoa numbers may be controlled by physical factors such as dilution of rumen contents resulting from eating, drinking and/or starvation. Quin (1943) found that sheep starved for 65 hours required 3 days to obtain their pre-starvation daily feed intake and that the glucose fermenting ability of the rumen (measured by in vitro gas production techniques) did not return to normal until the tenth day after feeding was resumed. He stated that starvation caused a rapid and practically complete suppression of the fermentative power of ruminal ingesta.

Meiske et al. (1958) indicated that reduced bacterial numbers and decreased protozoal activity occurred after the animal has been starved for 24 hours. They found that the ability of rumen samples to digest cellulose in vitro was reduced from 15 to 25 percent after 48 hours of starvation and from 68 to 90 percent after 72 hours. A decline in the number of microorganisms per unit of rumen fluid volume was also observed during the starvation period. However, in contrast to reports by Quin (1943) they reported that in vitro digestion of cellulose was approximately normal within 3 to 4 days after refeeding. Warner (1962) reported marked decreases in rumen microbial concentrations of sheep starved for 4 days and a variation in the period of time required for the different bacterial species to reach normal concentration after refeeding.

B. RUMEN pH

Factors Affecting Rumen pH

Reports on pH values of rumen ingesta in the literature seem to vary and Smith (1941), Myburgh and Quin (1943) and Clark and Lombard (1951) indicated that rumen pH values determined from sampled ingesta are subject to a variable and often large error. Myburgh and Quin (1943) and Clark and Lombard (1951) suggested that high values of pH recorded by earlier workers could be the result of salivary contamination when the ingesta was aspirated by stomach tube. Other workers reported higher values due to aeration and subsequent liberation of CO_2 from the ingesta. Monroe and Perkins (1939) and Smith et al. (1956) found variations in pH within the rumen itself and reported a definite difference between the samples collected from the upper and lower part of the rumen of a fistulated steer. Monroe and Perkins (1939) found differences in pH values among six samples from different locations in the rumen. Samples from the front portion of the rumen were the most acid and the variations among the samples from the six different locations ranged from 0.13 pH at 5:00 AM to 0.47 pH at 9:00 AM with an average difference of 0.32 for the day. However, once-a-day samples taken at 1:00 PM had a variation of only 0.14 pH units. They also found that when the initial samples were taken immediately following feeding at 5:00 AM and subsequent samples were taken at 2 hour intervals, rumen pH dropped until about 9:00 AM (6.20 pH), then gradually increased until it reached a peak at 8:00 AM (7.10 pH) immediately before the late afternoon feeding. The pH value dropped again after that feeding. However, they reported no such fluctuation in cows on pasture. Similar prefeeding highs and postfeeding drops in pH values of rumen samples were also reported by Briggs et al. (.957). Meiske et al. (1958)

observed a continuing increase of pH values from a normal of 6.8 to a maximum of 7.9 during a 3-day starvation period. During refeeding periods, pH dropped rapidly to a value as low as 5.6 to 5.9 before gradually returning to normal levels. Briggs et al. (1957) reported that the rumen pH values of sheep fed diets that did not cause digestive disturbance rarely fell outside the range of 4.5 to 7.4 and clinical digestive disturbances were often observed with levels in the lowest part of the pH range (4.5). Kick et al. (1938), working with rumen samples from rumen fistulated steers, reported a pH range of 5.5 to 7.7 and suggested that the variation depended on the ration. They also reported high values when alfalfa hay was fed to steers and that the addition of corn to rations caused a drop in pH. The fact that pH changes depend upon the type of feed consumed is apparent (Moir and Somers, 1957).

Moore (1964) reported a reduction of rumen pH when finely ground, pelleted roughage or high energy feed was fed. Balch and Rowland (1957), Raun et al. (1962), and Kromann and Meyer (1972) reported lower pH values in animals fed a high energy diet than those fed a low energy diet. Work by Latham et al. (1971) confirmed that a cereal ration produced slightly lower pH values than more fibrous ration. Low pH values of 4.1 and 4.2 were reported by Hungate et al. (1952) and Turner and Hodgetts (1955) respectively, when sheep were dosed with large quantities of wheat grain or glucose. Reid et al. (1957) and El-Shazly et al. (1961) suggested that decreased pH levels could be the result of acids produced from the starch fermentation. McAnally and Phillipson (1942) suggested that pH of the rumen may be a function of volatile fatty acid concentration.

Rumen Buffers

Researchers have long realized the importance of saliva in the buffering effect in the rumen. During the course of microbial digestion of carbohydrates, varying amounts of organic acids are produced causing acidification in the rumen. In order that the normal density of microbial activity is maintained, pH fluctuation would have to be limited by some mechanism. Therefore, normally, the alkaline saliva, containing a significant amount of sodium bicarbonate and phosphates, exerts a desirable buffering effect. Turner and Hodgetts (1955) indicated that if salivary secretions were ceased, volatile fatty acid production would lower rumen pH to 3.0. Coop (1949) observed that when sheep are re-fed after a period of starvation, rumen pH varied inversely with rumen VFA content. However, further work showed no such relationship and he concluded that short-term fluctuations in pH bear no obvious relationship to VFA content.

Phillipson (1942) suggested that rumen pH is probably a function of rumen VFA level and Briggs et al. (1957) confirmed these findings and also reported that lactic acid exerts a stronger effect on pH than VFA's. However, they also indicated that in ruminants fed different diets, variation in salvia secretion and accumulation of ammonia nitrogen in the rumen after feeding could considerably alter pH-VFA relationships.

Gray (1948) and Thorlacius and Lodge (1973) reported that the rate of VFA absorption was considerably lowered at pH values above 7.0. They indicated that at low pH conditions, the predominant order in which VFA's were absorbed from the ruminant was: Butyrate > Propionate > Acetate. On a hay diet, Thorlacius and Lodge (1973) found that decreasing ruminant pH from 6.5 to 5.5 increased acetate, propionate and butyrate clearance

by 70, 113 and 138 percent, respectively. However, Barcroft et al. (1943) reported the reverse order of absorption of these individual VFA's when the pH was alkaline. Pfander and Phillipson (1952) indicated that the concentrations of the individual VFA's has more influence than the pH value in VFA absorption. Work by Emmanuel et al. (1970) indicated that a bicarbonate buffer would increase VFA absorption by lowering the pH and increasing tissue uptake of CO_2.

Effect of Rumen pH on Microbial Activity

Reports have indicated that fluctuations in microbial numbers are related to pH values in the rumen. Hastings (1944) reported that the saliva is responsible for maintaining rumen pH at slightly below 7.0 which is favorable for the growth of bacteria and protozoa. Purser and Moir (1959) found that rumen pH is the main controlling factor in diurnal fluctuations of microbial numbers and Warner (1962) reported that minimal growth of rumen microorganisms occurred during the period 16 to 24 hours after feeding. At this time, pH values reached their highest diurnal values and maximal growth occurred at 8 hour after feeding when the pH values were still moderately low.

Myburgh and Quin (1943), in a study involving in vitro fermentation of glucose, found that gas production was most pronounced within a pH range of 6.8 - 7.8. They demonstrated that the addition of increasing amounts of HCl, when rumen pH was below 6.8, resulted in a sharp decline in pH and a progressive decrease in gas production. The end point was reached at pH 5.0 to 5.4. When NaOH was added beyond pH 7.8, progressive decreasing gas productions were observed and no gas production occurred above pH 8.35. Cheng et al. (1955) reported similar findings with respect

to the optimum pH range. However, they found that at pH 5 to 6 activity was only one-half that at optimum pH values. In in vitro studies where rumen ingesta was stored to long, acid formation and putrefaction occurs and these changes completely negated normal psychological conditions. McAnally (1943) stated that in in vitro fermentative studies, addition of buffer solutions to the ingesta is unsatisfactory since a quantity sufficient to alter pH would considerably lessen the fermentative power of the ingesta due to dilution of the media. She reported that the natural buffering power of the ingesta is appreciable and should suffice to hold pH within physiological limits except when large amounts of acids are being formed. Hungate et al. (1955) reported that the pH value at the beginning of the gas production experiment was 6.8 and that it was not lower than 6.5 at the end. However, El-Din and El-Shazly (1969a) found that the addition of sodium bicarbonate to the in vitro sample did not increase the rate of VFA production but did increase gas production.

C. RUMEN VOLUME

Relationship of Rumen Fluid Volume to Nutrition

Although the rumen does not secrete enzymes or digestive juices per se, it would be erroneous to consider its function merely as a large storage chamber in which food is kept until there is an opportunity for mastication and for the secretion of saliva to prepare it for digestion in the lower parts of the gastro-intestinal tract. Researchers have long realized that the rumen, with its vast populations of microbes, is responsible for providing the ruminant with a number of important intermediate steps which make certain nutrients available to the animal that

would otherwise be unusable. The ability of rumen microbes to utilize non-protein nitrogen from urea, a waste product of the metabolism of the animal, and digestion of cellulose contributed to the survival of the ruminant as a herbivore. This leads us to ponder the significance of rumen size in the nutrition of the ruminant.

