WATER KINETICS IN THE RUMEN

OF BEEF CATTLE

By

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CHAPTER I

INTRODUCTION

Animal performance, is a function of feed intake and ruminal digestion. Consequently, knowledge about rumen function and the factors affecting digestion and passage rate of feed particles and liquid digesta may help to maximize the utilization of feedstuffs by ruminant animals.

Digestive processes occurring in the rumen are very dynamic and complex; the system involves multiple pools of liquid and solid digesta that remain closely associated to each other. In addition, these pools are subject to different flow and passage rates through the rumen; such differences can have a dramatic effect on flux and efficiency of nutrient utilization.

Despite extensive research on ruminal digestion, critical basic information is lacking about how fluids and solids interact in the rumen during fermentation, digestion and passage of ingested feedstuffs. Level of feed intake and physical characteristics of the diet presumably are the two major factors regulating ruminal turnover and influencing digestibility, though saliva secretion and water intake can be influenced directly by these two factors.

Saliva production and presumably water intake might be expected to promote greater washout of soluble substances and small particles from the rumen. Moreover, saliva and solutes such as salts and volatile fatty acids have an impact on rumen volume, creating osmotic gradients that cause net outflux or influx of liquids through the rumen.

How flux of water in and out of the rumen changes with level of intake needs more definition, because salivary flow and influx through the ruminal wall can be altered by ruminal conditions which affect rumination and osmolality.

Previous experiments have demonstrated that ruminal osmolality has an impact on rumen function (Warner and Stacy, 1965; Ternouth and Beattie, 1971; Bergen, 1972; Warner and Stacy, 1977; Bennink et al., 1978; Phillip et al., 1981; Ferreiro, 1986; Carter and Grovum, 1989). The majority of these studies have used sheep as the experimental animal, therefore results from these studies may not be entirely applicable to cattle. Reviews on water intake by cattle (Leitch and Thomson, 1944; Winchester and Morris, 1956; Church, 1971; Castle and Thomas, 1975; ARC, 1980; Squires, 1988) are quite extensive, but data on water intake in digestion studies where liquid or solid markers are used is scarce.

Passage rate studies are abundant; however, available data on quantitative origin of water in the rumen, as well as percentages of liquids that never equilibrate with ruminal contents, remain limited. Surprisingly, changes in

rumen volume with time after feeding are not well defined. Liquid and solid passage rate have been reported to vary diurnally; unfortunately, relatively little is known about the interaction of diet type, level of feed intake, frequency of feeding and water intake on rumen volume.

The objectives of the research reported in this dissertation are:

1. To study diurnal variations in liquid and solid rate of passage using external markers.
2. To determine changes in osmolality in ruminal liquid and blood serum with time after feeding in beef cattle fed concentrate or hay diets.
3. To assess quantitatively the origin of ruminal water in beef cattle using external markers.
4. To test the effect of two feed additives (monensin and lasalocid) on water intake and liquid rate of passage.
5. To determine the effect of diet type and three different levels of feed intake on water consumption, liquid passage rate and rumen liquid volume as measured by evacuation of ruminal contents.
6. To compare the behavior of two liquid markers in the rumen when dosed in the drinking water.
7. To estimate the percentage of drinking water that evades the rumen, using two methods.

CHAPTER II

REVIEW OF LITERATURE

This chapter, will outline the literature relevant to the main topic of this dissertation.

This review is far from comprehensive, due to the enormous amount of information available in some of the areas being studied. Chapters are presented following the Journal of Animal Science style and format. Selected literature is addressed in each individual section. This review will discuss primarily those factors affecting water dynamics in the rumen. Among these factors, water intake, distribution of fluids in the rumen, osmolality of ruminal contents, ruminal volume, liquid passage rate and the methods used to estimate these variables will be reviewed.

Water and its Relation to Animal Productivity.

The role of water in the ruminant animal may be viewed from several different perspectives to understand its importance as a factor limiting animal production. From the agricultural standpoint, availability of water is essential for plant and animal production. When agricultural products are scarce due to lack of water, animal productivity is jeopardized. To emphasize this relationship, McMillan

(1965) reported that the total amount of water (including that needed to grow the feed) to produce one kg of meat or one liter of milk, was 110 metric tons and 3,300 liters, respectively.

Water supply becomes more critical as the level of production increases. High producing dairy cows increase their water intake by up to 30% during the last 4 months of pregnancy. One of the largest feedlots in the United States, uses 30,000 liters per minute just to water its cattle (Teeter, 1985). Waldo et al. (1965) indicated that water intake was 3.2 to 4.9 kg for every kg of dry matter consumed by Holstein heifers fed different diets. ARC (1965) recommended up to 6.5 kg of water/kg of dry matter intake for young calves. These figures briefly illustrate the role of water in livestock and agricultural production.

From the animal standpoint, drinking water typically represents the major source of liquid intake, usually accounting for up to 90% of the total fluid consumed (Waldo et al., 1965). Although 50 to 60% of the weight of an adult cow is water, this figure varies with age, nutritional status of the animal and sex (females have slightly less water than males do). MacFarlane, (1976) estimated that 12 to 20% of the body's water is present in the rumen, abomasum and intestines. Other observations (Warner and Stacy, 1968b) indicate that approximately 10% of the body weight of Merino ewes consuming alfalfa diets, is ruminal water. Percentages of water in the rumen vary with the type of

diet, rumination and salivation. Consequently, fluid entering the rumen includes saliva and drinking water in approximately equal amounts (Balch, 1958). Bailey (1961) estimated that saliva supplied 70 to 90 percent of the total fluid entering the reticulo-rumen of a mature cow.

Because ruminants consume fibrous material and feeds with a low content of moisture, they require a large amount of fluid for proper ruminal fermentation and digestion. Ruminal content weight can vary from 40 to 125 kg; and the dry matter percentage of the content could be as high as 17%. Liquids in the rumen are closely associated to solids; however, kinetics of liquids and solids within the rumen differ (Owens and Goetsch, 1986).

Because solids are transported from the rumen by fluid (Poutiainen, 1968), fluids play a role in passage of feed particles to the lower gastrointestinal tract. Because water is a major component of ruminal contents, the fate of liquids in the rumen are of interest. The passage rate of water from the rumen is a function of dietary factors that increase osmolarity (Faichney et al., 1980 1981). Solutes in feed, saliva or products of fermentation thereby enhance passage rate.

Water Intake and Drinking Patterns in Ruminants.

The importance of water intake has been described in a number of reviews (Schalk and Amadon, 1928; Leitch and Thompson, 1944; Winchester and Morris, 1956; ARC, 1965;

Church, 1971; MacFarlane, 1976; ARC, 1980; NRC, 1981; Shirley, 1986; Squires, 1988). These studies indicate that the proportion of water in the rumen is a function of diet composition, hence animals eating high moisture forages will have greater rumen volume when compared to animals fed dry hay. Although dry matter intake is the primary factor affecting water intake (Shirley, 1986) physiological conditions, stage of growth of the animal, water availability, quality of water, temperature of water offered, and ambient temperature all can alter the intake of free water (NRC, 1981). For yearling feedlot cattle in Iowa, water intake was almost doubled in summer vs winter (31.2 vs 19 liters/day; Huffman and Self, 1972). Recently Hicks et al. (1988) indicated that water intake by feedlot cattle fed in Oklahoma during summer was 38 liters per day, and that the amount of water consumed was influenced by both dry matter intake and by environmental temperature. The effect of temperature on water intake is dramatic. As ambient temperature increases, water intakes increase drastically. Winchester and Morris (1956) summarized data on water intake as influenced by environmental temperatures. Church, (1971) suggested that the data from Winchester and Morris may not be applicable under outdoor or farm conditions because their values were generated primarily from calorimetric chamber studies. Yet, most proposed values are lower than those suggested by ARC (1965; 6.5 kg of water per kg of dry matter intake for young calves).

Canadian workers (Degen and Young, 1984) tested the effect of ingestion of warm, cold and frozen water by steers. Ingestion of snow or frozen water reduced water intake, rumen volume and, consequently dry matter of ruminal contents. In addition, consumption of liquid water was preferred over the snow or frozen water. Although results of these studies illustrate the effect of cold temperature on water intake, animals may behave differently under field conditions. As demonstrated earlier by the same authors (Young and Degen, 1980), continuous access to snow or water resulted in similar water intakes.

The effects of cooling water in hot environments for feedlot cattle have been somewhat variable. Harris et al. (1967) indicated that cooling the drinking water of finishing steers maintained at 31°C daily temperature did not alter performance of animals. In contrast, Lofgreen et al. (1975) found that feed intake, body weight and energy utilization of British steers was increased when the water was cooled from 32°C to 18°C. Though intake tend to rise rapidly when temperature exceeded 30°C, variations among individuals make it difficult to characterize water needs (NRC, 1981). Shirley (1986) has suggested that water intake of a 450 kg steer under different temperatures (4, 21 and 32°C) will be 28, 46 and 66 liters per day, respectively.

Environmental factors, physical form of the feed and diet composition, have a direct effect on drinking behavior in cattle (NRC, 1981). Drinking patterns vary greatly from

animal to animal under farm, pasture or rangeland conditions. Reports by Leitch and Thompson (1944) on pregnant heifers fed either hay or concentrate diets showed that animals receiving free choice water drank more than did heifers watered once daily. Moreover, animals can drink all the water they needed for 24 h at one time. Similarly, animals under free range conditions in summer may drink water only once per day; during winter, animals can go up to 3 days without drinking (Squires, 1988). In contrast to these reports, Castle et al. (1950) observed that dairy cattle drank 2 to 5 times each day when water was available. Castle and Thomas (1975) showed that about 40% of the water consumed by dairy cattle was drunk between 1500 and 2100 h. Likewise, drinking time ranged 2 to 8 min per cow per day; rate of drinking ranged from 4.5 to 15 kg per minute. Drinking behavior of feedlot cattle appears to be similar to that of dairy cattle. Ray and Roubicek (1969), observed that the majority of the time, feedlot cattle drank in the late afternoon and at night. Recent studies by Sekine et al. (1989) with Holstein steers, demonstrated that frequency of drinking is closely related to the dry matter content of the diet; and the pattern of drinking was more variable in animals fed high moisture forage than in those fed hay. In contrast to the results of Castle and Thomas (1975), they found that steers fed hay drank primarily during the 3 h post-feeding period.

Dry matter content of the diet is the primary factor influencing water intake in cattle. However, other factors such as physical form of the diet, protein and mineral content of the diet also can alter water intake. Data by Utley et al. (1970), indicated that nitrogen retention of steers fed high concentrate diets tended to increase, when water was restricted to 60% of ad libitum intake; moreover, a significant negative correlation was observed between nitrogen retention and total nitrogen excretion in urine.

Ruminants typically have an alkaline urine when they are grazing or eating forages. This is due primarily to the high K^+ content in the cells of plants; in contrast, with high protein diets or during starvation, urine becomes acid as a response to high protein excretion by the kidneys, and animals tend to drink more water due to their increased urinary output.

The effects of Na^+, Cl^-, K^+ and bicarbonates on water intake of penned animals have received more attention than of other minerals. But under rangeland conditions, toxic minerals are extremely important.

In general, water intake increases as the level of salts in water or feed increases; however, cattle and sheep have a maximum tolerance level to salt. Weeth and Haverland (1961) measured water intake by heifers receiving either a 1.5 or 1.75% NaCl in the drinking water. In winter, water intake was depressed 24 and 42.4%, respectively, compared with animals consuming a 1.25% NaCl diet. But in summer,

water intake increased 46.6 and 69% when 1.0 or 1.2% of NaCl were included in the drinking water, respectively. Squires (1988) reported that sheep under free desert range can tolerate high loads of salt (200 g/day) only if water is available. Cattle on semi-arid areas, also can tolerate saltbush vegetation, if water is available (MacFarlane, 1976). In contrast, recent data (Hicks et al., 1988) indicated that increasing the dietary salt level from 0 to .5% in feedlot cattle tended to decrease water intake. Whether these results were due to a lowered dry matter intake rather than to salt intake alone is not clear, although dietary salt at this level had no effect on feed intake. Although animals are more tolerant to salt in the diet than in the drinking water, diet salt levels over 1% have major effects on water intake. One of the most evident effects of water intake on the physiology of the animal is water deprivation. Cattle show faster discomfort when deprived of water. Monozygotic twin beef steers have shown to decrease their feed intake 47% after restriction of water for 12 to 48 h periods (Bond et al., 1976). Warner and Stacy (1968b) indicated that sheep with restricted access to water, often drank all their water after eating. Observations with ruminal cannulated steers deprived in sequence of feed and water for 24 h periods, demonstrated that ruminal fermentation patterns were severely altered. It took 3 to 5 days for VFA levels to return to prefast values (Cole and Hutcheson, 1981). The effect of water

restriction in lactating dairy cows, was studied by Little et al. (1978). Dry matter intake and milk yield decreased by 24 and 16%, respectively; changes became apparent after the first 24 h of restriction. Further observations on Na^+, urea and osmolality of serum and urine, indicated that these parameters were increased in the deprived animals. Water restriction, also resulted in loss of body weight during the first week of deprivation. But after water was offered, animals recovered their lost weight within 4 days. In general, water deprivation in cattle results in reduced urine and feces, thus reduceing water loss by the body. If water is severely restricted, urine becomes more concentrated and osmolality increases. As a result of these conditions, plasma volume drops and hemoconcentration occurs. Packed cell volume (hematocrit), normally 33 to 40% can double to 60% in calves that are severely dehydrated. Moreover, these changes are associated closely with a decline in blood Na^+, Cl^- and occasionally K^+ (Watt, 1967). Because flux of water through the gastrointestinal tract plays a major physiological role in ruminants, factors affecting fluid dynamics in the rumen deserve attention.

Flow of Liquid through the Rumino-Reticulum.

Water ingested by the ruminant can disappear from the gastrointestinal tract by way of two main routes: absorption or passage. Controversy remains about the route taken by drinking water. In 1928, Schalk and Amadon in a

comprehensive review of the physiology of the ruminant stomach indicated that in animals deprived from water during drinking, water passes directly from the cardia into the ruminal cavity, and that none of the fluid flows through to the omasum or abomasum. Wise and Anderson (1939) reported that water offered to 3-6 month old calves from an open pail completely entered the rumen. In contrast, Ash (1962) observed a rapid flow of fluid through the reticulum omasum orifice, immediately after drinking cool water. They observed surges of liquid from 18 to 100 ml in the omasum of sheep during and immediately after drinking. Similar indications of water flowing directly to the omasum of sheep have been documented by Warner and Stacy (1968b). These authors indicated that after drinking, up to 800 ml of water might had passed down the reticular groove. Orskov and Benzie (1969) studied the destination of different liquid protein suspension drenched to sheep. They concluded that reticular groove closure is influenced more by the act of suckling than by the type of solution used. In addition, when animals were accustomed to suckle, solutions passed completely to the abomasum. In a similar experiment conducted with 22 month-old dairy heifers trained to suckle from a nipple pail, Huber et al. (1982) confirmed a high bypass of a glucose solution to the small intestine by elevated serum glucose levels after drinking. In the young calf, where the rumen is not functional, and liquid diets are the main source of nutrients for the animal, 95% of

consumed milk bypassed the rumen and reached the abomasum and small intestine (Smith, 1959).