Conrad et al. (1964) and Montgomery and Baumgardt (1966b) studied the importance of feed intake on cattle production and suggested that intake of low quality forages is controlled by the capacity of the gut, the pressure on the gut wall, the amount of bulky material ingested and by the weight of the ingested material. Freer and Campling (1963) reported that rumen volume had an effect on food intake and the ability of ruminants to digest food. Purser and Moir (1966b) reported that sheep with larger estimated rumen liquid volume had a greater propensity to consume feed than those with smaller rumen liquid volumes and that ad libitum intake of dry matter in the form of poor quality, bulky roughages is proportional to rumen volume.

Factors Influencing Rumen Fluid Volume

Many factors are known to influence rumen volume in cattle. The diet, water consumption, feed intake, retention time, digestibility, body fatness, pregnancy and lactation have all been described as factors affecting various rumen volumes. However a review of literature revealed that retention time, digestibility and intake are confounded. Campling and Balch (1961) reported that as the retention time increases, digestibility usually increases and intake decreases. Interactions among these three factors were reported also by Conrad et al. (1964) and Montgomery and Baumgardt (1965a, 1965b).

Bauman et al. (1971), in studies evaluating the polyethylene glycol method of estimating rumen volume in dairy cows, found that diet did not significantly affect rumen fluid volume although it drastically altered the rate of turnover of rumen fluid. They reported a 20.6 percent turnover of the rumen fluid per hour for cows fed liberal amounts of hay and grain and only 9.6 percent hourly turnover for cows fed high energy, low fiber diets. Topps et al. (1968) reported similar results of 5.6 percent hourly turnover rate in young steers fed a concentrate diet versus 14.3 percent for those fed a hay diet. Taylor (1959) and Crabtree (1967) suggested that as cattle fattened, the size of the organ chiefly concerned with its nutrition will decrease. Therefore estimates of liquid volume and the amount of feed it will consume per unit of body weight will be less. Diurnal variations in the liquid volume of the rumen has also been reported by numerous researchers. Purser and Moir (1966c) and Ulyatt (1964) reported that day to day changes in rumen volume amounted to approximately 22 percent of the fluid volume. They also suggested that the nature of feed influences the accuracy of rumen volume measurement and fast eaters tend to have larger rumen volumes than animals that eat slowly. Ulyatt et al. (1967) found that the volume of rumen fluid increased with increasing feed intake and starvation tends to cause a reduction in the rumen fluid volume.

Purser and Moir (1966b) also found a linear relationship between physiological rumen volume and weight in sheep. However, they suggested that the relationship must be curvilinear at lower sheep weights because the volume would be ridiculously small otherwise. In another study, Purser and Moir (1966a) found greater protozoa concentrations and below average

rumen amonia concentrations in sheep with smaller rumen volumes. Those sheep with large rumen volume exhibited above average dry matter digestibility.

Estimation of Rumen Fluid Volume

Various direct and indirect methods of determining the volume of the rumen have been described in the literature. The direct methods range from physically measuring the rumen contents to measuring the rumen itself. Measuring the rumen contents usually entails a rumen fistula and physical removal of ingesta; whereas the actual measuring of the rumen was done after slaughter. Indirect methods usually employ a marker for estimating rumen fluid volume. The use of soluble markers was critically appraised by Warner and Stacy (1968). Hyden (1960a) stated that in order for the results from the estimation of rumen fluid volume and the estimated flow rate of water and dissolved substances in the digestive tract to be reliable, the reference substance or marker should fulfill the following requirements:

1) The substance should be freely soluble in the fluid.

2) It must be well defined from a chemical point of view and it should be easily and accurately determinable chemically.

3) It should not be taken up, absorbed, produced or destroyed to any significant degree by microorganisms or by other parts of the contents in the gut.

4) It should not influence the normal digestive processes to any appreciable degree.

Polyethylene Glycol as a Rumen Marker

Polyethylene glycol (PEG), a highly polymerized long-chain compound of the general formula $HO\text{-}CH_2\text{-}(CH_2O\text{-}CH_2)_n\text{-}CH_2OH$ with a mean molecular

weight of 4,000, was first suggested as a suitable rumen marker by Sperber et al. (1953). Hyden (1955a, 1961) reported that its distribution in the rumen contents involves approximately 95 percent of the total water and can be accurately estimated turbidimetrically. Ulyatt (1964) reported that although polyethylene glycol fulfills the requirments as a water soluble marker, the main problems associated with its use is the overall theoretical concept of the marker method of estimating rumen fluid volume and flow rate. He suggested that problems are associated with all water soluble markers. Work by Tulloh et al. (1965) suggested that estimation of rumen fluid volume by PEG measurement compared favorably with other indirect estimates of fluid volume from body weights. Ulyatt et al. (1967) also found that PEG estimates of rumen volume agreed with rumen volume values obtained after slaughter. Alexander et al. (1969) reported that only 80-90 percent of the total rumen water was available to PEG and dry matter percentage of the rumen digesta affected the portion of total water available to PEG. They also reported that PEG concentration and refrigerated storage did not affect the accuracy of the method. However, in vitro work with rumen fluid by Alexander et al. (1969) and Czerkawski and Breckenridge (1969) indicated that PEG does not equilibrate with total rumen water. Ulyatt (1964) plotted the natural logarithm of PEG concentration against time and demonstrated that in most cases the relationship was not linear but concave. He found that the use of quadratic regressions resulted in improved statistical precision in estimating the initial marker concentration. He also suggested that the semi-logarithm plot of marker dilution curve was curvilinear and that in grazing sheep, uneven-patterns of intake and high rate of flow of

water contributed to the curvilinear nature of the curve.

Ration Effect on PEG as a Rumen Marker

Sperber et al. (1953) reported that the only disappearance of PEG from the rumen is via the reticulo-omasal orifice and Ulyatt (1964) suggested that the distribution of PEG in the rumen depends on the physical characteristic of the ingesta, the fraction of the ingesta it is associated with and its rate of flow through the digestive tract. Sinha et al. (1970) suggested that PEG moves through the digestive tract in the liquid phase and not in the solid phase.

Czerkawski and Breckenridge (1969) reported that PEG was excluded from a large portion of the water contained in sugar-beet pulp food particles and that this proportion of unavailable water for PEG is large enough to give erroneous estimates of rumen fluid volume in sheep. Bauman et al. (1971) calculated the PEG concentration at time of dosing by the least-squares regression method and found no statistical significant difference between actual rumen volume measurements by direct methods and the PEG estimates of rumen fluid volume. In seven out of ten animals the PEG volumes were within 3 percent of the gravimetric volumes. They noted that the largest variation was 10 percent for a cow receiving a high-grain, low-fiber ration. They also reported that during the conversion from a normal diet to a high-grain low-fiber diet, some cows went through a phase where the rumen contents were frothy and this could cause inadequate mixing of the marker and result in erroneous results. Clark et al. (1972) reported a 91 percent recovery of PEG from the ingesta of sheep fed ground alfalfa hay and that recovery from the ingesta of sheep fed 40 percent to 100 percent cotton seed hull (CSH) decreased as CSH

concentration increased. This indicated that the nature of diet could have a marked effect on the value of PEG as a rumen marker.

Lithium Sulfate as a Rumen Marker

Schou (1957) reviewed extensively the metabolism of the lithium ion by mammals but failed to provide much information on ruminants. Harrison et al. (1963) suggested that lithium is rapidly absorbed by the anterior portions of the digestive tract and small amounts are recirculated into the rumen via the saliva. They reported that this small amount of lithium is absorbed in the venous blood and saliva within 3 hours after dosing and suggested that the reticulo-rumen is the major site of absorption. Walker and Hawley (1965) compared the estimates of rumen fluid volume using lithium and PEG as markers and found that the lithium and PEG estimates agreed within the first day. However, after the first day, lithium tends to give low estimations of rumen volume.

CHAPTER III

EXPERIMENTAL PROCEDURE

A. OVERALL PROCEDURES USED IN SHIPPING FEVER RESEARCH

Before the onset of research, the series of studies of which the three trials reported herein were a part, various groups associated with the overall multi-region project conducted intensive surveys to determine the prevailing marketing procedures of the feeder-calf industry in the Southeast. They found that most Tennessee feeder-calf producers have small cow herds (approximately 28 cows per herd) and the calves are seldom shipped directly from the farm at which they were raised to feedlots or wheat pastures. Most of the calves are transported to the auction barn immediately after they are weaned by the feeder-calf producers. At the auctions they are purchased by orderbuyers and commingled with calves from other farms. The calves usually spend a period of about 18-36 hours (mean = 24 hour) in the auction barn without feed or water. They are then assembled in the orderbuyer's barn, commingled with calves from other auction sales and held until enough calves of a specific weight and grade have been assembled for a specific feedlot order. During the intensive marketing season (Fall), it normally takes 1 to 4 days for the orderbuyer to assemble a truckload (20,000 kg) of calves. However, it takes up to 10 days during other seasons. During the period in the orderbuyer barn (1 to 10 days, mean = 72 hours), hay and water are made available to the calves. Pen space at the auction barn was determined to be 0.65 to 0.95 m^2/steer and 1.42 to 2.80 m^2/steer at the orderbuyer barn.