Although the reticular groove reflex is almost absent in adult ruminants (Ruckebusch, 1988), a variety of compounds such as copper salts and sodium salts have been used to re-activate this reflex. Closure of the reticular groove has a number of clinical and nutritional implications.

In adult animals, manipulation of this reflex has been used primarily to deliver drugs intraruminally or to avoid ruminal fermentation by directing the compounds to the lower gastrointestinal tract (Ruckebusch, 1988). From the nutritional stand point, passage of liquids through the rumen is a vital process, because water serves as a vehicle to transport digesta out of the rumen. Unfortunately, very little work has been conducted to quantitate how much of the drinking water is naturally shunted past the rumen. The experiments of Warner and Stacy (1968a,b) probably are the benchmark in the study of the fate of water in the stomach of the sheep. Their observations in 13 of 20 different experiments indicated that detectable amounts of drinking water bypassed the rumen. Rogers et al. (1982), suggested that a considerable amount of drinking water never equilibrated with fluids in the rumen when studying the effects of mineral salts on rumen dilution rate in lactating dairy cows. They speculated, based on water intake and total ruminal outflow, that about 80% of the fluid passing

out the rumen was from drinking origin, but that a high amount of consumed water possibly moved directly to the omasum and abomasum. Comparable results on ruminal bypass of drinking water, were reported by Woodford et al. (1984). When water was withheld for 4.5 to 9 h postfeeding, 18% and 5% of drinking water was calculated pass directly to the lower gastrointestinal tract. Contrary to expectations, the highest passage of drinking water was observed when water was withheld for only 4.5 h; the authors attributed this finding to greater ruminal fill at this time. Though evidence of water passage through the reticular groove of adult cattle exist, our understanding of the primary mechanisms involved in this process remains vague.

Osmolality and Water Flux through the Ruminal Epithelium.

In addition to passage of water from the reticulo-rumen via the reticulo-omasal orifice, fluid movement across the ruminal epithelium has been considered to be an important route for water disappearance from the reticulo-rumen. Although the water absorption mechanisms from the rumen have not been completely identified, water flow through the rumen wall presumably is a net result of an osmotic gradient between fluids in the lumen of the rumen and the blood (Dobson, 1984).

Measurements of osmotic pressure are used to assess relationships between electrolytes and osmotic pressure. By definition a molar solution is a solution in which one mole

(gram molecular weight) of solute is dissolved in a solvent and both occupy 1 liter. A molal solution in contrast, is that solution which contains 1 mole added to 1 kilogram of solvent; thereby, the solute has one liter of free space for kinetic distribution. Hence, a molal solution is slightly more dilute than a molar solution.

Any substance, such as sodium salts or protein, exerts an osmotic pressure. Osmotic pressure generally is measured in osmols. One osmol is the amount of a substance that exerts 22.4 atmospheres of pressure at absolute temperature. Because one atmosphere is equal to 760 mm Hg, the pressure exerted by one osmol per kg (liter) can be calculated as (22.4 Atm)(760 mm Hg) = 17,000 mm Hg. More frequently tonicities are expressed as a 1000^{th} parts of the concentration, so units are milliosmolal concentrations (mOsm). Consequently 1 mOsm per liter or kilogram = 17 mm Hg at absolute temperature (273°K). Because body temperature is 37°C, 1 mOsm per liter exerts equals 19.3 mm Hg osmotic pressure. Normally, electrolytes and other substances dissolved in the body's fluids are maintained at relative constant osmolalities. Blood plasma exhibits an osmolality of 290-300 mOsmol/kg. Osmolality of saliva may vary among species. In ruminants, saliva is isotonic to plasma (290-300 mOsmol/kg) regardless of flow rate. In contrast, fluids in the gastrointestinal tract show great variations, because tonicities vary with type of diet and fermentation. Ruminal osmolality for roughage or silage-

based diets reaches a maximum between 350 to 400 mOsmol/kg (Warner and Stacy, 1965; Engelhardt, 1969; Bergen, 1972; Bennink et al., 1978). Engelhardt and Hauffe (1975) observed that before feeding, ruminal contents in sheep were hypotonic (247 ± 18 mOsmol/kg) to plasma. Soon after feeding, ruminal tonicity reached 500 mOsmol/kg when alfalfa and oats were consumed by sheep given no water (Warner and Stacy, 1968b). Likewise, Engelhardt (1969) reported that prefeeding osmolarity values in goats were hypotonic to blood (261 ± 23 mOsmol/liters), but two hours after feeding, contents were hypertonic reaching values of 420 mOsmol/liters. In addition, mean osmolarity values remained hypertonic for 6 h after feeding. Contrary to these reports, Bergen (1972) observed that changes in ruminal fluid osmolarity in sheep fed a silage and three concentrate rations varied from 250 to 300 mOsmol to maximum values of only 310 to 370 mOsmol by 2 h after feeding.

Reports regarding absorption of water across the ruminal epithelium are conflicting. Ternouth (1967) observed that ruminal volume increased immediately after feeding and that this increase could not be attributed to saliva secretion alone, but to a high transepithelial flux of water due to hypertonicity. He calculated that about 1 liter/h crossed the ruminal wall in Merino ewes. Other authors (Engelhardt, 1970) have considered that ruminal water absorption was 200 ml/h when rumen tonicity was 370 mOsmol/liter. However, at osmolalities between 260 and 340

mOsmol/liter net transfer of water was nil. In contrast, Martens (1985) indicated that changes in ruminal osmolality (240 vs 367 mOsmol/liters) markedly influenced absorption of water from a temporarily isolated ventral rumen of the sheep. With the hypertonic solution net influx of water into the rumen was 225 ml/h. In contrast, water was absorbed at a rate of 95 ml/h with the hypotonic solution. Contrary to these findings, Warner and Stacy (1968b) found little evidence of transepithelial water movement in esophageally cannulated sheep despite the different osmotic gradients between the blood and the ruminal contents. They concluded that the rate of water absorption from the rumen is very slow. However, when water absorption was estimated over the whole day, water absorbed across the ruminal epithelium and the quantity of water drank was approximately equal. Reports by Harrison et al. (1975) indicated that net absorption of water throughout the ruminal wall of two sheep was 10.8 and 10.1 liters/day after 12 liters of water was infused daily. Warner and Stacy (1972) indicated that net water absorption was over high ranges of osmolalities (295 to 360 mOsmol/kg).

Under normal feeding conditions ruminal contents may not reach osmolalities outside this range. Thus, influx of water into the rumen associated to feeding may not be significant. However, ruminal water influx might be important as it increases ruminal dilution rate.

Under certain pathological conditions such as acidosis, the ruminal ingesta becomes hypertonic to plasma which causes flux of water from the extracellular space into the rumen. In acute cases of acidosis in sheep, Huber (1976) reported that osmolalities of ruminal contents increased to 400 mOsmol with lactic acid accounting for approximately 61% of the increased osmolality (89.2 mOsmol).

Altering tonicities of ruminal fluid, with hyper or hypo-molal solutions has been used to manipulate the ruminal environment. Intraruminal infusions of artificial saliva or sodium bicarbonate into sheep markedly increased ruminal fluid dilution rate (Harrison et al., 1975). Likewise, Rogers et al. (1979) studied the effect of sodium chloride or sodium bicarbonate on liquid dilution rate, water intake and ruminal osmolality in Holstein steers fed either a high concentrate or high roughage diet. Intraruminal infusions of water, plus .5 or 1.0 kg of sodium chloride, and water plus .36 or .72 kg sodium bicarbonate were compared. Ruminal liquid dilution rate as measured by marker dilution, and the total amount of water leaving the rumen via the reticular-groove was increased by both levels of sodium chloride and sodium bicarbonate. But molar proportions of propionate in ruminal fluid were decreased. Water intake increased markedly in steers dosed with either additive. In general, hypertonic solutions increased ruminal osmolality, with higher values being found in concentrate-fed animals. In another study with dairy cows using sodium chloride,

sodium bicarbonate or limestone in the rations, Rogers et al. (1982) indicated that water intake and liquid dilution rate increased only when cows received sodium chloride or sodium bicarbonate. Ruminal fluid osmolalities were not altered by supplementation of the mineral salts, but authors did not attempt to explain these results. Chase et al. (1988) found similar increments in ruminal liquid dilution rate after dosing bicarbonate solutions into the rumen of beef heifers. Increasing liquid turnover rate will force ruminal bacteria to have faster growth rates and thereby may influence the outflow of nutrients to the lower tract. But experimental data, are not conclusive about why liquid dilution rate is altered. Under certain conditions, ruminal osmolality may play an important role, but changes in blood or rumen osmolalities are not always detectable in animals given supplemental dietary minerals. Water intake consistently increases with salt supplementation, but when water was infused directly into the rumen, liquid dilution rate was not altered (Harrison et al., 1975; Rogers et al., 1979). This difference suggests either that drinking water is more stimulatory toward ruminal structures and enhances liquid outflow, or that some drinking water passes directly into the postruminal digestive tract through the reticular groove without mixing with the ruminal contents.

Rumen volume and Ruminal Evacuation.

The rumen is a very dynamic organ in which digestion and fermentation processes occur in a liquid environment. According to Bailey (1961), saliva supplied some 70 to 90% of the liquid entering the rumen while only 13 to 24% was supplied by drinking water (Poutiainen, 1968). In cattle, the total liquid volume ranges from 15 to 21% of body weight (MacFarlane, 1976; Owens and Goetsch, 1988); however, ruminal volume varies with age, diet type, level of intake and pattern of feeding. In general, as level of feed intake and percentage of roughage in the diet increase, rumen volume increases proportionately. Physical and chemical composition of the diet also have an impact on ruminal volume; large particle size, greater bulk, and higher cell wall and lignin content promote more rumination and salivation thereby increase fluid and mineral inputs into the rumen.

Distribution of liquid within the rumen, and its relationship with solids particles has been discussed by Owens and Goetsch (1986). Although there are two main pools (solids and liquids) within the rumen, each pool has several subpools that may behave independently.

Because solids and liquids move continuously and leave the rumen at different rates (Van Soest, 1982), rumen volume estimates do not describe dynamics of fluids; thus, turnover and passage of ruminal contents, need discussion.

Rumen volume and passage rate of liquid from the rumen are not simple parameters to measure. In the past, direct and indirect methods have been used.

One direct method to measure rumen volume, is to slaughter animals at various intervals after feeding, as outlined by Makela (1956). Although this method permits complete sampling of the rumen and has unquestionable accuracy (Van Soest, 1982), its use requires a large number of animals and has the disadvantage that volume can be only measured once in each animal.

A more frequent approach to estimate ruminal volume is to totally remove contents from the rumen of cannulated animals (Reid, 1965). This procedure permits one animal to be used repeatedly to quantitate ruminal volumes, and causes limited disturbance. Nevertheless, the approach requires labor and there is always a question of how the physiology of the animal is affected by cannulation and evacuation. Exposure of ruminal digesta to oxygen, handling, cooling and mixing of digesta and stimulation of ruminal epithelium may alter motility and secretion. Nevertheless, the results of Towne et al. (1986) have shown that emptying of ruminal contents in cattle did not significantly alter the microbial populations, VFA concentrations and liquid passage rate.

Manual removal of total ruminal contents in grazing sheep, did not impact health of the animal; rumination began immediately after contents were returned to the rumen, and no reduction of rumen fill was observed throughout the

measurement periods (Cruickshank, 1986). However, one of the main disadvantages of the procedure is that researchers assume, based on a single measurement, that rumen volume and that rates of inflow-outflow are constant throughout the day. Warner and Stacy (1968a) suggested that small quantities of fluid may move in and out the ruminal epithelium, during the evacuation process resulting in an under or an over-estimation of the rumen volume; in addition they indicated, that ruminal liquid volume is changing continuously and volumes might vary within or across days.

Goetz et al. (1988) studied the effects of ruminal evacuation on intake and recovery of dry matter in Angus steers. Ruminal contents were evacuated on sequence every 3, 2, 1 days or two evacuations on consecutive days were followed by 1 day interval. Individual evacuations tended to increase feed intake in contrast to pre-evacuation feed intake, but consecutive evacuations depressed feed intake. Two evacuations separated by 1 day and followed by a 12 day recovery period appeared to be a practical maximum frequency that had no appreciable adverse effect on the animals.

Markers and Liquid Rate of Passage.

The complexity of ruminal evacuation encouraged researchers to develop indirect methods to estimate ruminal volume. Reference substances or "markers" can mimic the flow of digesta throughout the gastrointestinal tract. Among the most common water soluble markers used in

digestion studies are Polyethylene glycol (PEG) of a high (>3,000) molecular weight and, the ethylenediaminetetraacetic acid (EDTA) chelates of chromium and cobalt. Markers that attach to particles include some rare earth elements such as ytterbium and erbium (Teeter, 1981). and Cr mordants of fiber. Stained particles, plastic particles and non-attaching chemicals, e.g. Cr_2O_3 and Fe_2O_3 also can be used. Markers can be dosed orally or intraruminally, and its concentration within the rumen is measured at time intervals after dosing. Concentration decreases over time yielding a dilution curve. The natural logarithm of the concentration when regressed against time, yields a decay or a dilution rate (Van Soest, 1982). Initial pool size or rumen volume is calculated by relating the initial marker dose (g) to the extrapolated rumen concentration (g/h) at time zero. The slope of the concentration line represents the dilution rate (/h), and the reciprocal of this slope represents the turnover or retention time (Teeter, 1981; Van Soest, 1982). Half life represents the time for half of the marker to disappear from the gastrointestinal tract and is calculated by multiplying the natural logarithm of 2 (.639) by the turnover or retention time (Van Soest, 1982).

Indirect estimates of ruminal volume often are similar to direct measurements (Bauman et al., 1971), but a series of additional factors must be considered (Faichney, 1975; Teeter and Owens, 1983). Kotb and Luckey (1972) reviewed

the characteristics of natural and external markers in digestibility studies and concluded that markers offered economical advantages, but they should not be used without some caution. They enumerated the basic criteria for an ideal marker. Faichney (1975) summarized these criteria, and proposed a continuous-dose method using a liquid and a solid marker simultaneously. Engelhardt (1974) stated that none of the available markers fulfill these criteria, and Van Soest (1982) indicated some of the problems in calculating and interpreting results of marker studies, when some of the basic criteria are not met. Teeter and Owens (1983) examined some of these properties and used markers in ruminant nutrition studies.

Mathematical models have been developed (Grovum and Williams, 1973; Ellis et al., 1979) to estimate passage rate of digesta from sequential concentrations of a marker in feces. Problems such as lag time and mixing time in the rumen are not easy to interpret from these models.