For this study, a commercial marketing facility at Algood, Tennessee, which was used as a weekly auction market and an intermediate assembly point by several orderbuyers, was provided by the owners. The time spent by the calves in the auction and orderbuyer barn phase, and the pen space allotments were based on the means obtained from the surveys in order that normal industry environments were duplicated as closely as possible.

During the 1976 through 1979 Fall marketing seasons, 14 groups (shipments) of weanling feeder steer calves weighing 136 kg to 303 kg were purchased from several Tennessee and Kentucky feeder-calf producers. The calves on each farm within each shipment were allotted to at least one of three pre-shipment treatments 30 days prior to transporting to the auction barn (Table 1). A combination of the same three pre-shipment treatments were used in all trials. These pre-shipment treatments were: 1) Steers subjected to normal industry environment and stresses without being fed concentrates (NI); 2) Steers fed a high-energy, high-antibiotic concentrate diet during the orderbuyer-barn phase (HE); 3) Steers weaned at the farm of origin 30 days prior to transportation and fed a 65 percent concentrate diet until marketed (PW). The diet compositions are presented in Table 2. The NI and HE calves were weaned on the day they were shipped to the auction barn. At the end of the 30 day period, the calves were transported 40 to 100 km from the farm to the auction barn at Algood, where they were subject to auction barn environments for 24 hours (0.65 - 0.85 m^2/steer space without feed or water). For the next 72 hours, orderbuyer-barn environment was simulated (1.8 - 2.3 m^2/steer space with access to feed and water). Dry hay and water were made available to the PW calves and the NI calves. At the end of the orderbuyer

Table 1. Description of pre-shipment managment treatments.

Treatment Designation	Management and Diets	
	Farm of Origin[a] (30 days)	Orderbuyer Barn[b] (72 hours)
Normal Industry	with dam, no concentrates	mixed grass hay, free choice
High Energy	with dam, no concentrates	high-energy, high antibiotic ration (3.4 kg/steer/day)
Preweaned	weaned and fed a 65% concentrate ration + hay or pasture during last 30 days[c]	mixed grass hay, free choice

[a]All calves were allowed .65 to .75 m^2 of pen space without feed or water during the 24 hour in the auction barn.

[b]Pen space was increased to 1.8 to 2.3 m^2 during the 72 hour in the orderbuyer barn.

Table 2. Ingredient composition of pre-transit diets[a].

Ingredient	IRN[b]	Diet[c] PW	Diet[c] HE
Corn, grain, dry rolled	4-02-931	31.2	32.3
Cotton, seed hulls	1-01-599	42.0	--
Peanut, shells, grnd.	1-03-629	--	30.2
Soybean, seed, solv-extd., grnd., mx 7 fbr.	5-04-604	15.0	7.0
Sugarcane, molasses	4-04-696	5.0	9.0
Alfalfa, Aerial part, dehy meal	1-00-023	5.0	--
Alfalfa, leaves dehy grnd.	1-00-137	--	10.0
Animal-fat, heat rendered	4-00-375	1.0	--
NaCl, trace mineralized	6-04-152	.5	.5
Limestone, grnd mn 33 Ca	6-02-632	.3	.7
Calcium phosphate dibasic, commercial	6-01-080	--	.3
Propylene glycol		--	5.0
Vitamin A palmitate, commercial[d]	7-05-143	+	--
Oxytetracycline[d]		+	--
Vitamin-oxytetracycline premix[e]		--	5.0

[a]Percentage, dry matter basis

[b]International reference number

Table 2. (Continued)

[c]PW = preweaned diet, HE = 3 day high-energy high-antibiotic diet fed at OBB

[d]To supply 5,000 IU of vitamin A and 20 mg of xytetracycline/kg of diet

[e]To supply 1,100 mg of oxytetracycline and 5,000 IU of Vitamin A/kg of diet

barn phase, the calves were transported to feedlots at the Highland Rim Experiment Station, Springfield, TN, or Southwest Great Plains Research Center, Bushland, TX, or to wheat pasture at the Southwestern Livestock and Forage Research Station, El Reno, OK. Upon arrival at the feedlot or wheat pasture, the calves were assigned to post-shipment treatments involving the addition of potassium, B-vitamins and sodium bicarbonate at various levels to the feedlot receiving diet, or varying periods of adaptation prior to receiving a high-energy finishing ration or exposure to wheat pasture.

B. DATA COLLECTED

Data were collected and samples were taken at the following points: 1) at the farm of origin 30 days before moving into the marketing chain (FO); 2) upon arrival at the auction barn (AAB); 3) 24 hours after arrival at the auction barn or the beginning of the orderbuyer barn period (AOBB); 4) departure from the orderbuyer barn (DOBB); 5) arrival at the feedlot or when unloaded from the trucks after 18 to 34 hour haul (AFL); and 6) at various sampling dates at the feedlot.

Data collected include body weight, health (packed cell volume, rectal temperature, and clinical evaluation), body measurements (for calculating size and shape indexes); serological, pathological, immunological and rumen fluid samples. Gas producing (cellulose digestion) potential and pH of the rumen fluid were determined immediately upon collection. A 10 ml aliquot was preserved by diluting with 10 ml of 40 percent Formalin and stored for protozoa counts. The remaining rumen fluid was frozen for future determinations of volatile fatty acid, rumen ammonia

and osmolarity. The rumen fluid volume of each calf was also estimated using either polyethylene glycol or lithium sulfate as markers.

CHAPTER IV

ESTIMATING RUMEN VOLUME IN FIELD STUDIES

A. SUMMARY

In this study, 172 weanling steer calves (54 from each of shipment 7 and 11, and 64 from shipment 12) involved in a cooperative USDA-SEA University of Tennessee shipping fever studies were used. Upon arrival at the auction barn, the calves were dosed with either lithium sulfate (LIT) or polyethylene glycol (PEG) and rumen fluid samples were collected at 1 to 3 hour (1 hour) and 16 to 24 hour (24 hour) post dosing. The marker content of the 1 hour and a subsequent 24 hour sample were transformed to natural logarithms and regressed on time by individual animal. The resulting regression coefficients (b values) were used to predict marker concentration at time of dosing (time-0) for each animal. A common b value within each shipment was also calculated. The error associated with using the marker concentration in the sample collected 1 hour after dosing with LIT or PEG to estimate rumen fluid volume (V2) was evaluated. A comparison of this estimate with rumen fluid volume calculated from predicted marker concentration at time-0 using individual b values (V20) and that using the common b value (V20B) were also made.

Mean V2, V20 and V20B in shipment 7 were 34.6 $\pm$ 2.01ℓ, 31.6 $\pm$ 1.88ℓ and 31.9 $\pm$ 2.01ℓ respectively. In shipment 11, mean V2, V20 and V20B values were higher than those in shipment 7 but the mean difference between V2 and V20 were similar even though different markers were employed. Standard error or mean V2, V20 and V20B were also similar in the 2 shipments though the coefficients of variation (CV) of the estimates

of rumen fluid volume in shipment 7 were larger than the respective CV values in shipment 11. Mean estimates of V2, V20 and V20B in shipment 12 were slightly higher than those in shipment 7 but lower than the respective estimates in shipment 11. Difference between V2 and V20 in shipment 12 was only 1.01ℓ compared to 3.00ℓ and 2.92ℓ in shipments 7 and 11, respectively. Comparison of the analysis of the three estimates V2, V20 and V20B in each of the shipments suggested that the method of calculation had no effect on the statistical interpretation of the results. The calculation of rumen fluid volume from predicted rumen marker value at time-0 removes the bias introduced in studying parameters expressed in terms of rumen fluid volume.

B. INTRODUCTION

Estimates of rumen fluid volume are essential to certain aspects of rumen studies. They are especially important in studying rumen bacterial or protozoal population changes (El-Shazly and Hungate, 1965) in studies dealing with feed intake and rumen ingesta turnover rate. Satisfactory estimates of rumen fluid volume by direct (physical measurement of the rumen content) or indirect (use of water soluble markers) methods have been reported in controlled experiments by Hyden (1960a), Ulyatt et al. (1964), Tulloh et al. (1965), Sinha et al. (1970) and Bauman et al. 1971. Hyden (1955b) postulated the criteria by which a substance would fulfill requirements as a satisfactory rumen marker, and Warner and Stacy (1968) critically appraised the use of water-soluble markers. Both reports suggested that lithium sulfate and polyethylene glycol were satisfactory rumen markers and the results using these markers compared

favorably with concurrent direct estimates.

Although the first samples used in determining rumen fluid volume usually are not collected until 1 hour after dosing, estimates of rumen volume reported in the literature were generally adjusted to volume at time of dosing by regressing the marker content on post-dosing sample time. This procedure requires a minimum of 2 samples which are generally collected at 1 and 24 hour after dosing. Constant volume was assumed when the regression analysis was performed by Ulyatt (1964). In large field studies involving changes in rumen parameters, especially changes occurring during some phases of the feeder calf market-transit chain, collection of samples 24 hour after dosing is not feasible or is often impossible. Complication with respect to an extended sampling period following dosing could occur in studies where the calves are loaded on trucks and transported to another location shortly after initial samples are taken and the calves dosed with the rumen marker. In addition, the collection of 1 hour samples at equal time intervals after dosing may be a problem when large numbers of animals are involved Field studies involving rumen parameters expressed on a per unit rumen fluid volume basis could be conducted using a large number of animals if satisfactory estimates of rumen volume could be based on the marker content of one sample.