Researchers when using markers routinely assume that rumen volume is constant over time. Yet, fluid passage rate studies would be expected to vary under certain conditions, e. g., within a day. Warner and Stacy (1968ab) emphasized that the mathematical approaches to estimate ruminal volume and passage rate are based on steady state conditions. They suggested that diurnal changes in rumen volume can account for some erroneous interpretations. Sampling time and site must be considered when using markers.

Chemical analysis and characteristic of markers also have been studied extensively (MacRae, 1974; Ellis et al., 1979; Udén et al., 1980; Teeter and Owens, 1983). However, problems with absorption, marker recovery, marker migration or exclusion from specific phase remain of concern.

Chromium and cobalt complexes with EDTA have been investigated as liquid markers by Udén et al. (1980). Although both markers gave similar results, 2 to 3% of Cr was recovered in urine indicating that it had been absorbed from the gastrointestinal tract.

Results in the literature are conflicting about the value of PEG for estimating rumen volume and liquid passage rate. Czerkawski and Breckenridge (1969) based on in vitro studies with PEG and feed particles suspended in buffers solutions, suggested that PEG may not be distributed equally in all the ruminal water space, especially in high concentrate diets. Likewise, Alexander et al. (1969) indicated that PEG is excluded from intratissue water of feedstuff, due to its large molecular weight. In contrast, Bauman et al. (1971) indicated that PEG accurately predicted ruminal volume of cows when compared to direct evacuation. Teeter and Owens (1983), suggested that recovery of PEG is depressed by tannins or some other water-soluble substances present in cottonseed hulls. Other factors such as absorption, lack of marker equilibration and poor analytical techniques, also may have contributed to systematic errors reported in the literature.

One alternative approach to estimate both rumen volume and liquid passage rate, is to combine a pulse or a continuous dose of a water soluble marker with direct ruminal evacuation. Teeter and Owens (1983) reported that rumen volume was underestimated by 4% by liquid marker measurements as compared to direct ruminal evacuation 5 days later. Similar results have been reported by Colucci et al. (1982). They compared values of rumen volume, obtained by direct evacuation and marker dilution technique. They concluded that at low levels of intake, the marker dilution technique over estimated ruminal volume, but at high levels of intake, ruminal volume was greatly underestimated. Kansas workers (Del Curto et al., 1990) suggested that ruminal liquid volumes estimated with Co-EDTA were always 10 to 20% greater than those based on ruminal evacuations. However, ruminal evacuations 4 h after vs before feeding yielded larger ruminal volumes.

In conclusion, the use of external markers to estimate rumen volume and kinetics of digesta along the gastrointestinal tract (GIT) offer a reliable alternative, but their application and accuracy is conditioned to particular experimental conditions, therefore the need to validate its use under different circumstances.

CHAPTER III

DIURNAL VARIATION IN RUMINAL FILL AND IN MARKER FLOW IN BEEF HEIFERS LIMIT FED A HIGH CONCENTRATE DIET

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ABSTRACT

Six Angus x Hereford heifers (588 kg) with permanent ruminal and T-type duodenal cannulas fed an 80% concentrate diet twice daily (0830 and 1630) at 1.7% of BW were used to investigate diurnal changes in ruminal volume, passage rate of liquid and solid digesta, DM digestibility, and intestinal transit time were also studied. Markers included in the diet ytterbium-labeled alfalfa and Cr_2O_3-mixed with cottonseed hulls and Co-EDTA. Although heifers consumed an average of 3.1 liters/kg DM, animals and day influenced ($P<.05$) water intake. Weights and composition of ruminal contents were evacuated 3.5 h after a morning feeding and 4 h after an evening feeding were similar. Animals differed ($P<.05$) in ruminal liquid volume, DM% and solids volume. Compared to daily duodenal flow of DM and liquid estimated from the mean of the three markers, values for Yb were 14 to 15% low ($P<.05$) vs 2 to 3% high for Cr and 12% high for Co.

Fecal DM output followed a similar pattern being 9% low for Yb, 4% low for Cr and 13% high for Co. Marker concentrations in feces varied by 15% being lowest at 2000 and 2400. Transit time (t), measured as the time lapsed between from marker withdrawal from the diet and the time at which fecal concentrations exhibited their decay, tended to be fastest for Co-EDTA (10 h), followed by Yb (13 h) and Cr_2O_3 (19 h). Regressed ruminal dilution was lower ($P<.02$) for Yb (5.7%/h) than for Cr_2O_3 (8.9%/h) with Co-EDTA being intermediate (7.3%/h). Estimates of ruminal passage rate, duodenal flows, DM digestibility, and fecal output differed with markers. Ruminal volumes taken at equal intervals postprandially did not change.

(Key Words: Diurnal Variations, Passage Rate, Markers, Beef Cattle.)

Introduction

Changes in rumen capacity during the day may alter passage rate of digesta and digestibility of consumed feedstuffs (Warner and Stacy, 1968b; Thomson et al., 1985; Cruickshank, 1986; Galyean et al., 1986). Available techniques to study flow of digesta throughout the gastrointestinal (GI) tract include the use of reference materials (markers) that remain either in solution or intimately associated with the particulate matter (Faichney, 1975; Warner, 1981; Teeter, 1981.). Unfortunately the majority of research conducted in ruminal kinetics rely upon

the assumption of constant rumen volume, i.e. "steady state" conditions, rapid and complete marker equilibration, minimal absorption of markers from the GI tract, and no adsorption of marker to digesta or microbes (Jaques et al., 1989). However, the rumen is not a static organ; changes in volume might be expected from day to day (Warner and Stacy, 1968a).

Diurnal patterns of ruminal fill in sheep grazing either white clover or prairie grass have been reported by Thomson et al., (1985) and Cruickshank, (1986). They observed maximal ruminal capacities at the end of the afternoon grazing period. Nycterohemeral rhythms in feed and water intake in dairy cattle fed several times daily were documented by Nocek and Braund (1985). Recently, Deswysen et al. (1989), supported the idea of nycterohemeral rhythms on rumination in cattle fed corn silage-based diets. Goetsch and Owens (1985c) suggested that if rate of passage and digestibility vary diurnally, marker dilution also would change diurnally, so that digestibility estimates are inaccurate. A continuous-dose method in which the use of a liquid and a solid marker are fed continuously for a period of time sufficient to establish equilibrium in marker concentration at any sampling site has been proposed by Faichney (1975). This procedure may partially overcome some problems of under- or over-estimation of ruminal volume, digesta (fluid and solids) passage rate, and fecal output when non-representative grab samples are collected. However, diurnal excretion of the marker, and frequency of

dosing and sampling remain major points of concern when using the continuous marker dose method. Dual markers will not automatically correct for diurnal variation in flow of separate phases.

The objectives of this research were 1) to determine daily water intake, 2) to investigate diurnal changes in ruminal volume, and 3) to estimate duodenal flow, DM digestibility, daily fecal output and total transit time, using Ytterbium and Cr_2O_3 as particulate markers, and Co-EDTA as a liquid marker fed continuously. Daily variations in fecal marker concentrations were also studied.

Materials and Methods

Animals and Diet.

Six mature Hereford x Angus cattle (588 kg) fitted with large (10 cm i.d.) ruminal and duodenal cannulas (T-type), housed in individual pens, were assigned randomly to treatments (ruminal evacuation time) in a crossover experiment. Animals received a concentrate diet (Table 3.1) twice daily (0830 and 1630) at 1.7% of body weight (DM basis); daily water intake was recorded using a water meter throughout the 21-d trial.

Marker Preparation and Dosing.

Ytterbium chloride[1] (Yb) and chromic oxide[2] (Cr_2O_3) were used as indigestible external markers to estimate

[1]Ytterbium chloride ($YbCl_3 \cdot 6H_2O$) Research Chemicals. R. C. PHO, Az.
[2]Chromic Oxide (Cr_2O_3) Fisher Scientific Co. New Jersey.

Table 3.1 Composition of concentrate fed (DM Basis)

Ingredient	%
Corn, dry rolled (IFN 4-02-931)	63.10
Cottonseed hulls (IFN 1-00-599)	14.10
Soybean meal, (IFN 5-04-600)	10.05
Alfalfa pellets, dehydrated (IFN 1-00-023)	6.00
Cane molasses (IFN 4-04-696)	5.00
Salt (trace mineralized)[a]	.50
Ground limestone (IFN 6-02-632)	.50
Dicalcium phosphate (IFN 6-01-080)	.50
Aurofac-50[b]	.15
Urea (42% N)	.10
TOTAL	100.00

[a]Trace min, Carey Salt, Mission Kansas, contained: NaCl, 92-97%; Mn, .250%; Fe, .200%; Cu, .033%; I, .700%; Zn, .005%; Co, .0025%; white mineral oil.

[b]Aurofac-50, CADCO, Inc., DesMoines, Iowa. Contained: 50 g of chlortetracycline per 454 g.

particle passage rate and fecal output; Co-EDTA[3] served to estimate liquid passage rate.

Prior to the experiment, 20 kg of alfalfa hay were labeled with Yb by the immersion washing procedure as outlined by Teeter et al. (1984); 150 g of Yb-labeled alfalfa (1.65 mg Yb/g DM) were fed to provide a total daily dosage of 248 mg Yb/hd. A mixture of cottonseed hulls (83.9% of DM), and molasses (14.0% of DM) served as a vehicle for daily administration of chromic oxide (6.13 Cr g/hd; 2.1% of DM). Complexes of Co-EDTA were prepared as specified by Udén et al. (1980) except that cobalt crystals were not diluted in water but were held in a desiccator for daily dosage (1.5 g Co-EDTA crystals).

Daily dosage of each marker was split in two equal portions and hand mixed with the concentrate at feeding time during the first 15 days of the study; thereafter, daily oral marker dose was discontinued to follow the declines in concentration in fecal DM.

Sample Collection and Ruminal Evacuation.

A schedule of events during the experiment is described in Table 3.2. The first 6 days of the trial were used as an adaptation period to the diets; this period also allowed the markers to equilibrate with digesta in the gastrointestinal tract. Marker adaptation times of 7 to 10 d have been used routinely by past researchers. On d 7 and d 12 of the study,

[3]Cobalt acetate complexed with ehylenediaminetetraacetic acid. Fisher Scientific Co., New Jersey.

Table 3.2 Sampling schedule during the experiment

Day of trial	Topic of interest	Sampling time (h) Location: Rectum	Duodenum	Rumen[a]	Measurement/Sampling
7	Steady	0800,1400,2000,0200	1000,2200	-	Fecal grab
8	state	1000,1600,2200,0400	1000,2200	-	samples, and
9	& digestion	1200,1800,2400,0600	1000,2200	-	duodenal fluid
10	Evacuation		-	1130,1930	Rumen evacuation
11					
12	Steady	0800,1400,2000,0200	1000,2200	-	Fecal grab
13	state	1000,1600,2200,0400	1000,2200	-	samples, and
14	& digestion	1200,1800,2400,0600	1000,2200	-	duodenal fluid
15	Evacuation		-	1130,1930	Rumen evacuation
16					
17	Dilution	0800,1400,2000,0200	-	-	
18	rate and	0800,1400,2000,0200	-	-	Fecal grab
19	excretion	0800,1400,2000,0200	-	-	samples
20	time	0800,1400,2000,0200	-	-	
21	lag	0800,1400,2000,0200	-	-	

[a]Three heifers were evacuated in the morning and 3 in the evening; in the second period, evacuation times for each animal were reversed.

fecal grab samples and duodenal fluid samples (250 ml) were collected at specified times for 3 consecutive days; pH was measured immediately. Duodenal samples were composited within animal, hour, and period, whereas fecal samples were kept frozen individually for subsequent chemical analysis. In a crossover design, approximately 3.5 h after the am feeding or 4 h after the evening meal (Table 3.2), total ruminal contents were removed mechanically on d 10 and d 15 of the experiment using a vacuum device. Ruminal contents were screened twice (.63 X .63 cm and .31 X .31 cm square pore mesh) manually to separate the particle matter from the liquid phase; each phase was weighed, mixed thoroughly and sampled. After sampling the remaining ruminal contents were returned into the rumen. Approximately 25 min per animal were used for the entire ruminal evacuation procedure.

Simultaneously, a subsample (1 liter) of the liquid phase was used immediately to determine the density and pH of ruminal liquid. All samples were kept frozen until chemically analyzed.

Chemical Analysis.

Feed, fecal, duodenal composites, ruminal liquid and solid contents were thawed, dried at 55°C for 48 h, air equilibrated and ground through a Wiley mill equipped with a 2 mm screen; thereafter, a 1 g sub-sample was dried for 24 h at 90°C used to determine DM.

Dried fecal, duodenal, and ruminal samples were analyzed for Yb and Co concentration by atomic absorption spectrophotometry (Hart and Polan, 1984). Chromium was analyzed colorimetrically after acid digestion as outlined by Fenton and Fenton (1979).

Ruminal fluid samples were thawed in a water bath (37°C) and centrifuged at 3,500 X g for 15 min. The supernatant fluid was analyzed for Co concentration (Hart and Polan, 1984). Standards were prepared simultaneously from rumen liquid samples taken prior to marker dosage, and adjustments in dilution were made, as necessary to ensure that the marker concentration was in the detection range of the spectophotometer.

Calculations.

To estimate ruminal fluid associated with solids (bound liquid), and the amount of free liquid in the rumen, the formulas presented in the Appendix A were employed.

Total rumen liquid volume was calculated as the weight of volatiles in bound liquid plus free liquid divided by the density ruminal liquid (Appendix A). Ruminal liquid density was estimated by weighing 1 liter of rumen fluid. Total DM in the rumen was calculated by adding DM present in the liquid phase to that in the solid phase. Therefore, total ruminal DM includes solids from both the liquid and solid phases.

Daily fecal output was calculated by dividing daily marker intake by marker concentration in feces (mg/kg) (Prigge et al., 1981) using values for marker on the plateau (Steady state). Dry matter digestibility, was calculated as presented in Appendix A.

After the markers were removed from the diet (day 15, Table 3.2), the decline in fecal marker concentration was estimated by regressing the natural logarithm of Yb, Cr, and Co-EDTA concentration against time. Mean fecal marker concentrations within one standard deviation from the plateau mean which would include feces derived from digesta in transit were ignored in this calculation.

The time at which the plateau intercepted the regressed decline was calculated. Marker transit time (t) for the whole gastrointestinal tract was computed as the time that elapsed after markers were withdrawn from the diet to the time point of intersection at which extrapolated decay line and the extrapolated plateau line met. This method of estimating transit time is similar to determining the time of first appearance of fed markers in feces.

Duodenal digesta flow was calculated by assuming that all of the oral marker was recovered at the duodenum and by dividing daily dose of marker (mg/day) by marker concentration in the composited duodenal contents. Duodenal DM flow was calculated multiplying duodenal digesta flow times DM concentration of duodenal sample.