The objectives of this study were: 1) to evaluate the error associated with using the marker concentration in a sample collected 1 hour after dosing with lithium sulfate (LIT) or polyethylene glycol (PEG) as markers to estimate rumen fluid volume, and to compare this estimate with rumen volume calculated from predicted marker concentration

by standard procedures at the time of dosage (0 hour), and 2) to develop methods of predicting 0-hour rumen fluid volume from a single rumen fluid sample taken 1 to 3 hours after dosing with the rumen marker.

C. EXPERIMENTAL PROCEDURE

During the Fall seasons of 1977 and 1978, three trials were conducted, using steer calves involved in cooperative USDA-SEA University of Tennessee shipping fever studies, to evaluate the methods of estimating rumen fluid volume in field studies. The general animal management, experimental diets and experimental design were described in detail by Billingsley (1979), Cole et al. (1979) and Orr (1979). Upon arrival at the auction barn (AAB), the animals were dosed with either 1 g of lithium sulfate (LIT) or 100 g of polyethylene glycol (PEG) at the time samples were collected for determining various rumen parameters. The use of the lithium sulfate as a marker in rumen fluid volume estimation was described by Walker and Hawley (1965), and PEG was used as a marker in rumen studies by Hyden (1960b), Ulyatt (1964), Czerkawski and Breckenridge (1969) and Bauman et al. (1971). Samples of rumen fluid were taken 1 to 3 hours after dosing (1 hour sample), and a second sample was taken 16 to 24 hours later (24 hour sample).

Sampling was done by orally inserting a stainless steel strainer connected to a tygon tube deep into the rumen via a speculum and suction was supplied by a 50 ml syringe. The strainer and tubing arrangement procedure and equipment were similar to those developed by Raun and Burroughs (1962). Oral sampling by the stomach tube and strainer method was used in this study because rumen fistulated calves are atypical

of calves used in many field studies and it is not feasible to include fistulated calves in these studies. El-Din and El-Shazly (1969a, 1969b) reported that variation between rumen fluid samples collected via stomach tube and samples taken through a rumen fistula was no greater than 3.5 percent.

The rumen fluid samples were frozen immediately after collection, and analysis for PEG concentration was done according to the turbidimetric method described by Hyden (1955a). LIT analysis was done according to the flame emission method described by Emmel et al. (1977). PEG and LIT concentrations were converted to logarithmic values and these value were regressed on time as described by Ulyatt (1964). The individual regression coefficient (b) values were used to predict PEG and LIT concentration at the time the coefficients of regression:
used to calculate of the coefficients of regression:

$$b_i = \frac{[(\log(L2_i) - \log(L3_i)]}{VT_i}$$

where: b_i = Log value of the regression coefficient of PEG or LIT on time for the i^{th} steer.

L2 = PEG or LIT concentration in the rumen fluid sample collected from the i^{th} steer at 1 to 3 hour after dosing (1 hour sample).

L3 = PEG or LIT concentration in the rumen fluid sample collected from the i^{th} steer at 16 to 24 hour after dosing (24 hour sample).

VT = Time between the 1st and 2nd collection of rumen fluid samples from the i^{th} steer in hour.

The intercept (a) which represents predicted PEG or LIT concentration

at time-0 was calculated with the following equation:

$$a_i = \text{antilog}[\text{Log}\ (L2_i) + b_i\ (VT2_i)]$$

where: a_i = The predicted PEG or LIT concentration in rumen fluid of the $i^{\underline{th}}$ steer at time-0.

$L2_i$ = PEG or LIT concentration in the 1 hour rumen fluid sample from the $i^{\underline{th}}$ steer.

$VT2_i$ = time for dosing of the $i^{\underline{th}}$ steer to collection of the 1 hour sample in hour.

An overall coefficient of regression (b_{ct}) was calculated within each shipment by least squares procedures. PEG or LIT concentration in the fluid and estimations of rumen fluid volume were made from the unadjusted samples (1 hour and 24 hour samples) on time of sampling. PEG or LIT concentration in the rumen fluid at time-0 was predicted by the following equation:

$$a2_i = \text{antilog}[\text{Log}(L2_i) + b_{ct}(VT2_i)]$$

Three estimates of rumen fluid volume were calculated. These three estimates were: 1) estimated rumen fluid volume based only on PEG or LIT concentration in the 1 hour sample (V2), 2) estimated rumen fluid volume based on the predicted PEG or LIT concentration at time-0 (a_i), using separate coefficient of regression for each steer (V20) as described by Ulyatt (1964), and 3) estimated rumen fluid volume based on predicted PEG or LIT concentration at time-0 ($a1_i$) using the average regression coefficient within a shipment (V20B). In all cases, the following general equation was used to calculate the rumen fluid volume:

$$\text{Volume} = \frac{D}{L}$$

where: Volume = rumen fluid volume in liters.

D = the grams of PEG or mg of LIT used to dose the steers

L = the amount of marker PEG (g/l) or LIT (PPM) in the rumen fluid sample.

D. RESULTS AND DISCUSSION

Comparison of Mean Values of Estimates

Upon arrival at the auction barn (AAB), all calves in shipment 7 were dosed with a rumen marker (100 g of PEG) and rumen fluid samples were collected at 1 to 3 hours after dosing (1 hour sample). The mean estimated rumen fluid volume calculated directly from the polyethylene glycol (PEG) content of a single rumen fluid sample (V2), was 34.6 + 2.01ℓ (Table 3). The PEG content of that sample (1 hour) and the PEG content of a subsequent 24 hour (16 to 24 hour post dosing) sample were transformed to natural logarithms and regressed on time by individual animal. The resulting individual coefficients of regression were used to predict PEG concentration at the time of dosing (time-0) for each animal, similar to procedures described by Ulyatt (1964), Purser and Moir (1966a) and Ulyatt et al. (1967). Individual rumen fluid volumes (V20) were estimated from the predicted time-0 PEG concentrations and the mean V20 was 31.6 ± 1.88ℓ . The difference between the mean of V2 and V20 (3ℓ) was considered by Ulyatt (1964) to represent the hourly rumen turnover rate. However, in their study rumen fluid volume, except for diurnal variations, was considered to be constant. In this study, the steers were fasted for 24 hours after dosing with PEG, and the assumption of constant rumen fluid volume may be invalid. If rumen fluid volume decreased during this fasting, as observed by Ulyatt et al. (1967),

Table 3. Means and their standard deviation for estimates of rumen volume upon arrival at the auction barn calculated by different procedures.

ITEM	V2[a]	V20[a]	V20B[a]
Shipment 7 - 54 steers, shipped in October, 1977			
Mean, kg	34.55	31.63	31.90
SD	14.69	13.72	14.37
CV	42.52	43.38	45.05
Range	8.67-72.69	8.18-72.25	7.89-73.60
Shipment 11 - 54 steers, shipped in October, 1978			
Mean	45.49	42.51	42.42
SD	13.48	12.57	12.62
CV	29.64	29.57	29.75
Range	19.86-71.50	25.70-67.3	18.80-66.82
Shipment 12 - 64 steers, shipped in November, 1978			
Mean	38.53	37.54	36.72
SD	23.29	23.73	22.19
CV	60.44	63.21	60.43
Range	2.16-95.14	1.73-96.47	2.06-90.67

[a]V2 was calculated directly from PEG or LIT in 1 hour samples. V20 represents the predicted volume at time-0 based on individual b values derived by regressing PEG or LIT concentration in the 1 hour and 24 hour samples on time. V20 represents predicted volume at time-0 based on a mean b value derived by regressing PEG or LIT concentration in the 1 hour and 24 hour samples on time in a single analysis.

subsequent to dosing with PEG, the PEG concentration of the 24 hour sample would be larger than expected under the assumption of constant volume. The higher PEG concentration in the 24 hour would result in a lower predicted time-0 PEG concentration and higher estimates of V20.

Ulyatt (1964) fitted both linear and quadratic dilution curves for rumen marker concentration in samples collected at 1.5, 3, 6, 12 and 24 hours post dosing. The quadratic regression gave lower predicted rumen fluid volume at time-0 than the linear regression. However, evaluation of data collected by Hyden (1961) suggested that no improvement in accuracy of estimating rumen volume was realized when a quadratic regression was used to predict marker concentration at time-0.

The ratio of mean rumen fluid volume (V20 = 31.6ℓ) to mean steer body weight (275 kg) at AAB (.147) was slightly higher that some values for this ratio reported in the literature but was similar to the .12 ratio reported by Purser and Moir (1966a).