Ruminal evacuation data were analyzed using a general linear models procedure (GLM) for a crossover experiment as indicated by Cochran and Cox (1957). The statistical model included animal, period, hour and marker. Differences between the decline in (slopes) fecal marker concentrations were calculated using a GLM procedure (SAS, 1985ab); means were separated by the least square method. Data from duodenal fluid and fecal grab samples were analyzed as a randomized complete block design with a split plot over time, using animal X marker interaction as an error term (error A) for the main plot; the animal*hour interaction was used to test hour effects (split plot error B). The least significant difference test was used to compare means.

Results and Discussion

Individual feed and water intakes are presented in Table 3.3 Water intake varied ($P<.05$) between animals and days. Heifers consumed an average of 3.1 liters per kg of DM consumed. This average represents only the drinking water without taking into account water from feed (which averaged generally 10%). Mature cattle, typically consume 2 to 4 kg of water per kilogram of DM eaten (Leitch and Thomson, 1944). Water intake values in the present experiment are quite similar to data presented by Waldo et al.(1965) for Holstein heifers fed different rations. They indicated that water intake was 3.3 kg per kilogram of DM on mixed diets of grain and hay. Our mean value is slightly

Table 3.3 Feed and water intake of individual heifers

Item	PEN #						Mean
	1	2	3	4	5	6	
Body weight, kg	660.4	575.6	565.2	613.7	519.3	596.5	587.5
Daily feed intake	10.5	10.2	10.0	11.2	8.6	9.4	9.9
Kg DM % BW	1.6	1.7	1.7	1.8	1.6	1.6	1.7
Water intake, liters/d	32.4	35.6	29.5	33.1	25.0	30.0	30.9
liters/kg DM Consumed	3.1	3.5	2.9	2.9	2.9	3.2	3.1

lower than those (3.5 to 5.5 kg of water per kg of DM) suggested by ARC (1965). Despite similarities in average water intakes between our data and other reported data, animal to animal variation is of interest. Table 3.3 shows how individual water intake varied. Some heifers (2,4,6) drank slightly more water regardless of their daily dry matter intake (Table 3.3).

Ruminal liquid volume and solid dry matter in the rumen were similar for the am vs the pm evacuation time (39 vs 40 liters and 9.1 vs 9.8 kg) (Table 3.4). Nevertheless, total ruminal contents tended to be greater in the evening (50.1 vs 47.9 liters). Percentage of free liquid and liquid associated with the solid fraction, ruminal liquid pH and density of the ruminal fluid, did not differ for the am vs the pm estimates (Table 3.4).

Animal to animal variation, was large and significant for several of these measurements (Table 3.4). Rumen fluid volume of individual heifers averaged from 31 vs 48 liters despite similar feed and water intakes and was repeatable for the two periods (r=.96). Similar animal effects were noted by Teeter and Owens (1983). Variation in rumen volume in this study was not related to body weight of the animal. Large differences (42%) in rumen volume of sheep, were reported by Purser and Moir (1966). They suggested that apparent changes in rumen volume within an animal and during the day might change. However, Warner and Stacy (1968b) questioned the methodological approach of Purser and Moir

Table 3.4 Rumen liquid and solid volumes estimates by direct ruminal evacuation in beef heifers fed concentrate diets[a]

Item	Time of evacuation 1130	1930	SEM	Animal effect, P<
Heifers	6	6	-	-
Solid volume, kg DM	9.1	9.8	0.48	.06
Liquid volume, liters	38.7	40.3	1.37	.02
Free liquid, liters	24.4	27.0	0.78	.01
%	62.4	66.3	0.45	.01
Bound liquid, liters	14.3	13.2	0.63	.01
%	37.6	33.7	0.45	.01
Total contents, kg	47.9	50.1	1.80	.05
DM, %	19.4	19.7	0.40	.001
	Ruminal liquid characteristics			
Ruminal pH	5.64	5.62	0.05	.01
Liquid density	0.98	0.99	0.01	.30

[a]Least squares mean.

(1966). Nevertheless, data presented by Warner and Stacy (1968b) showed a similar tendency in ruminal volume to change during the day for sheep that were fed a concentrate diet.

One explanation for the equal ruminal volumes for our am vs the pm evacuation, is based on the reports of Warner and Stacy (1968b). They indicated that during feeding there is a considerable increase in rumen volume, but 2 to-4 h after feeding or drinking, the rumen volumes tend to return to resting values, which are larger than pre-feeding ruminal volumes. In the present experiment, ruminal evacuations were conducted approximately 3.5 h post feeding. At this time, the rumen may have returned to a resting volume as stated by Warner and Stacy, (1968b); prefeeding ruminal volumes are not known because rumens were not evacuated at that time. In our experiment, in contrast to other studies water was always available, so animals could have drunk water before the ruminal emptying. Frequency of drinking was not monitored in this study, but this concern led us to measure drinking frequency in subsequent experiments, (Chapter VIII).

Ruminal evacuations were conducted at 0 and 4 h postfeeding to determine DM fill and indigestible ADF in steers fed either a protein or energy supplement, by (Del Curto et al., 1990). Ruminal DM fill tended (P=.11) to be larger 4 h postfeeding with the high protein supplement fed once a day. In addition, liquid volume and dilution rate

were not affected by either source of supplement in two studies. However, the same authors in a third study found an increase (P<.10) in ruminal liquid volume, with the high protein supplement. Overall, results from those trials indicated that DM ruminal fill varied with post-prandial time. Because the times of evacuation used in our study were 3.5 and 4 h after feeding our results suggest that ruminal fill does not change diurnally independent of meal timing and quantity of feed consumed. As feed and water intake may exhibit diurnal patterns, such patterns may explain why previous workers have observed diurnal variations in rumen volume.

Percentages of ruminal DM were similar (19.4 vs 19.7%) for the am and pm emptying (Table 3.4). Our values are slightly higher than those reported (14%) by Owens and Goetsch (1988) and (15%) by Del Curto et al. (1990). These differences probably are due to the type of diet used and level of intake. Owens and Goetsch (1988), indicated that ruminal liquid volume increased as the level of roughage in the diet increases. In our study animals consumed an 80% concentrate diet, whereas in Del Curto et al. steers were fed dormant tallgrass diet.

Duodenal DM flow, calculated on the basis of the three external markers, are presented in Table 3.5 DM. Flows were similar between the am (4.8 kg/d) vs pm (4.9 kg/d) sampling time. Comparisons of DM among the three markers indicated that (P<.05) DM flow was higher for Co (5.5 kg/d) and Cr_2O_3

Table 3.5 Duodenal digesta flow based on particle and liquid markers in heifers fed concentrate diets

Item	Markers			
	Yb	Cr	Co	SEM
Dry matter flow, kg/d	$4.14^{a}\pm.31$	$5.04^{b}\pm.60$	$5.50^{b}\pm.58$	0.30
Duodenal flow, liters/d	$57.19^{a}\pm4.2$	$68.45^{b}\pm6.0$	$74.74^{b}\pm6.2$	2.46
Ruminal dilution rate %/h	$5.8^{a}\pm.002$	$8.6^{b}\pm.01$	$7.3^{ab}\pm.003$	.008
	Sampling time			
	1130	1930		
Duodenal pH	2.3±.05	2.3±.05		0.05
DM Flow Kg/d	4.8±.47	4.9±.41		

^{a,b}Mean values in a row with different superscript differ (P<.05).

(5.04 kg/d) than for Yb (4.14 kg/d). Harris and Phillipson (1962) reported that digesta OM flow, and flow of Cr_2O_3 exhibited similar duodenal diurnal variations in sheep fed a hay diet twice daily. However, MacRae and Ulyatt (1972) demonstrated that diurnal variations in duodenal and ileal Cr_2O_3 flow were independent of OM flow when Cr_2O_3 dosing do not coincide with feeding. Differences in DM duodenal flow estimates between Cr_2O_3 and Yb probably are due to the degree of association of the marker to the particle phase. In the present study, duodenal flow was calculated assuming 100% recovery of either marker. Van't Klooster et al. (1969) detected only 91% of fed Cr_2O_3 at the duodenum of sheep continuously sampled over a 24 h; extension of the collection period to 72 h, gave better recoveries of the marker (99 and 98%). MacRae (1975) indicated that recovery of Cr_2O_3 at the duodenum varied from 56 to 100%.

Failure to fully recover dosed Cr_2O_3 could be attributed to several factors. First, if sampling retards ruminal outflow, Cr_2O_3 would temporarily accumulate within the rumen, indicating that steady state conditions were not achieved. Secondly, the method of accumulating duodenal samples, typically on a wet, not a DM basis, will underestimate mean flow if the pattern of markers passage does not match the pattern of digesta flow. Finally, non-representative sampling at the duodenum could yield erroneous flow values.

The use of Cr_2O_3 in duodenal sampling has been questioned due to variability in recoveries (MacRae, 1975). Zinn et al. (1980) however observed little variation in Cr flow. Faichney (1975) suggested that the Cr_2O_3 is not a precise index to estimate digesta flow rate, since its behavior in the gastrointestinal tract appears to be independent to the solids and liquid phases.

The dual phase marker technique with spot sampling (Faichney, 1975) yielded similar results to Cr_2O_3 in continuous collections (MacRae, 1975). The dual phase marker system as described by Faichney (1975) and simplified by Armentano and Russell (1985) though correcting adequately for non-representative sampling, does not correct for pattern of flow effects. Further marker migration among phases after sampling but before analysis may cause gross errors in flow estimates. Results from the present trial, suggest that Yb and Cr_2O_3 flowed independently from each other; however Co was more similar to Cr_2O_3 than to Yb. Similar results have been reported by Andersen et al. (1985) with steers grazing wheat pasture. Their estimates of duodenal flow were less variable when based on ytterbium than in chromium.

Estimates of fecal output were higher ($P<.05$) when based on cobalt than chromium or ytterbium concentrations (Table 3.6). Although in this experiment total feces were not collected to compare with marker fecal output estimates, expected DM digestibility based on NRC (1984) values for

Table 3.6 Fecal output and DM digestibilities in heifers fed concentrate diets based on particle and iquid markers

Item	Markers			SEM
	Yb	Cr	Co	
Fecal output kg DM/d	$2.52^{a}\pm.03$	$2.68^{b}\pm.08$	$3.13^{c}\pm.04$	0.05
Apparent digestibility, %				
Ruminal	$58.53^{d}\pm3.1$	$49.45^{e}\pm6.1$	$44.97^{e}\pm5.8$	2.00
Total tract	$74.75^{a}\pm.31$	$73.10^{b}\pm.85$	$68.64^{c}\pm.44$	0.57

^{a,b,c}Mean values in a row with different superscript are different ($P<.05$).

^{d,e}Mean values in a row with different superscript are different ($P<.01$)

feeds fed was 78.1%. Thus, results of this trial suggest that cobalt overestimated fecal output and consequently underestimated digestibility. Relative to NRC calculated values for digestibility Yb tended to underestimate fecal output and slightly overestimate, DM digestibility. Collection of fecal grab samples every two hours, indicated that marker concentration in fecal DM varied between animals and hours of sampling (Figure 3.1).

Chromic oxide, exhibited more diurnal variation in excretion as indicated by its higher coefficient of variation (35 vs 17.2 and 16.3% for Cr, Yb and Co; Figure 3.1). Data from Vogel et al. (1985), in contrast, showed that Cr_2O_3 estimates of fecal output in cattle grazing wheat pasture were less variable than Yb or Co estimates. Prigge et al. (1981) compared twice-daily dosing of Yb vs Cr_2O_3 to assess diurnal variations in marker excretion in fecal grab samples collected at 4 h intervals in cattle fed forage diets. They indicated, that twice-daily dosing reduced diurnal variation in fecal marker excretions. Timing of the collection period, rather than differences in marker excretion across animals may have been involved. Phar et al. (1970) found no differences in Cr_2O_3 concentration in fecal DM of cattle fed once daily and dosed with marker every 8 h due to time of sampling when Angus steers were sampled every 2 h over a 48 consecutive hours. In contrast, our results indicate that time of sampling is important because fecal marker concentration varied during the day

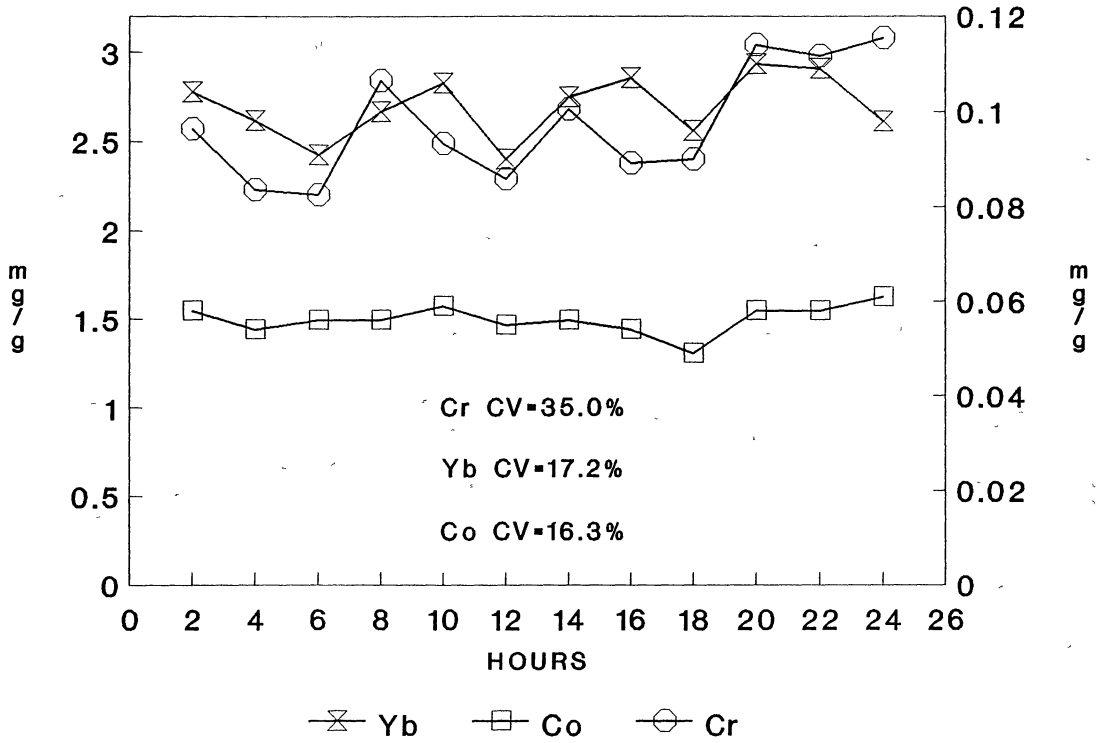

Figure 3.1 Diurnal changes in fecal marker concentration

(Fig 3.1). Similar results have been reported (Hopper et al., 1978) in lactating Angus grazing cows dosed with markers at 9 h intervals where diurnal patterns in Cr_2O_3 concentration in fecal DM, were observed. Marker concentration was highest at 0900, and lowest at 2000. Faichney (1972) stated that when Cr_2O_3 is given continuously in the diet of sheep, fecal marker concentrations did not change over time and concluded that estimates of digestibility were reasonable when marker is dosed continuously and representative fecal samples are collected.