Least-squares procedures were used to calculate a coefficient of regression (common b) based on the PEG concentration in all 54 1 hour and 24 hour rumen fluid samples. When the predicted PEG concentration at time-0 (calculated using the common b value) was used to estimate individual time-0 rumen volume (V20B), the mean V20B was 31.9 $\pm$ 1.97ℓ The 8.5 to 9.4 percent higher rumen volume estimates (V2) from a single 1 hour PEG concentration, compared to time-0 estimates (V20 and V20B), could significantly affect interpretation of results in studies involving rumen microbe population changes over time or other rumen parameters expressed on a total rumen basis. However, this indicated individual error due to using a single 1 hour sample to estimate V2 (8.5 to 9.4

percent) is less than the 14 percent and 19 percent error which Hyden (1961) and Ulyatt (1964) suggested were intrinsic to the water soluble marker method of measuring rumen fluid volume.

The calves used in Shipment 11 were from the same two farms which supplied the calves used in Shipment 7. However, lithium sulfate was used as the rumen marker rather than PEG as in the first shipment. Mean V2, V20 and V20B were higher in Shipment 11 than they were in Shipment 7. These differences could be due to differences in rumen fill when the calves arrived at the auction barn and/or differences in rumen volume change between the 1 hour and 24 hour sampling. However, the 2.98 ℓ mean difference between V2 and V20 was similar to the 2.92 ℓ difference observed in Shipment 7. The similarity of these values suggests the rumen turnover rate was similar during the two trials.

Mean V20 and V20B were also similar in Shipment 11. Although different rumen markers were used in the 2 trials, standard errors of mean V2, V20 and V20B values were similar to the standard errors in Shipment 7, but the coefficient of variation (CV) of the estimates of rumen fluid volume in Shipment 7 were larger than the respective CV values in Shipment 11.

Mean estimates of V2, V20 and V20B in Shipment 12 were slightly higher than those estimates in Shipment 7, but were lower than the respective estimates in Shipment 11. The overall coefficients of regression of the log of rumen marker (b_{ct}) concentration on time in Shipment 11 (-0.11) include and in Shipment 12 (-0.11) were similar, but they were slightly lower than the value (-.16) reported by Clark et al. (1972). However, the difference in V2 and V20 was only 1.01ℓ compared to those values of Shipments 7 and 11. This variation is difficult to

explain. The animals in Shipment 12 were not purchased from the same farm from which the calves in Shipments 7 and 11 were purchased. It is possible that either greater animal variation with respect to rumen volume existed at these four farms, or the error of estimating rumen volume may have been greater. The steers used in Shipment 12 were also used in a study which required serial bleeding, and the time between dosing and the 1 hour sample was exactly 60 minutes. The similarity of trends between V2, V20 and V20B mean values within each shipment are encouraging, with respect to the validity of estimating rumen liquid volume from a single post-dosing rumen fluid sample. Purser and Moir (1966a), who dosed sheep with 8 g Cr_2O_3 1 hour before collecting rumen volume, used the single rumen fluid sample (1 hour post-dosing) to estimate rumen volume and compared these estimates to physical volume at slaughter. They suggested that the estimated rumen volumes have meaning as a relative measure of rumen volume because of the significant relationship between the indirect (one sample) and direct (measurement of rumen content at slaughter) estimates of rumen value.

Evaluation of Analysis of the Estimates of Rumen Volume

The calves in Shipments 7 and 11 were purchased from 2 different farms, and those in Shipment 12 were purchased from 4 different farms. In the analysis of variance for each shipment, farm of origin (FO) was included in the statistical model. Calves in Shipment 7 had been subjected to different pre-shipment diets during the last 30 days at FO. Subsequent planned post-shipment treatments were included in the statistical model in order to evaluate initial differences among animals destined to be subjected to various levels of the post-shipment factors. However, the

major interest in this particular study was evaluation of the interpretation of the effect of these factors on estimated rumen volume, when estimates were made directly from the 1 hour rumen fluid sample and when predicted volumes at time-0 were calculated using the overall b value. Analyses of variance of Shipments 7, 11 and 12 are presented in Tables 4, 5 and 6, respectively. V20 was considered to be the most accurate estimate of initial rumen volume, since it was calculated by the method most frequently reported in the literature (Ulyatt, 1964, Hungate et al. 1955 and El-Din and El-Shazly, 1969b).

Comparison of the analysis of the three estimates in each of the shipments suggests that the method of calculation had no effect on statistical interpretation of the results. In most instances the mean square, F value and probability of a higher F value were similar for the three estimates. The error components of variance were similar within each shipment and between Shipments 7 and 11. However, the error variances in Shipment 12 were higher than those in Shipments 7 and 11.

Table 4. Analysis of variance for the effects of farm of origin, pre-shipment treatment and post-shipment treatment on rumen fluid volume (Shipment 7).

		Mean squares, F value and probabilty								
		V2[a]			V20[a]			V20B[a]		
SOURCE[b]	df	MS	F	P	MS	F	P	MS	F	P
FO	1	4644.89	37.04	0.0001	3102.76	24.57	0.0001	3973.24	31.89	0.0001
NA	2	92.28	0.74	0.4868	56.12	0.37	0.6968	47.55	0.38	0.6858
FO x NA	2	6.35	0.05	0.9507	24.63	0.19	0.8238	15.42	0.12	0.8840
PC	2	100.93	0.80	0.4557	184.09	1.46	0.2473	221.14	1.77	0.1853
FO x PC	2	16.44	0.13	0.8776	51.76	0.41	0.6670	60.63	0.49	0.6191
NA x PC	4	213.34	1.70	0.1732	219.74	1.74	0.1647	214.27	1.72	0.1692
FO x NA x PC	4	70.49	0.56	0.6917	77.24	0.61	0.6572	72.54	0.58	0.6777
Residual	33	125.40			126.27			124.60		
CV		32.42			35.52			34.99		
R^2		61.64			55.75			60.19		

[a]V2, V20 and V20B are defined on page 37.

[b]FO = Farm of origin, NA=0, 40 or 80 g $NaHCO_3$/steer/day beginning on feedlot day 1, PC=NI, HE and PW (these levels are explained on page 24).

Table 5. Analysis of Variance for the effects of farm of origin, special treatment and post-shipment treatment on rumen fluid volume (Shipment 11).

		Mean squares, df and values								
		V2[a]			V20[a]			V20B[a]		
SOURCE[b]	df	MS	F	P	MS	F	P	MS	F	P
FO	1	754.44	4.66	0.0381	688.48	5.46	0.0261	687.44	4.90	0.0337
PC Tmt	2	86.05	0.53	0.5928	52.14	0.41	0.6655	79.29	0.56	0.5736
FO x PS	2	20.30	0.13	0.8827	44.41	0.35	0.7060	18.53	0.13	0.8768
Spec. Tmt.	2	419.55	2.59	0.0898	298.44	2.37	0.1106	377.63	2.69	0.0823
FO x ST	2	687.49	4.24	0.0227	617.98	4.90	0.0142	622.06	4.43	0.0195
PS x ST	4	44.87	0.38	0.8910	68.81	0.55	0.7935	39.52	0.38	0.8879
FO x PS x ST	4	106.90	0.66	0.6243	145.63	1.15	0.3496	88.39	0.63	0.6446
Residual	34	162.06			126.11			140.35		
CV		27.99			26.42			34.91		
R^2		40.55			48.44			50.59		

[a]V2, V20 and V20B are defined on page 37.

[b]FO - Farm of origin, NA=0, 40 or 80 g $NaHCO_3$/steer/day beginning on feedlot day 1, PC=NI, HE and PW (these levels are explained on page 24).

Table 6. Analysis of variance for effects of farm of origin, days in the orderbuyer barn and orderbuyer barn ration on rumen fluid volume (Shipment 12).

		Mean squares, df and F values								
		V2[a]			V20[a]			V20B[a]		
SOURCE[b]	df	MS	F	P	MS	F	P	MS	F	P
FO	3	2074.7	4.88	0.0049	2090.0	4.80	0.0055	1883.8	4.88	0.0049
OBB	1	1409.8	3.31	0.0751	1847.9	4.24	0.0451	1279.5	3.31	0.0752
FO x OBB	3	447.5	1.05	0.3786	590.7	1.36	0.2681	405.0	1.05	0.3801
PC	1	4013.4	9.43	0.0035	3700.6	8.49	0.0055	3645.5	9.44	0.0035
FO x PC	3	236.4	0.56	0.6469	190.4	0.44	0.7277	214.4	0.56	0.6473
OBB x PC	1	28.9	0.07	0.7954	14.4	0.03	0.8563	26.2	0.07	0.7958
FO x OBB x PC	3	64.3	0.15	0.9285	109.0	0.25	0.8608	59.0	0.15	0.9275
Residual	47	425.5			435.7			386.3		
CV		53.5			55.6			53.5		
R^2		40.5			41.6			40.5		

[a]V2, V20 and V20B are defined on page 37.

[b]FO = Farm of origin, OBB = either 4 or 10 days in the orderbuyer barn and PC = hay or a 65 percent concentration ration at the orderbuyer barn.