Dry matter digestibility for the diet used in our study, was 78.1% as calculated from individual feed ingredients from the NRC (1984) tables. Dry matter digestibility appeared to be slightly underestimated with Cr_2O_3 and overestimated with Yb (Table 3.6). However this merely reflects differences in output of feces. When fecal output is overestimated, DM digestibility is underestimated. Table 3.6, also shows that estimates of DM digestibility with Co-EDTA are lower when compared to Cr_2O_3 or Yb. Reasons for these differences may be due to some cobalt absorption through the gastrointestinal tract. Although, calculated DM digestibilities with markers were slightly lower than estimated digestibility value from NRC tables, it appears that Yb predicts this parameter better than Cr_2O_3 or Co. Zinn and Owens (1980) measured DM digestibility of this diet at 75.2% in Hereford heifers.

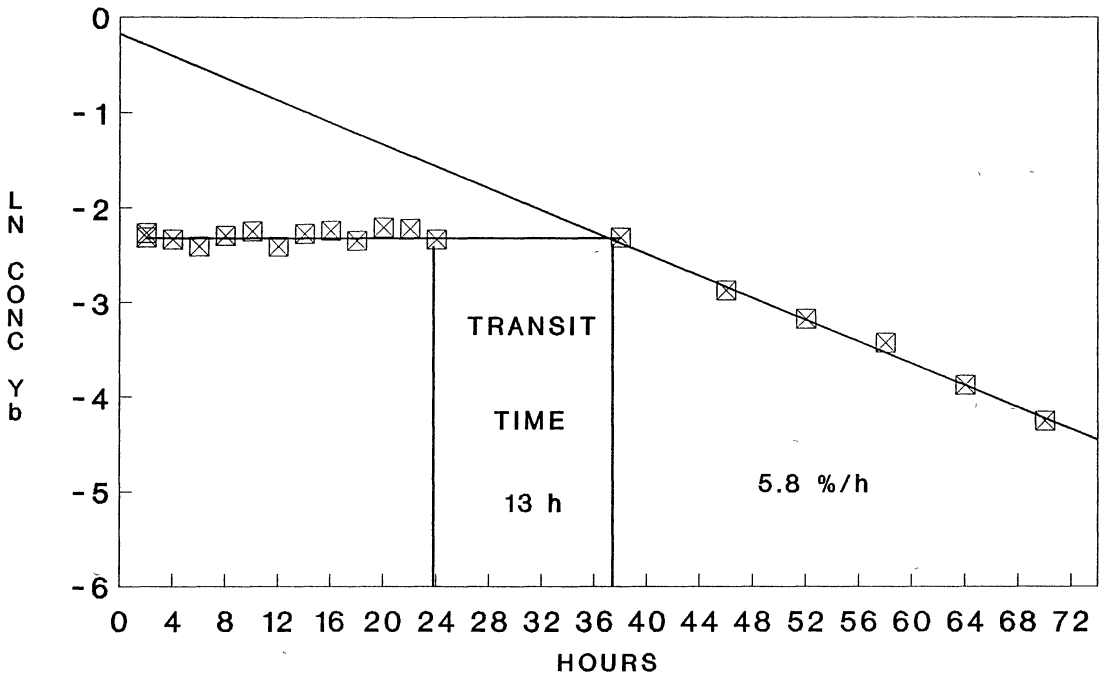

Figure 3.2 Diurnal rate of passage (ytterbium dilution rate)

Transit time (t, considered as the time (h) taken by the marker to travel from the mouth to the rectum) was estimated from the time of marker withdrawal from the diet to the decline in fecal marker concentration. Transit time differed between markers as illustrated in Figs. 3.2, 3.3, and 3.4. Transit time was faster (10 h) for Co, 13 h for Yb and 19 h for Cr_2O_3. These differences may reflect relative degrees of association of markers to either the solid or liquid fraction of digesta. Hence, Co, being water soluble, should have been cleared from the reticulo-rumen more rapidly than Yb or Cr_2O_3, differences in transit time between Yb and Cr_2O_3 may be due to the lack of association of Cr_2O_3 to the particulate phase of digesta, as suggested by Faichney (1975), or to closer association of Cr_2O_3 with the surface of the intestinal mucosa.

Passage rates differed ($P<.03$) between Yb and Cr (5.8 vs 8.6%/h) with Co being intermediate (7.3%/h) as shown in Figures 3.2, 3.3, and 3.4.

These results suggest that the rumen is acting as a major pool in delaying passage of particles to the hindgut. The slower passage rate and longer transit time for Yb than Co supports the concept that particles leave the rumen less rapidly than liquids do. Previous studies have indicated that fine, soluble and liquid matter leaves the rumen faster than fibrous material (Van Soest, 1982; Merchen, 1988, Owens and Goetsch, 1988). Passage rates estimated with Cr_2O_3 are more variable (CV=10.32 vs 26.2 and 12.27 for Yb, Cr and

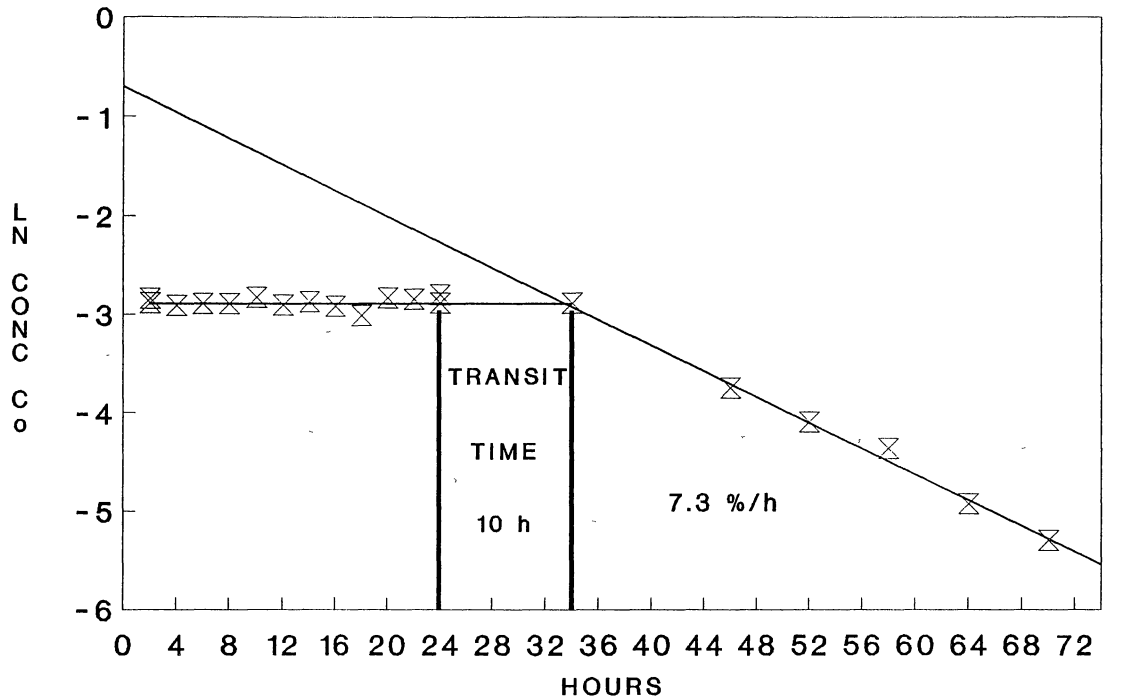

Figure 3 3 Diurnal rate of passage (cobalt dilution rate)

Co). Although transit time of Cr_2O_3 was the longest among markers, which might reflect longer particle retention time in the rumen, estimates of a faster passage rate were not expected. Estimates for Yb and Co seem to be more reasonable, however Co-EDTA dilution rates were much slower than estimated from the decline in ruminal Co concentrations (3.5 to 6%/h; Teeter and Owens, 1983).

One can speculate as to why Cr_2O_3 behaved different than Yb if they both mark solid phase in the rumen. Previous reports (Faichney, 1972; Faichney, 1975) indicated that Cr_2O_3 due to its physical characteristics travels at an independent rate than liquid or solid digesta. Consequently, Cr_2O_3 excretion in feces would be subject to daily variations (MacRae, 1974). Also, once Yb reaches the acidic conditions of the abomasum, it probably disossiates from particulate matter (Crooker et al., 1982) and thereafter flows with liquids and small particles, not particles.

The coefficient of variation in Cr_2O_3 concentration in feces was highest among the markers (35 vs 17.2 and 16.3% for Cr, Yb and Co) suggesting a different excretion pattern.

According to Faichney (1975), the use of Cr_2O_3 for measuring flow rates in animals fitted with single T-type cannulas is questionable because of the special movement of Cr_2O_3 throughout the gut. Whether or not postruminal mixing pools as indicated by Goetsch and Owens (1985) or

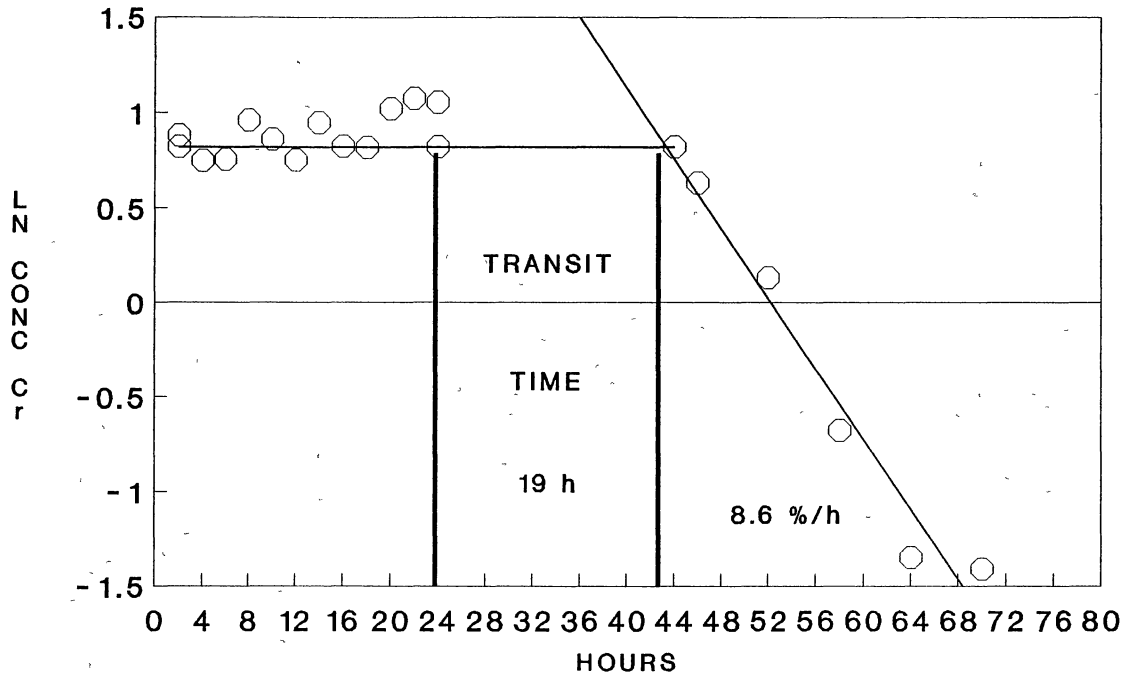

Figure 3.4 Diurnal rate of passage (chromium dilution rate)

association with the mucosal surface of the intestine contribute to some delay in Cr_2O_3 excretion is not known.

In summary, estimates of ruminal passage rate, duodenal flow, DM digestibility and fecal output differed among Yb, Co and Cr_2O_3. But ruminal volume measured at equal times postprandially in am vs pm did not differ. Concentrations of Yb and Co in DM of fecal grab samples collected at 2 h intervals throughout the day, were less variable than Cr_2O_3 concentrations. Although equilibrium of markers in the gastrointestinal tract was achieved, estimates of transit time and passage rates for the three markers differed markedly.

CHAPTER IV

EFFECTS OF DIET ON RUMINAL LIQUID AND ON BLOOD SERUM OSMOLALITY AND HEMATOCRIT IN FEEDLOT HEIFERS.

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ABSTRACT

Eight ruminally cannulated beef heifers (550 kg) were used in a crossover experiment to examine osmolality changes in ruminal liquid and blood serum. Blood hematocrit and ruminal pH also were determined. Heifers were adapted for 20 days to ad libitum intake of either an 80% concentrate or a prairie hay diet. After the adaptation period, ruminal and blood samples were obtained for three consecutive days at -2, 1, 2, 4, and 6 hours after feeding. Ruminal pH varied ($P<.05$) with diet and postprandial time, being higher ($P<.01$) prefeeding than postfeeding. Ruminal osmolality peaked 1 and 2 h postfeeding for the hay and concentrate diets at 265 and 296 mOsm/kg, respectively. Serum osmolality remained consistently higher than ruminal osmolality. Hematocrit was higher ($P<.004$) for heifers fed the hay diet, but postprandial changes were minor. Ruminal

liquid and serum osmolality ranged within normal physiological values with values peaking between 2-4 h postfeeding, both tending to be higher with the concentrate diet.

(Key Words: Ruminal Osmolality, Serum Osmolality, Hematocrit, Beef Cattle)

Introduction

Dietary constituents and metabolites can influence the ruminal environment markedly. Ruminal pH, because of simplicity of measurement has been associated by regression to rates of ruminal fermentation, rumination time, microbial population and volatile fatty acid production and concentration (Merchen, 1988; Owens and Goetsch, 1988; Owens and Zinn, 1988; Welch and Hooper, 1988; Yokoyama and Johnson, 1988). Another important ruminal variables which might influence ruminal fermentations, is osmotic pressure. Previous experiments, primarily with sheep, have demonstrated that osmolality in the rumen is important (Van Weerdeen, 1961; Warner and Stacy, 1965; Ternouth and Beattie, 1971; Bergen, 1972; Warner and Stacy, 1977; Phillip et al., 1981 a,b; Carter and Grovum, 1989; Peters et al., 1989; Teller et al., 1989). High ruminal fluid tonicity has been associated with reduced feed intake and influx of water from blood into the

rumen. Likewise an increased (380-400 mOsm/kg) osmolality of ruminal contents seemed to depress rumination and motility of the rumen (Martz and Belyea, 1986; Welch and Hooper, 1988). High osmolality can damage the ruminal epithelium during severe acidosis (Dirksen, 1970). Ruminal osmolality is basically dependent upon diet constituents and their fermentation within the rumen. Feeding high levels of fermentable carbohydrates such as grains, will increase osmolality. Prior to feeding, ruminal liquid normally is hypotonic to blood, but tonicity rises (350-380 mOsm/kg) soon after feeding. According to Phillip et al. (1981), high ruminal osmolalities (525 mOsm/kg) depressed feed intake of ruminally cannulated lambs within 30 min after feeding. These authors concluded that ruminal osmolality inhibited short term feed intake. Regression analysis (Carter and Grovum, 1990) suggested that tonicity was important, but some measurements of tonicity, as when access to water is prohibited, may be abnormal physiologically. A report by Teller et al. (1989) indicated that voluntary feed intake of Holstein heifers fed either direct cut or wilted grass silage was not altered by ruminal osmolality. The purpose of our experiment was to measure the change in osmolality in ruminal liquid and in blood serum with time after

feeding beef heifers different types of diets. Packed red cell volume (hematocrit) and ruminal pH also were monitored.