CHAPTER V

RUMEN PARAMETER CHANGES IN MARKET-TRANSIT STRESSED STEERS

A. SUMMARY

During 1977 and 1978, 3 trials were conducted using 172 weanling feeder-steer calves purchased from 7 Tennessee feeder-calf producers. The calves were allotted to at least 1 of 3 pre-shipment treatments 30 days prior to transportation to the auction barn (AB). The 3 pre-shipment treatments (PC) were: 1) Normal Industry conditions (NI) [calves were left with their dam and not fed concentrates during the last 30 days at the farm of origin (FO), and fed a mixed grass hay ration at the order-buyer barn (OBB)]. 2) High-energy - high-antibiotic ration at OBB (HE) [calves left with their dam and fed no concentrates at FO but fed a high-energy - high-antibiotic ration (3.34 kg/steer/day) at the OBB]. 3) Preweaned (PW) [calves weaned and fed a 65 percent concentrate ration plus hay or pasture during the last 30 days at FO and fed a mixed grass hay ration at the OBB].

At the end of the 30 day FO phase, all calves were subjected to the stress of normal AB and OBB environments before being shipped to the feedlot (FL). Rumen samples were collected at FO, arrival AB (AAB), arrival OBB (AOBB), departure OBB (DOBB), arrival at feedlot (AFL), and at days 4 (DAY 4), 11 (DAY 11), 32 (DAY 32) and 144 (DAY 144) at the feedlot. Rumen fluid volume was estimated using the polyethylene glycol (PEG) or lithium sulfate (LIT) marker techniques, and cellulose digestion potential (CDP) was determined by the *in vitro* gas production potential method (GP).

In vitro GP of the rumen fluid and rumen fluid volume followed similar trends during the market-transit (M-T) phase in all trials. Increases in GP and slight increases in rumen fluid volume were observed at AAB, and *in vitro* GP and rumen fluid volume decreased after a 24 hour starvation period in the AB. The rumen parameters returned to pre-shipment levels within 11 days after arrival at the FL. The factors affecting GP during the M-T chain include FO, PC and the length of time calves spent in the OBB. Rumen fluid volume was significantly affected by FO when measured at the farms 30 days prior to marketing, during transit and at most sampling points at FL. The addition of $NaHCO_3$ to the FL receiving diet did not affect rumen fluid volume. PC had a significant effect on rumen fluid volume only at AAB and DOBB in trial 3.

B. INTRODUCTION

During the chain of market-transit events, feeder calves are constantly subjected to various stresses. Economic losses from the effects of morbidity and mortality represent one of the major problems faced by feeder-calf producers and feedlot operators. Numerous hypotheses concerning the factors influencing the incidence of Bovine-Shipping-Fever complex have been reported, but little information is available on the nutritional status of the calves during market-transit. Sinha and Abinanti (1962), in a review of shipping fever in cattle, described inflammation in the rumen, reticulum and intestines as being associated with the Bovine-Shipping-Fever complex. Thus, the effects of starvation and dehydration on the rumen parameters would suggest an influence on the nutritional status of the animal. Purser and Moir (1959) and Esdale and Satter (1972)

reported that during stress, the microbial activity in the rumen decreases, and Meiske et al. (1958) and Warner (1962) demonstrated a reduction in the microbial population during starvation. A reduction in the rumen fluid volume was also reported by Ulyatt et al. (1967), when animals were subjected to starvation.

The objectives of this study were to monitor various rumen parameters [rumen fluid volume, cellulose digestion potential (measured by the gas production of rumen fluid) and pH] in steers during market-transit, and to evaluate factors that affect these parameters.

C. EXPERIMENTAL PROCEDURE

During the Fall seasons of 1977 and 1978, 3 trials were conducted involving 172 weanling steer calves purchased from 7 Tennessee Feeder-calf producers. Thirty days prior to transportation to the auction barn, the calves on each farm in each trial were randomly allotted to at least 1 of 3 pre-shipment treatments (Table 1, p. 25). The ingredients in the pre-transit are presented in Table 2, p. 26.

Each year, the calves were transported 40 to 100 km to an auction in Algood, TN, where they were subjected to AB environment (0.65 - 8.52 m^2 of space per calf without feed or water) for 24 hours. Then they were subjected to OBB environment (1.8 - 2.3 m^2 of space per calf with access to feed and water) for 72 hours. At the end of the 72 hour period, the calves were loaded on trucks and transported to the respective feedlots.

The nutrient composition of the feedlot diets in trials 1 and 2 is given in Table 7. Trial 3 steers grazed in wheat pasture during the post-transit phase. In trial 1, sodium bicarbonate ($NaHCO_3$) was fed

Table 7. Nutrient composition of feedlot ration[a].

Ingredient	IRN[b]	Composition				
		Crude Protein	Crude Fiber	Ether Extract	NFE	Ash
Corn, aerial pt, ensiled, mature, well eared mn 30, mx 50 dry matter, (3)[c]	3-08-153	9.2	21.1	2.4	62.9	4.4
Corn, dent yellow, grain, gr 2 US mn 54 wt, (4)	4-02-93	10.2	2.1	4.9	81.7	1.3
Cotton, seed w some hulls, mech-extd grnd, mn 41 prot mx 14 for mn 2 fat, (5)	5-01-617	44.8	13.8	2.3	33.0	6.1

[a]percent, dry matter basis

[b]international reference number

[c]33 percent dry matter, as fed

at 0, 40 and 80 g/calf/day at the feedlot to one third of the calves from each farm that had received the NI, HE and PW diets respectively, at the FO and OBB. In trial 2, all calves were fed hay at OBB and were assigned to post-treatment $NaHCO_3$ levels (0, 40 and 80 g/calf/day) at the feedlot. In trial 3, one half of the calves were fed hay at OBB and the rest were fed the HE diet described in Table 2, p. 26. One half of the calves subjected to each PC treatment stayed in the OBB for 4 days while the remaining calves stayed for 10 days.

Sampling

At each of the sampling points (FO, AAB, AOBB, DOBB, AFL and other dates at the feedlot), weight was taken and rumen fluid samples were collected via a stainless steel strainer connected to a tygon tubing, with suction supplied by a 50 ml syringe. This apparatus was similar to the one developed by Raun and Burroughs (1962). The pH of the rumen fluid was taken immediately after sampling by means of a Fisher portable pH meter with a glass electrode, and two 20 ml aliquots were used in a gas production experiment to determine the cellulose digestion potential of the rumen fluid. A 10 ml aliquot was preserved by diluting with 10 ml of 40 percent Formalin and stored for protozoa counts. Another 10 ml sample was collected and frozen immediately until volatile fatty acid (VFA) analysis could be performed. The calves were dosed with either 1 g of lithium sulfate (LIT) or 100 g of polyethylene glycol (PEG) and at 1 to 3 hours after dosing with the marker (PEG or LIT), aliquot samples of rumen fluid were collected and frozen for analysis of marker concentration and the estimation of rumen fluid volume.

The method used in measuring gas producing (cellulose digestion) potential of the rumen fluid was similar to the one developed by Hungate and modified by El-Din and El-Shazly (1969b). Aliquots of 20 ml rumen fluid were incubated with 1 g of substrate (composed of feedlot receiving ration) and the amount of gas produced in 1 hour was recorded. Carbon dioxide was employed to maintain anaerobic conditions.

D. RESULTS AND DISCUSSION

In Vitro Gas Production (GP)

The mean gas production (GP) or cellulose digestion potential are presented in Table 8. The GP of the rumen fluid increased between the FO sample and AAB. In trials 1 and 2, the dehydration of the calves when hauled from the farm to the auction barn could have resulted in the increase in concentration of the microbial population in the rumen fluid and thus increased GP. However, in trial 3, no such increase in GP between FO and AAB was observed. One possible explanation could be that the calves in trial 3 had access to water at the farm prior to transporting to AB and were less dehydrated. The shorter distance traveled from the FO to the AB could also be a factor in reducing dehydration. This is especially true in calves from FO 42 which was located near the AB. An analysis of variance for the effects of FO on the GP indicated that FO was a significant factor ($P<.05$) influencing GP in all 3 trials (Table 9). With the exception of the GP at AOBB, FO significantly affected GP during the entire market-transit feedlot phase. The 24 hour starvation of the calves prior to AOBB could have resulted in an extremely rapid decrease in GP which tends to mask the FO effect. This is in agreement with findings

Table 8. Overall means and standard error of *in vitro* gas production[a] (cm^3).