Materials and Methods

Eight crossbred beef heifers (550 kg), fitted with 10 cm i.d. ruminal cannulas, were used in a crossover design with two 23-d experimental periods. Animals were randomly assigned to individual pens and diets and received a concentrate diet (Table 4.1) or chopped prairie hay supplemented daily with 1.5 kg/hd of a 50% protein concentrate. During the first 20 days of each period animals were given ad libitum access to their diet. Feed intake was recorded daily. Feed was provided twice daily (0830 and 1630) during the entire trial at 120% of the previous days intake. After day 20, intake was restricted to 90% of the mean intake on days 14 to 19. Water and a mineral premix were available at all times. Ruminal and blood samples were collected sequentially during the last 3 days of each experimental period, at -2, 1, 2, 4, and 6 h after the 0830 feeding. A 30 ml blood sample was withdrawn at each time via jugular venipuncture. Blood was placed in siliconized tubes to harvest serum. Immediately after collection, 10 ml of blood were transferred into heparinized tubes for hematocrit determination. Blood

Table 4.1 Composition of concentrate fed (DM Basis)

Ingredient	%
Corn, dry rolled (IFN 4-02-931)	63.10
Cottonseed hulls (IFN 1-00-599)	14.10
Soybean meal, (IFN 5-04-600)	10.05
Alfalfa pellets, dehydrated (IFN 1-00-023)	6.00
Cane molasses (IFN 4-04-696)	5.00
Salt (trace mineralized)[a]	.50
Ground limestone (IFN 6-02-632)	.50
Dicalcium phosphate (IFN 6-01-080)	.50
Aurofac-50[b]	.15
Urea (42% N)	.10
TOTAL	100.00

[a]Trace min, Carey Salt, Mission Kansas, contained: NaCl 92-97%, Mn .250%, Fe .200%, Cu .033%, I .700%, Zn .005%, Co .0025%, white mineral oil.

[b]Aurofac-50, CADCO, Inc., DesMoines, Iowa. Contained: 50 g of chlortetracycline per 454 g.

serum samples were frozen at -20°C until osmolality was analyzed. Aliquots from heparinized blood samples were transferred to microhematocrit capillary tubes and hematocrit was determined in triplicate within 1 h after blood sampling.

Ruminal liquid samples were taken prior to each blood sample. Approximately 250 ml of fluid were withdrawn from the ventral ruminal sac with a suction flask and a manual pump. Immediately after collection, ruminal liquid was filtered through two layers of cheesecloth and pH was determined with a glass electrode. Thereafter, the samples were centrifuged[1] at 10,000 x g for 15 min; aliquots of the supernatant fluid were frozen and stored at -70°C until analyzed. At the time of analysis, the serum and ruminal samples were thawed and osmolalities were determined in duplicate in an osmometer[2] using the freezing point depression procedure.

Data were analyzed as a crossover experiment with a split plot in time (days in period and hour in day). Treatment x animal x period was used as the whole plot error term, and sampling time within day was the error term to calculate sampling time effects. Differences

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[1] Sorvall RC2-B, Du Pont Co.,Wilmington, Delaware.
[2] OSMETTE model 2007, Precision Systems, Inc., Sudbury, Mass.

between means were analyzed using an LSD procedure as indicated by Steel and Torrie (1980). Time effects were divided into for comparison into -2 vs the other times (pre- vs post-feeding) and linear and quadratic effects of post-feeding time.

Results and Discussion

Ruminal pH was altered ($P<.05$) by diet type (Table 4.2). Heifers fed the high concentrate diet had overall lower ruminal pH. Ruminal pH at -2 hours was higher ($P<.01$) than the mean pH postprandially (Figure 4.1). In addition, a post-prandial quadratic ($P<.07$) effect of time on pH was detected.

Mean values for ruminal pH were within the range expected (Owens and Goetsch, 1988) for concentrate (5.5 and 6.5), and roughage (6.2 and 7.0) diets. They indicated that pH usually is lowest between .5 and 4 h after a meal; this agrees with our data. In this study, pH tended to be lowest two hours after feeding for the concentrate diet vs 4 hours postfeeding for the hay diet (Figure 4.1; Table 4.3).

Osmolalities of ruminal liquid and of serum were similar ($P<.05$) between the hay and concentrate diets (Figure 4.2); however values tended to be higher with the concentrate diet (Table 4.3). Postprandial values for ruminal and serum osmolalities were higher ($P<.05$)

Table 4.2 Effect of diet on ruminal pH, hematocrit, ruminal liquid and serum osmolalities of heifers fed hay or concentrate diets.

	Diet		
Items	Concentrate	Hay	SE
Ruminal pH	6.2[b]	6.7[a]	0.09
Hematocrit %	34.4[b]	37.0[a]	0.43
	-osmolality (mOsmol/kg)-		
Ruminal Liquid	284.0	250.4	13.60
Serum	303.0	296.0	4.61

[a,b]Mean values in a row with different superscript are different ($P<.05$).

SE = Standard Error.

than the preprandial level (Table 4.4), however peak values never reached those reported by others (Warner and Stacy, 1965; Ternouth, 1967; Engelhardt, 1975; Phillip et al., 1981; and Teller et al., 1989). This may be attributed to the fact that most of these studies measured osmolality in sheep and the sheep had been deprived of food or water for different periods of time; in some studies, salt loads were infused into the rumen. Such experimental procedures may alter dramatically ruminal liquid osmolality. Presumably, the preprandial (-2 h) osmolality values in the range of 200-280 mOsm/kg, as we observed, are more physiological as mentioned by Mackie and Therion (1984). Postprandial ruminal osmolality values, however, can vary considerably depending upon the type of diet (Warner and Stacy, 1965; Bergen, 1972; Bennink et al., 1978) and the concentration either of the dissolved substances in the feed or the products of microbial activity (Schwartz and Gilchrist, 1975; Martens, 1985). Lower ruminal osmolality values for the roughage diet presumably were due to lower production of solutes and greater dilution of these solutes (VFA and mineral salts) by saliva. Bennink et al. (1978) indicated that VFA and minerals are the major contributors to the rise in osmolality, but their

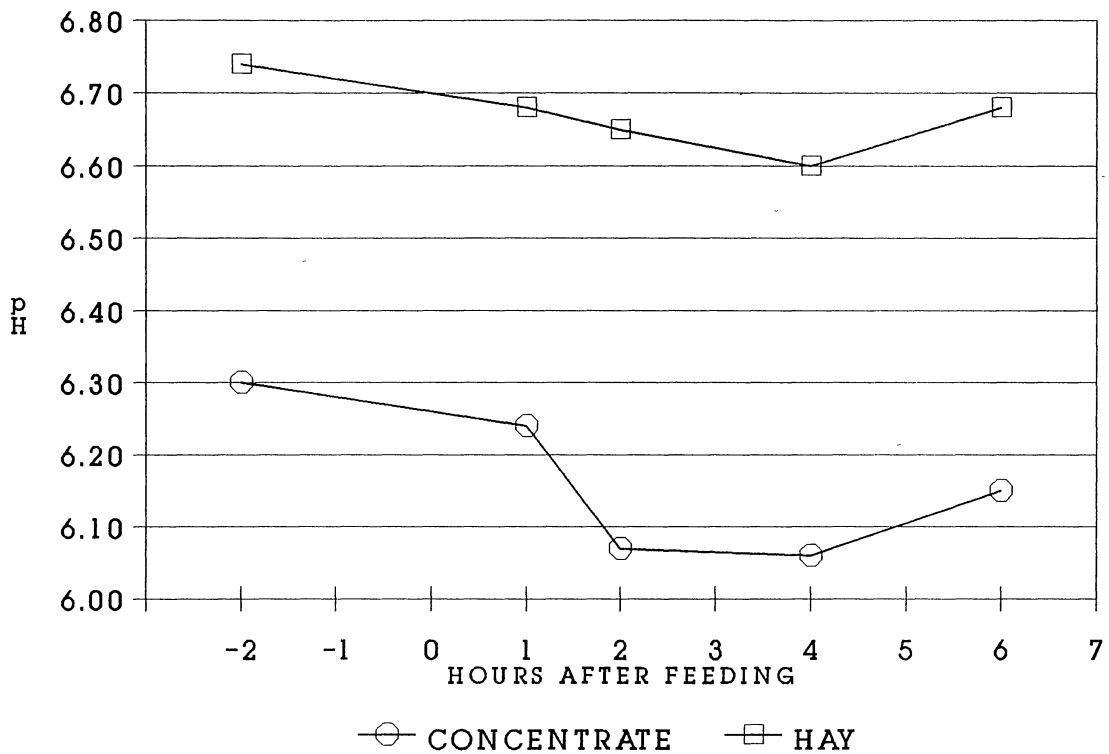

Figure 4.1 Ruminal pH vs time in beef heifers fed concentrate and hay diets

relative contribution varies with diet. These authors, reported that mean ruminal osmolalities were 312 and 332 mOsm/kg for Holstein steers fed alfalfa hay and concentrate diets, respectively. Those values tended to exceed ours, but they restricted water intake while we did not. In Bennink's experiment Ruminal osmolalities changes at 2 h postprandially, expressed as a percentage, were 19% and 15% for the alfalfa and concentrate diets, respectively. In our study, values increased only 6% and 9% for the concentrate and prairie hay, respectively.

Intraruminal infusions of water or hypertonic solutions can alter the osmolality of both ruminal fluid and blood (Warner and Stacy, 1977). Kato et al. (1979) showed that infusions of water into the rumen of sheep, decreased osmolality by 33%. The concentrations of Na^+ and K^+ in ruminal fluid was reduced by 35% and 31%, respectively when compared to the control animals. In contrast, the addition of a highly concentrated solution of electrolytes to the rumen, drastically increased ruminal osmolality. Similar results <u>in vitro</u> have been documented by Ferreiro (1986) in that additions of artificial saliva, carbohydrates or molasses increased ruminal liquid osmolality. He concluded that molasses had a greater effect upon

Table 4.3 Postprandial changes in ruminal pH, hematocrit, ruminal liquid and serum osmolalities of beef heifers fed hay or concentrate diets

		Time before or after feeding (h)					
Items	DIET*	-2	1	2	4	6	SE
Ruminal pH	C	6.3^{b}	6.2^{b}	6.1^{b}	6.1^{b}	6.2^{b}	
	R	6.7^{a}	6.7^{a}	6.7^{a}	6.6^{a}	6.7^{a}	.09
Hematocrit(%)	C	34.8^{b}	34.8^{b}	34.0^{b}	34.1^{b}	34.2^{b}	
	R	37.2^{a}	36.7^{a}	36.8^{a}	37.5^{a}	37.0^{a}	.53
		--------- osmolality (mOsmoles/kg)---------					
Ruminal Liq	C	278.5	288.9	296.0	280.2	277.8	
	R	241.2	264.7	256.9	247.2	241.9	14.26
Serum	C	296.5	302.5	303.9	304.4	308.7^{a}	
	R	285.6	301.1	299.5	304.9	288.9^{b}	6.11

*C = Concentrate diet; R = Prairie hay.

^{a,b}Mean values in a column with different superscript are significantly different ($P< 0.05$).

SE = Standard Error.

ruminal liquid osmolality due to its high mineral content.

When the rumen becomes hyperosmolar, direct addition of water to the rumen will lower the ruminal osmolality (Ternouth and Beattie, 1971). Yet, Engelhardt (1969) stated that hypotonicity of the rumen contents can not be explained either by the inflow of saliva or by influx of water into the rumen. He concluded that one major cause for ruminal liquid hypotonicity was absorption of VFA through the ruminal epithelium. Similarly, Ternouth (1967) indicated that 2 h after feeding VFA concentration and osmolality dropped simultaneously even though that ruminal osmolality always remained higher than serum levels. Contrary to these suggestions, Warner and Stacy (1972) indicated that sodium absorption from the rumen is one of the major causes of ruminal liquid hypotonicity in fasted sheep.

Serum osmolality (Figure 4.2) increased after feeding and remained elevated for 4 h ($P<.006$). Thereafter, osmolality decreased slightly to return to its preprandial level with the roughage diet (Table 4.4). Ternouth (1967) reported similar results in Merino ewes fed an alfalfa hay diet and given unrestricted access water. In our study, serum

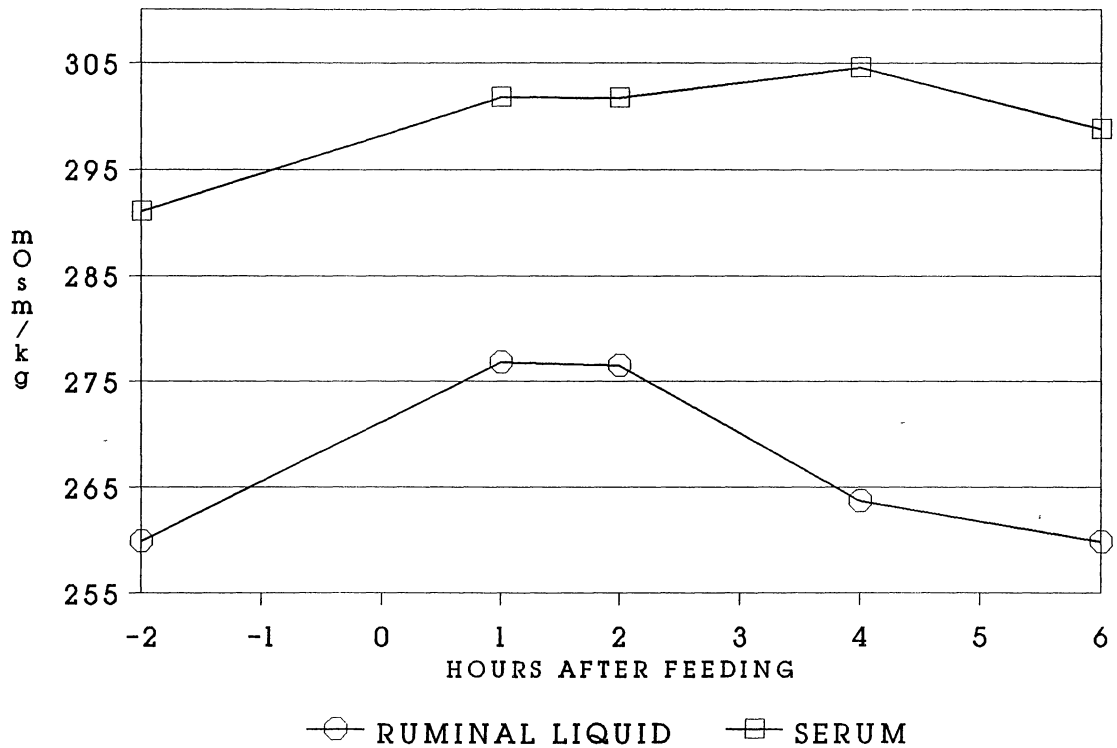

Figure 4.2 Serum and rumen osmolalities vs time in beef heifers fed concentrate and hay diets

osmolality values never were lower than the ruminal fluid osmolalities as had been reported by Ternouth (1967). However, plasma tonicities continued to increase by 12 mOsm/kg after 6 h postprandially; this linear increase ($P<.07$) was obvious only for animals fed concentrate (Table 4.4). In contrast, animals consuming hay reached a maximum serum osmolality value of 304 mOsm/kg 4 h postprandially. Thereafter, at 6 h postfeeding, serum osmolality dropped. Based upon these findings, one can speculate that VFA uptake because influx of water across the ruminal wall cannot explain this change because water flux should be in the opposite direction. Whether changes in salivary flow were associated with the lower osmolality of ruminal contents of heifers fed the hay diet is not known. However, Blair-West and Brook (1969) reported that plasma volume decrease at 20 to 60 min postfeeding. Differences in saliva production during eating or to net transfer of water into the rumen may explain the differences in ruminal tonicity between diets.

Warner and Stacy (1977) showed that total saliva production decreased as the tonicity of plasma or ruminal fluid were increased with various solutes infused intraruminaly. Moreover, flow rate was decreased in both parotid glands. According to Carter

Table 4.4 Changes in Ruminal pH, Hematocrit, Ruminal liquid and serum osmolalities of beef heifers related to time of feeding

	Time before or after feeding (h)						
Items	-2	1	2	4	6	Eff*	SE
Ruminal pH	6.5	6.5	6.4	6.3	6.4	Q	0.03
Hematocrit (%)	36.1	35.8	35.4	35.8	35.6		0.50
	--------osmolality (mOsmoles/kg)--------						
Ruminal Liquid	259.9^{b}	276.8^{a}	276.5^{a}	263.7^{b}	259.8^{b}	L	6.80
Serum	291.1^{b}	301.8^{a}	301.7^{a}	304.6^{a}	298.8^{ab}		6.35

*L= linear ($P< 0.05$); Q= quadratic ($P< 0.05$). Effects on post-feeding values.

^{a,b}Mean values in a row with different superscript are significantly different ($P< 0.05$).

SE = Standard Error.

and Grovum (1990) there is a negative relationship between saliva production and osmolality of body fluids, which helps to maintain fluid and electrolyte homeostasis in blood. Further studies should consider both how saliva production alters osmotic status of the rumen and the converse, i.e., how osmolality of serum influences salivary flow.

Packed red cell volume (hematocrit) was consistently higher ($P<.004$) for heifers fed hay than for heifers fed concentrate (Figure 4.3). No differences between preprandial and postprandial times or between postprandial times proved significant. Hematocrit levels were close to the normal physiological values (32 to 35%) reported in the literature (Swenson, 1984). But published information on the effects of different types of diets on hematocrit is very limited.

Warner and Stacy (1965) indicated that high ruminal osmolalities (near 400 mOsm/kg) were accompanied by hemoconcentration in sheep. They attributed these changes to transfer of body water from the blood into the rumen. Likewise, Ternouth (1967) with Merino ewes, observed that packed cell volume and serum proteins in blood increased during the first hour postfeeding. In contrast, we observed no postprandial

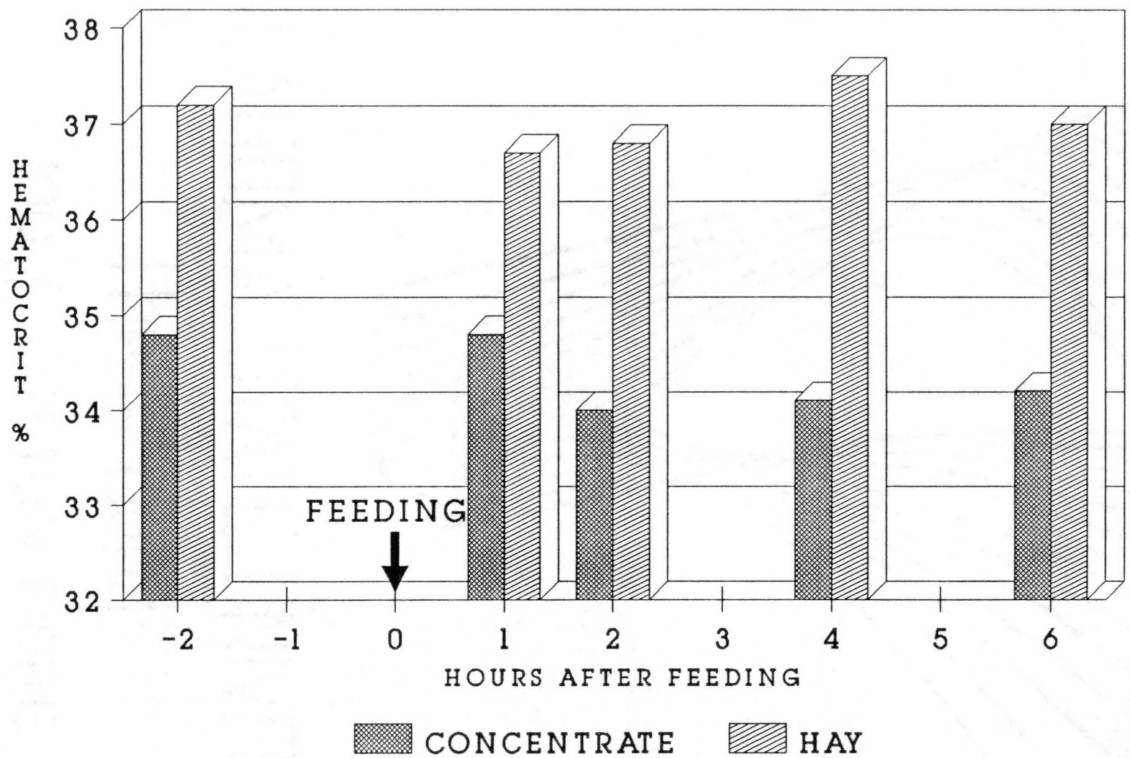

Figure 4.3 Hematocrit vs time in beef heifers fed concentrate and hay diets

shifts but ruminal tonicities remained low, also. Ferreiro (1986) reported average hematocrit values of 42% and 45% in cattle fed high molasses diets, suggesting that his animals were dehydrated. He suggested that a high hematocrit may be one of the reasons why animals fed molasses-based diets drink more water/kg DM than the control animals do. Engelhardt (1970) found very little net movement of water across the ruminal epithelium when rumen osmolalities remained between 260 to 340 mOsm/kg. As ruminal fluid osmolality averaged about 240 mOsm/kg with the hay diet (Figure 4.4), water should be absorbed from, not diffuse into the rumen. This should cause hematocrit to be lower with roughage, not higher as we observed.

Ruminal liquid osmolalities (Figure 4.4) never attained the high values (500 mOsmol/kg) reported by Warner and Stacy (1965). Hematocrit was higher ($P<.05$) and ruminal osmolality (250.4 mOsm/kg) was lower for heifers fed the hay diet.

Serum osmolality remained relatively constant for both diets. Hence, hematocrit changes might be due to differences in saliva production. Barring differences in mineral absorption with the hay diet or clearance between diets, the higher hematocrit, might be explained by greater salivary flow providing saliva is

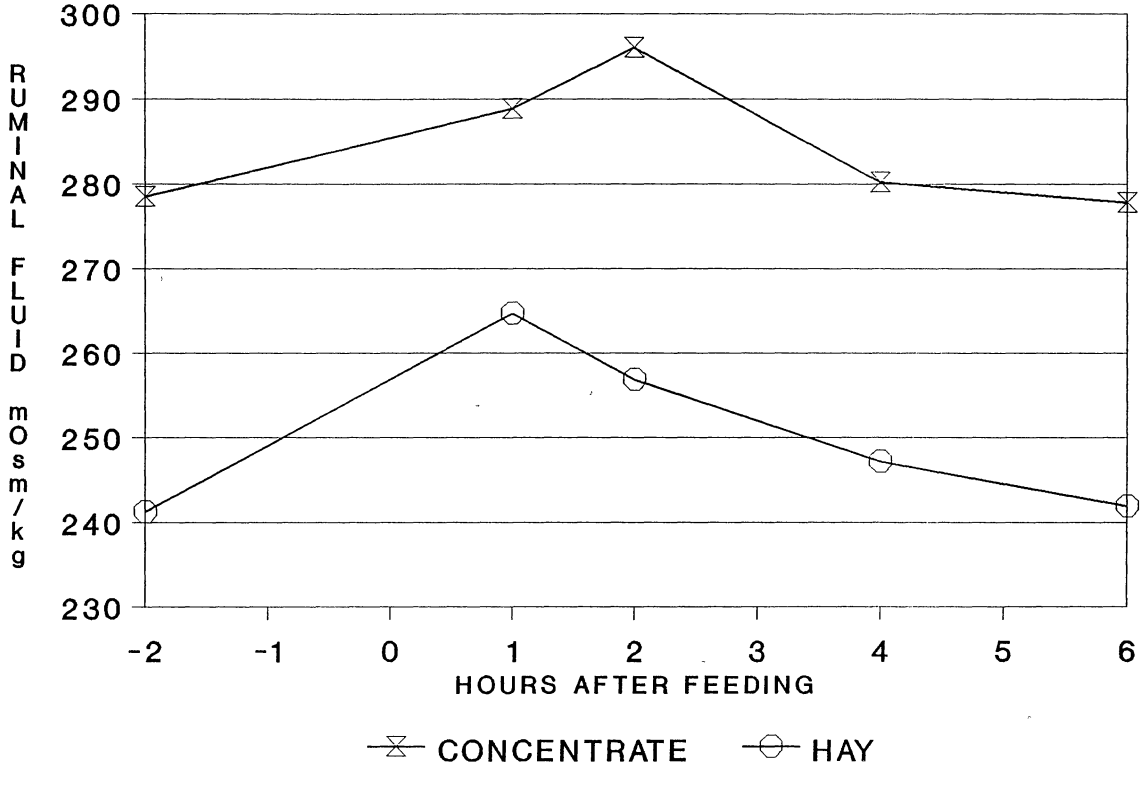

Figure 4 4 Rumen osmolality vs time in beef heifers fed concentrate and hay diets

hypotonic. The osmolality of plasma has been suggested to be one of the most important factors of salivary flow rate (Warner and Stacy, 1977). Hypertonic solutions infused intravenously reduced parotid salivation. According to Bailey (1961), salivation rate immediately before feeding is higher than salivation after feeding; postprandial salivation rate was the lowest for the day. Heretofore, these findings were attributed to ruminal distension, as noted by Wilson (1963) in which direct infusions of water into the rumen inhibited parotid secretion. Yet, Warner and Stacy (1977) indicated that hypotonic solutions infused into the rumen decreased plasma osmolality and stimulated salivation. Whether, the reduced ruminal and serum osmolalities observed in our study for the animals consuming hay, was due to increased salivary secretion or an increased ruminal distension is not known. However, these results indicated that animals consuming hay presumably had greater total saliva input into the rumen.

As indicated previously, little water moves across the ruminal wall when osmolality is isotonic to blood (260 to 340 mOsm/kg). If true, we cannot attribute our higher hematocrit to net transfer of water from blood into the rumen. Red blood cell size variations also

can alter hematocrit. Increased serum osmolality would cause red blood cells (RBC) to shrink which in turn would decrease hematocrit. As diet did not alter serum osmolality, altered size of RBC alone is not a tenable explanation for the differences in hematocrit. Nevertheless, this assumption deserves scrutiny.

In conclusion, our results suggest that osmolality values of ruminal contents are maximum between 1 and 2 h after feeding; tonicity was higher with grain than low quality forage diet. Serum osmolality peaked later (4 to 6 h or later after feeding). Hematocrit was greater with the low quality forage diet than the concentrate diet; differences were not expected and have not been reported previously. Diet effects and postprandial changes in osmolality of the blood serum and ruminal fluid do not support the idea that flux across the rumen wall is extensive under normal feeding conditions. Flux across the rumen wall may be more evident when water is restricted or salt is fed or infused into the rumen. High Hematocrit and low ruminal osmolality may reduce salivary flow. Whether a high hematocrit could depress salivary flow and forage intake in vivo deserves study.

CHAPTER V

EFFECT OF DIET AND LEVEL OF INTAKE ON RUMEN LIQUID AND SOLID VOLUMES, PASSAGE RATES, AND WATER CONSUMPTION OF BEEF CATTLE.

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ABSTRACT

Twelve Hereford x Angus heifers (604 kg) fitted with rumen cannulas were used to determine the effect of diet and feed intake level on rumen liquid and solid volumes and passage rates. Animals were adapted to either an 80% concentrate diet or to an isonitrogenous (60% alfalfa : 40% prairie) hay diet and fed once (0800) daily at one of three levels of intake (1.0, 1.4 and 1.8% BW on a DM basis) for a minimum of 14 d, in four (two replicated) 3 x 3 latin squares. Ruminal contents were evacuated, and screened to separate liquids from solid digesta at the end of each period. Rumen liquid volumes were larger ($P<.01$) for the hay diet (71.3 vs 46.5 liters). Outflow varied ($P<.01$) between diets (132 vs 75 liters/d, for the hay and concentrate diets, respectively). Water consumption tended

(P<.10) to be higher for the hay diet, and consumption increased linearly (P<.01) with the level of DM intake (3.0 ± .15 liters/kg roughage diet, 2.4 ± .15 liters/kg DM concentrate diet). Level of intake did not altered fluid volume. Likewise, liquid passage rate changed linearly (P<.05) with DM intake level. Blood hematocrit declined as feed and water intake increased. Treatment x level of feed intake interactions were not significant for rumen volume, water intake and hematocrit, however, interactions were detected for outflow and liquid passage rate. Positive correlations between rumen volume and outflow (r=.81; P<.001); water intake and level of DM intake (r=.66; P<.001, across diets) were detected. Whereas, negative correlations were apparent between rumen volume vs hematocrit (r=-.63; P<.004); and liquid dilution rate (r=-.46; P<.05). Our results indicate that ruminal pool size is less flexible (CV=8.74) than dilution rate (CV=11.55).

(Key words: Intake Level, Liquid Passage Rate, Rumen Evacuation, Water Intake).