Location[b]	Trial 1		Trial 2		Trial 3	
	Mean	SE	Mean	SE	Mean	SE
FO	6.52	0.256	6.80	0.625	6.70	0.306
AAB	10.26	0.747	9.09	0.672	5.10	0.458
DOBB	4.05	0.393	3.29	0.422	5.73	0.582
AFL	4.44	0.331	1.89	0.274	--	--
Day 4	7.95	0.535	6.61	0.473	--	--
Day 11	8.00	0.438	5.55	0.516	--	--
Day 32	7.44	0.396	8.36	0.695	--	--
Day 144	10.83	0.654	11.46	0.410	--	--

[a] as a measure of cellulose digestion potential

[b] sampling locations are defined on page 45

Table 9. Mean gas production[a] (cm^3) by FO

Farm of Origin[c]	Sampling location[b] and times after AFL								
	FO	AAB	AOBB	DOBB	AFL	Day 4	Day 11	Day 32	Day 144
Trial 1									
3	6.94	13.71[d]	--	4.98[d]	4.94	8.46	7.79	7.94	10.53
12	6.10	7.78[e]	--	3.12[e]	3.94	7.44	8.19	6.99	11.13
Trial 2									
3	10.02[d]	10.53[d]	3.07	--	1.89	7.72[d]	6.20	7.39	11.84
12	3.58[e]	5.65[e]	3.50	--	1.61	5.45[e]	4.87	9.32	11.07
Trial 3									
41	6.84[e]	7.73[d]	--	5.96[d,e]					
42	5.13[f]	5.53[c]	--	7.04[d]					
43	5.80[e,f]	3.05[f]	--	4.11[e]					
44	9.13[d]	3.56[f]	--	5.65[d,e]					

[a] as a measure of cellulose digestion potential

[b] sampling locations are defined on page 45

[c] the farm of origin number

[d,e,f] means in the same column and trial superscripted with different latters are different ($P<.05$)

by Meiske et al. (1958) and Warner (1962) who reported that microbial activity decreases rapidly during starvation. After the 72 hours in the OBB, where feed and water were available and where rumen fill probably increased, the effect of FO was again significant. The GP was about "normal" (pre-shipment levels) by the fourth day in the feedlot (approximately 7 days from the onset of the 24 hour starvation period). This is similar to the findings by Quin (1943) that glucose fermenting ability (measured by in vitro gas production techniques) of the rumen fluid from sheep starved for 65 hours did not return to normal until about the tenth day after refeeding.

PC also had a significant effect on the GP of the rumen fluid during market transit (Table 10). The GP of rumen fluid from HE calves was significantly higher than that from NI and PW calves at DOBB, AFL and DAY 4 suggesting that feeding a high-energy-high-antibiotic concentrate ration during the 72 hours in the OBB prevented the drastic decrease in microbial activity following the 24 hour starvation period. It suggest also that pre-transit concentrate diets tend to increase the recovery rate of rumen microbial activity after the 24 hour starvation. In trial 3 the rumen fluid from the calves that stayed 10 days in the OBB had significantly higher ($P<.05$) mean GP at DOBB than that of calves that stayed only 4 days (6.65 vs 4.80 respectively). (Table 11)

The higher mean GP of calves that were in the OBB for 10 days could be the result of the longer time available for the microbial activity to return to "normal" after the 24 hour starvation that occurred in the AB. This interpretation would agree with findings by Quin (1943).

Rumen Fluid Volume

The mean estimated rumen fluid volumes are presented in Table 12. In trials 1 and 3 the mean difference in the rumen fluid volume at

Table 10. Mean gas production[a] (cm^3) by pre-shipment treatment (Trials 1 and 3)

	Sampling locations[b] and times after AFL								
	FO	AAB	AOBB	DOBB	AFL	Day 4	Day 11	Day 32	Day 144
Trial 1									
NI[c]	6.37	9.62	--	2.97[e]	3.31[e]	6.09[e]	7.20	8.53	12.42
HE[c]	6.65	9.98	--	5.72[d]	5.73[d]	9.49[d]	8.38	6.61	10.17
PW[c]	6.54	11.17	--	3.46[e]	4.30[d,e]	8.27[d,e]	8.37	7.31	10.04
Trial 3									
NI[c]	6.33	4.68	--	2.61[e]	--	--	--	--	--
HE[c]	7.08	5.53	--	8.84[d]	--	--	--	--	--

[a] as a measure of cellulose digestion potential

[b] sampling locations are defined on page 45

[c] NI = Normal Industry, HE = fed a 50 percent high-energy high-antibiotic ration at the orderbuyer barn, PW = Preweaned

[d,e] means in the same column and trial superscripted with different letters are significantly different ($P<.05$)

Table 11. Mean gas production[a] (cm^3) by post-shipment supplementation (Trials 1 and 2) and the number of days in the OBB (Trial 3).

	Sampling location[b] and times after AFL								
	FO	AAB	AOBB	DOBB	AFL	Day 4	Day 11	Day 32	Day 144
Trial 1[c]									
1	6.52	10.25	--	4.05	4.44	7.23	9.00	7.01	8.91[f]
2						9.49	7.49	8.15	10.49[f]
3						7.13	7.46	7.14	13.56
Trial 2[c]									
1	6.8	9.09	3.29	--	1.75	5.70	4.91	8.05	11.79
2						7.66	6.07	9.10	11.13
3						6.45	5.68	7.92	11.48
Trial 3[d]									
1	6.71	3.40	--	4.80[f]					
2				6.65[e]					

[a] as a measure of cellulose digestion potential

[b] sampling locations are defined on page 45

[c] 1 = nonsupplemented control, 2 = 40g $NaHCO_3$/steer/day in the FL ration and 3 = 80g $NaHCO_3$/steer/day

[d] 4 days in the OBB, 2 = 10 days in OBB

[e,f] means in the same column and trial superscripted with different letters are significantly different ($p<.05$)

Table 12. Overall means and standard error of estimated rumen fluid volume (ℓ).

Location[a]	Trial 1		Trial 2		Trial 3	
	Mean	SE	Mean	SE	Mean	SE
FO	34.1	1.79	34.5	3.17	37.7	3.81
AAB	34.5	2.06	45.5	1.87	38.7	2.93
DOBB	31.9	1.14	--	--	24.8	1.64
AFL	32.2	1.42	32.1	1.56	21.3	0.93
Day 4	32.7	1.48	37.6	1.63	--	--
Day 11	35.4	1.50	36.9	1.80	--	--
Day 32	41.0	2.45	38.8	1.10	17.7	0.76
Day 144	--	--	43.0	1.28	--	--

[a]sampling locations are defined on page 45

FO and AAB was approximately ℓ. In all cases AAB rumen fluid volume was greater than the FO volume. One explanation would be that during the 30 day period on the farm, the rumen size increased due to growth of the young calves and therefore they had a higher rumen capacity. A significant ($P<.05$) decreased in rumen fluid volume was observed in the calves at AOBB after spending 24 hours in the AB, and at DOBB after 72 hours in the OBB. In trial 3 there was a further decrease in rumen fluid volume when the calves were hauled over a distance of 1360 km to the feedlot. However, no such decrease between DOBB and AFL was recorded in trial 1. This could be explained in that the calves in trial 3 were hauled over a much longer distance than those in trial 1 (1360 km vs 640 km respectively). Calves hauled longer distances probably underwent more dehydration. Estimates of the rumen fluid volume from samples obtained on DAY 4 showed "normal" volume, indicating that the calves had recovered sufficiently from the market-transit stress to return to normal daily fluid intake by feedlot DAY 4. Quin (1943) reported that sheep starved for 65 hours required 3 to 4 days to regain pre-starvation feed intakes.

The analysis of variance of data by trial for the effect of FO on estimated rumen fluid volumes indicated that FO had a significant effect on rumen fluid volume at FO and AAB in all trials (Table 13). In trial 1 there was a consistent significant effect of FO on rumen fluid volume throughout the market-transit events and at the feedlot until DAY 4. Non-significant differences were then observed until DAY 144. The effect of FO on the rumen fluid volume in trial 2 was significant at FO, AAB, DAY 11 and DAY 144 at the feedlot. In trial 3, FO was a significant factor at all sampling points except DOBB.

Table 13. Mean estimated rumen fluid volume (ℓ) of calves raised at different farms.

	Sampling location[a] and times after AFL							
	FO	AAB	DOBB	AFL	Day 4	Day 11	Day 32	Day 144
Trial 1 (1977)								
3[b]	27.38[c]	24.34[c]	26.81[c]	--	27.40[c]	29.36	33.92	39.58
12[b]	40.73[d]	44.36[d]	37.05[d]	--	36.74[d]	35.63	36.63	42.36
Trial 2 (1978)								
3[b]	43.82[c]	41.74[c]	--	30.75	35.61	32.38[c]	38.96	39.74[c]
12[b]	22.58[d]	49.23[d]	--	33.58	39.76	41.02[d]	38.64	46.13[d]
Trial 3 (1978)								
41[b]	34.80[d,e]	32.38[d,e]	22.92	20.07[d,e]			18.88[c]	
42[b]	39.78[c,d]	51.56[c]	26.73	25.59[c]			19.89[c]	
43[b]	51.37[c]	45.57[c,d]	24.77	23.05[c,d]			19.36[c]	
44[b]	25.56[e]	25.43[e]	24.67	16.45[e]			12.36[d]	

[a]Sampling locations are defined on page 45

[b]Farm of origin number

[c,d,e]Means in the same column and trial superscripted with different letters are significantly different ($p<.05$).

The addition of $NaHCO_3$ to the post-transit diet did not affect the estimated rumen fluid volume significantly ($P>.05$). The number of days the calves spent in the OBB was significant only in the DOBB estimations (Table 14). Those calves that stayed in the OBB for 10 days had a larger mean rumen fluid volume at DOBB than those that stayed for only 4 days. This was probably due to the fact that a longer recovery period following AB starvation occurred during the 10 days in the OBB.

In trial 3, pre-shipment treatments were significant only at AAB and DOBB (Table 15). At AAB the HE calves had a larger rumen fluid volume than the NI calves; however, the NI calves had a significantly larger rumen fluid volume at DOBB. At the OBB the NI calves were fed had instead of a concentrate ration. Therefore, the bulkiness of the ration may have contributed to the distention of the rumen.

pH

The pH values in trial 1 by FO, $NaHCO_3$ treatment at the OBB and pre-shipment treatments are presented in Table 16. The overall mean pH values are presented in Table 17.

Analysis of variance showed that FO had a significant effect ($P<.05$) on rumen pH at AAB, DOBB and AFL. Rumen pH upon arrival at AB exhibited a definite decrease from the value at FO (7.21 to 6.86). After the 24 hour starvation period in the AB, calves from farm 3 had completely recovered from the resulting pH decrease by DOBB. The rumen pH recovery rate of calves from farm 3 was significantly higher than that of calves from farm 12 upon arrival at the feedlot. An increase in pH after a 3-day starvation was reported by Meiske et al. (1958), but the increase in pH in this trial was not as great. However, the animals in

Table 14. Mean estimated rumen fluid volume (ℓ) by post-shipment $NaHCO_3$ supplementation (Trials 1 and 2) and number of days in OBB (Trial 3).

	Sampling locations[a] and times after AFL							
	FO	AAB	DOBB	AFL	Day 4	Day 11	Day 32	Day 144
Trial 1[b]								
1	34.06	34.63	31.93	--	36.03	33.74	31.98	45.64
2					30.22	31.38	35.46	38.73
3					30.11	32.97	38.58	38.42
Trial 2[b]								
1	34.80	45.56	--	32.01	37.29	35.92	37.02	44.97
2					41.69	34.35	38.09	42.12
3					33.85	40.38	41.05	42.00
Trial 3[c]								
1	37.76	38.60	21.27[d]	21.14	--	--	18.23	--
2			28.16[e]	21.46	--	--	17.21	--

[a]Sampling locations are defined on page 45

[b]1 = nonsupplemented control, 2 = 40g $NaHCO_3$/steer/day in the FL ration and 3 = 80g $NaHCO_3$/steer/day

[c]1 = 4 days in the orderbuyer barn, 2 = 10 days

[d,e]means in the same column and trial superscripted with different letters are significantly different ($P<.05$)

Table 15. Mean estimated rumen fluid volume (ℓ) by pre-shipment treatment[a] (Trials 1 and 3).

	Sampling location[b] and times after AFL							
	FO	AAB	DOBB	AFL	Day 4	Day 11	Day 32	Day 144
Trial 1								
NI	32.27	31.79	32.17	--	35.01	32.17	35.53	51.32[c]
HE	33.92	35.79	29.10	--	28.63	31.63	39.01	44.51[c]
PW	35.98	36.29	34.52	--	33.00	34.27	32.33	38.40[d]
Trial 3								
NI	39.36	31.09[c]	30.98[c]	21.09		17.40		
HE	36.16	46.21[d]	18.44[d]	21.50		18.03		

[a]NI = Normal Industry, HE = fed a 50 percent high-energy high-antibiotic ration at the orderbuyer barn, PW = preweaned

[b]Sampling locations are defined on page 45

[c,d]Means in the same column and trial superscripted with different letters are significantly different ($P<.05$).

Table 16. Mean pH values and their standard error for Trial 1

Location[a] and times after AFL	Mean	SE
FO	7.21	0.024
AAB	6.86	0.053
DOBB	6.83	0.046
AFL	7.19	0.023
Day 4	6.80	0.030
Day 11	6.77	0.039
Day 32	6.38	0.037
Day 144	7.09	0.051

[a]Sampling locations are defined on page 45

Table 17. Mean rumen pH of calves raised at different farms, subjected to different pre-shipment treatments and fed various levels of $NaHCO_3$ at the feedlot.

	Sampling locations[a]						
	FO	AAB	DOBB	AFL	Day 4	Day 11	Day 32
FO							
3[b]	7.22	6.95[c]	7.02[c]	7.25[c]	6.81	6.74	6.35
12[b]	7.20	6.77[d]	6.64[d]	7.12[d]	6.80	6.80	6.41
$NaHCO_3$, level[e]							
1	7.21	6.86	6.83	7.19	6.80	6.76	6.29
2					6.81	6.71	6.40
3					6.80	6.85	6.47
PC[f]							
NI	7.13[c]	7.04[c]	6.86	7.18	6.81	6.81	6.35
HE	7.28[d]	6.97[c]	6.72	7.17	6.82	6.86	6.35
PW	7.22[c,d]	6.58[d]	6.90	7.21	6.78	6.65	6.44

[a]Sampling locations are defined on page 45

[b]farm of origin number

[c,d]Mean in the same column and trial superscripted with different letters are significantly different ($P<.05$).

[e]1 = control, 2 = 40g $NaHCO_3$/steer/day at the feedlot, 3 = 80g $NaHCO_3$/steer/day

[f]NI = nonsupplemented control, HE = fed a 50 percent high-energy high-antibiotic ration at the OBB, PW = preweaned

this trial were starved for a period of only 24 hours. No significant effect on rumen pH due to the addition of 0, 40 and 80 g of $NaHCO_3$/steer/day to the feedlot ration was observed.

The effect of pre-shipment treatments on the pH at FO and ABB was significant. This significant effect was not observed upon departure from the OBB or at any of the sampling points at the feedlot. The pH value of PW calves which were fed a concentrate diet at FO decreased more between FO and ABB sampling than that of calves left with their dams and not fed concentrates. Since the PW calves were fed a concentrate diet during the last 30 days at FO, these results are in agreement with the report by Kick et al. (1938) that addition of corn to the rations caused a decrease in pH. Work by Latham et al. (1971) also confirmed that cereal rations produced a slightly lower pH value in the rumen than fibrous diets. Reid et al. (1957) and El-Shazly et al. (1961) also suggested that decreased pH values could be the result of acids produced during starch fermentation.

E. CONCLUSION

The results of this study indicate that there is a definite reduction in the magnitude of rumen parameters cellulose digestion potential (measured by in vitro gas producing potential) and rumen fluid volume between the auction barn to arrival at the feedlot of the market-transit chain. This tends to suggest that the nutritional status of the animals during this period was subnormal and thus could result in greater susceptibility to diseases, with the result being expressed in terms of high morbidity and mortality in calves immediately after arrival at the feed-

lot. The addition of high-energy concentrate feed at the farm of origin or during the orderbuyer barn phase appeared to improve the nutritional status of the animal, especially during the orderbuyer barn period. The results also showed that farm of origin is a significant factor in rumen parameter changes and should be taken into consideration in all future studies involving market-transit changes and feedlot adaptation in feeder-steer calves.

CHAPTER VI

SUMMARY

One of the objectives of this study was to evaluate the error associated with estimating rumen fluid volume (V2) in field studies using the marker concentration in the rumen fluid sample collected 1 hour after dosing with either lithium sulfate or polyethylene glycol. A comparison of this estimate with the rumen volume (V20) calculated from predicted marker concentration at time of dosing using individual regression coefficients, and that (V20B) calculated from predicted marker concentration at time of dosing using a common regression coefficient were also made. Rumen parameter (rumen fluid volume, _in vitro_ gas producing potential and pH) changes of 172 weanling feeder-steer calves subjected to the marker-transit chain were evaluated and factors affecting these changes were also examined.

Mean V2, V20 and V20B in Shipment 7 were 34.6 $\pm$ 2.01ℓ, 31.6 $\pm$ 1.88ℓ and 31.9 $\pm$ 2.01ℓ, respectively. In Shipment 11, mean V2, V20 and V20B values were higher than those in Shipment 7 but the mean difference between V2 and V20 were similar even though different markers were employed. Standard error of mean V2, V20 and V20B were also similar in the 2 shipments though the coefficients of variation (CV) of the estimates of rumen fluid volume in Shipment 7 were larger than the respective CV values in Shipment 11. Mean estimates of V2, V20 and V20B in Shipment 12 were slightly higher than those in Shipment 7 but lower than the respective estimates in Shipment 11. Difference between V2 and V20 in Shipment 12 was only 1.01ℓ compared to 3.00ℓ and 2.92ℓ in Shipments 7 and 11, respectively. Comparison of the analysis of the 3 estimates V2, V20 and V20B in each of the shipments suggested that the method of calculation had no

effect in the statistical interpretation of the results.

In vitro gas production (GP) of the rumen fluid and rumen fluid volume follow similar trends during the market-transit phase in the 3 trials. Increases in GP and slight increases in rumen fluid volume were observed on arrival at the auction barn, and GP and rumen fluid volume decreased after a 24 hour starvation period in the auction barn. The rumen parameters returned to pre-shipment levels within 11 days after arrival at the feedlot.

The factors affecting GP during the market-transit chain include the farm of origin, pre-shipment treatments and the length of time the calves spent in the orderbuyer barn. Rumen fluid volume was significantly affected by the farm of origin differences when measured at the farms 30 days prior to marketing, and at most sampling points at the feedlot. The addition of sodium bicarbonate to the feedlot receiving diet did not have any significant effect on rumen fluid volume. Pre-shipment treatment had a significant effect on rumen fluid volume only at the arrival auction barn and departure orderbuyer barn phase of the market-transit chain in trial 3.

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