Introduction

Addition of forage to a grain diet, shifts site of digestion from the rumen to the intestines (Cole et al., 1976; Teeter, 1981) and increases turnover rate of both fluid and particulate digesta from the rumen (Grovum and Williams, 1979). Level of feed intake presumably is one of the major factors regulating rumen turnover; however, other

factors, such as physical characteristics of the diet (bulk, particle size), also have an impact on rumen volume and gut fill. Little change in liquid dilution rate but a reduced rumen volume were observed (Sutton, 1980) when concentrate replaced 50% or a 100% hay diet. Colucci et al. (1982) indicated that passage rate was slower both for hay and concentrate when the level of concentrate was increased from 17 to 68% in non-lactating dairy cows. In contrast, Bernal (1989) found that liquid turnover in early lactating dairy cows fed a mixed diet was faster at lower levels of feed intake. Poore et al.(1990) reported that dietary concentrates did not influence ruminal passage rate of alfalfa hay relative to sorghum grain when concentrate was increased from 30 to 60%; but when concentrate was included at 90%, passage rate of alfalfa hay decreased. Increasing the fraction of concentrate in the diet decreases ruminal fluid rate of passage (Evans, 1981 a,b; Owens and Goetsch, 1986), probably by altering mastication and rumination time. Roughages stimulate saliva production which in turn may increase in fluid passage rate. Both saliva secretion and water consumption can be affected by the nature of the diet, and might be expected to promote washout of small particles and soluble substances from the rumen.

Research concerning the effects of intake level on liquid passage rates of diets containing high levels of concentrate have received little attention. The objectives of this study were to determine the effect of diet type

(concentrate vs hay) and three different levels of feed intake on water consumption, blood hematocrit, liquid rate of passage, and solid and liquid volumes measured directly by ruminal evacuation.

Materials and Methods

Twelve mature (7 years old) Hereford x Angus beef heifers (604 kg) fitted with large ruminal and T-type duodenal cannulas were fed either a concentrate or a mixed roughage (60% alfalfa : 40% prairie hay) diet at three intake levels (1.0, 1.4, and 1.8% of individual BW/d; DM basis). Animals were housed individually, adapted to their diets for a minimum of 14 d, and fed once daily at 0800. Water was offered in individual troughs. Water intakes were recorded daily using a water meter. Within each 3 x 3 latin square, each animal received three levels of intake but remained on the same diet. So 6 animals chosen randomly received each diet, continuously.

Blood and ruminal liquid samples were collected two hours after feeding, 3 days before ruminal evacuation; pH was determined and blood was sampled for hematocrit measurement. At 21 h prior to ruminal evacuation, Co-EDTA was pulse dosed (250 ml) into the rumen (Co-EDTA acetate containing 1 g of Co) of each animal. An equal volume of tap water was used to rinse any residual marker left in the graduated cylinder and funnel into the rumen. Total rumen contents were removed mechanically using a vacuum device at

the end of each experimental period 21 h after the cobalt was dosed. Ruminal contents were screened twice through a screen with 63 by 63 mm openings and 31 by 31 mm square pore mesh. Procedures to separate the liquid and the solid phases and marker analysis were similar to those described in Chapter III. Calculations for liquid dilution rate, dry matter, bound liquid to the solid phase, free liquid, and ruminal volume are presented in the Appendix A. Hematocrit and pH determinations were described in Chapter IV.

Data were analyzed using a general linear models (GLM) procedure for a split plot experiment using a complete randomized design for the main units. The Animal within diet interaction was used as an error term for the main plot (diet). The subunits (intake levels) were arranged in a 3 X 3 latin square design and orthogonal contrasts were used to test for linear and quadratic effects of intake. The diet by intake interaction was tested by using the square by intake interaction.

Results and Discussion

Daily water intake tended to be greater ($P<.10$) with the hay diets (Figure 5.1) and increased ($P<.001$) with levels of feed intake for both the concentrate and the hay diets almost doubling as intake increased by 80%. No water intake by diet interaction was detected ($P>.54$). Daily water intake values are in agreement with those of animals fed concentrate diets reported in Chapter III. Likewise,

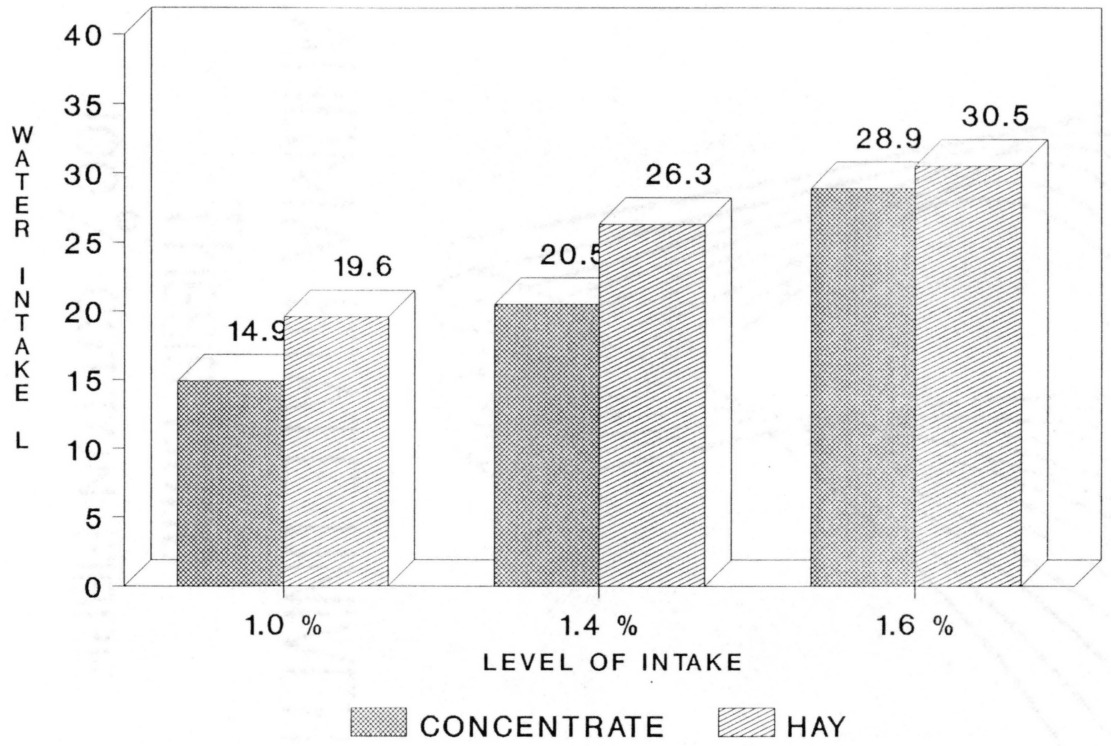

Figure 5.1 Daily water consumption in beef heifers fed concentrate vs hay diets

Leitch and Thompson (1944), Winchester and Morris (1954), Kay and Hobson (1963) and Hicks et al. (1988) indicated that feed intake was closely related to water intake. The high correlation between water intake and DM intake in this study ($r=.71$; $P<.001$) was similar to the correlation ($r=.84$; $P<.04$) for the same parameters observed in heifers consuming concentrate in Chapter III.

Total weight of DM in the rumen is shown in Figure 5.2. No differences were detected ($P>.19$) between the concentrate and the hay diet; however, as level of intake increased, total weight of solids in the rumen increased (7.3 vs 8.6 and 9.5 kg for the low, medium and high level of concentrate). No intake by diet interaction was detected for ruminal solids. Previous reports (Campling et al., 1961; Campling, 1966; Grovum and Williams, 1977; Evans, 1981a; Owens and Goetsch, 1986, Poore et al., 1990) all have indicated that quantity of DM in the rumen increased as the level of DM intake increases. Campling et al. (1961) fed hay at 4.5 or 7.0 kg/d or ad libitum_to mature cows and noted that intake markedly affected the weight of DM in the reticulo-rumen. Likewise, total rumen solids in the rumen of sheep increased by 81% when daily intake of chopped hay was increased from 400 to 1,300 g/d (Grovum and Williams, 1977).

Addition of forage to concentrate diets, generally has increased the amount of DM in the rumen, however the inclusion of different levels of concentrate (2.5, 5.0, and

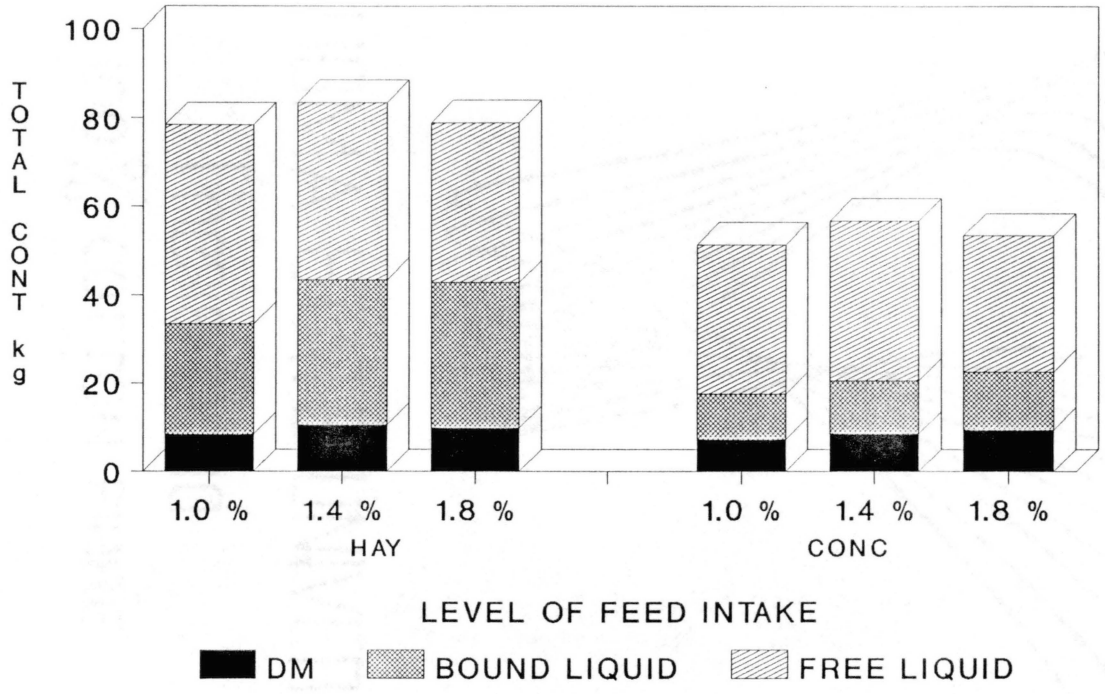

Figure 5.2 Total ruminal contents, kg

7.5 kg/d) to ad libitum hay diets did not affect the amount of DM in the rumen of cows immediately after feeding (Campling, 1966). Similarly, reports by Poore et al. (1990) support previous work, indicating that total DM in the rumen of steers fed an alfalfa-wheat straw based diet supplemented with 30, 60 or 90% of concentrate, was not influenced by dietary concentrate percentage.

Ruminal liquid volumes were larger ($P<.001$) for the hay than the concentrate diet (71 vs 46 liters; Figure 5.3), indicating that intake of fibrous materials increased liquid rumen volume markedly. However, level of feed intake did not affect ruminal liquid volume and no interaction was detected ($P>.63$). Jacques et al. (1989), indicated that at equal intake levels, ruminal liquid volume in Jersey cows increased as percentages of forage in the diet increased. However, our results concerning effects of diet type on liquid volume contrast with those of Grovum and Williams (1977). They found that increasing the level of alfalfa in the diet of sheep, markedly increased ruminal liquid volume. However, intake of CP and minerals increased with added alfalfa in their study. Whereas CP and mineral intake in our study for the concentrate vs hay diet were similar.

Data on ruminal liquid volumes as influenced by feed intake and concentrate level were summarized by Owens and Goetsch (1986). Ruminal liquid volumes observed in our study are higher for the hay diet fed at all levels of intake when compared with regression values they reported

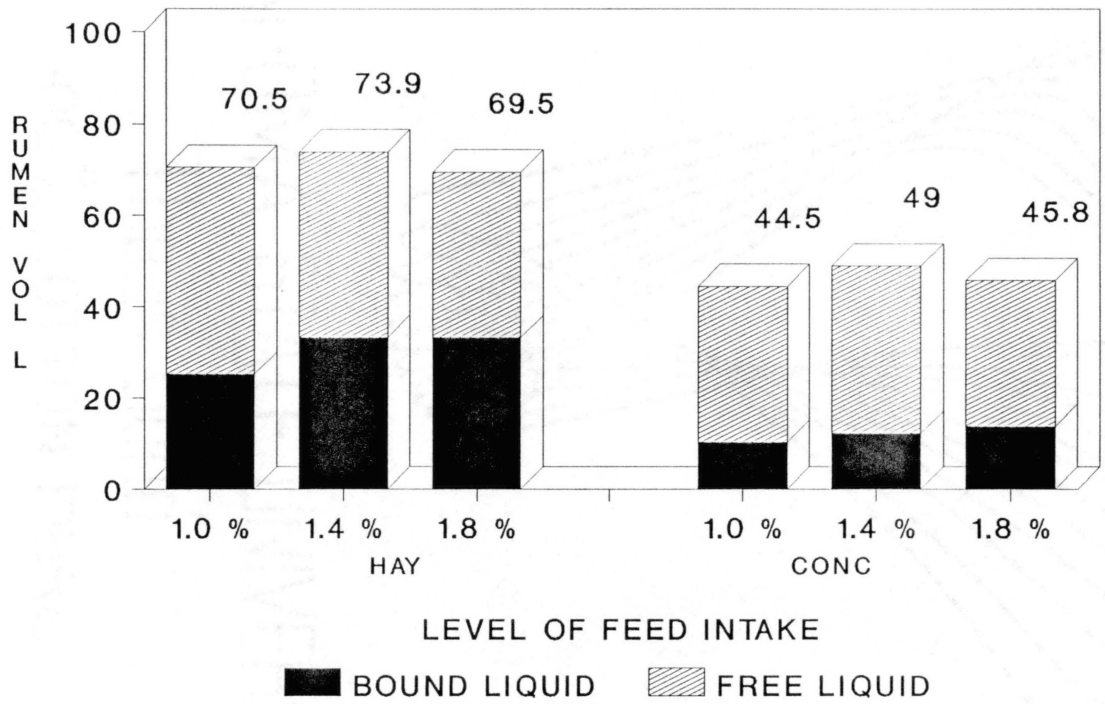

Figure 5.3 Rumen liquid volume in heifers fed concentrate vs hay diets

(69.6 vs 58.4 liters at low intake; 74.0 vs 52.4 at medium intake; 70.0 vs 45.7 at high intake). Conversely, ruminal liquid volumes for our concentrate diet were slightly lower than their estimates for the low and medium level of feed intake (44.6 vs 58.4 and 49.12 vs 52.4 liters) but similar at the high level of feed intake (45.7 vs 45.7 liters).

Liquid passage out of the rumen is measured and reported either as fractional passage (%/h) or outflow (liters/d). The former ignores ruminal volume differences but may regulate bacterial dilution rate. The later is more likely to be subject to physiological control. Liquid passage rate (%/h) increased linearly ($P<.001$) with level of intake for the hay diet (6.15 vs 8.10 and 8.8 %/h) and the concentrate diet ($P<.06$; 6.4 vs 6.6 and 7.4 %/h) for the low, medium and high levels of intake, respectively (Figure 5.4). Total ruminal liquid outflow from the rumen was higher ($P<.001$) for animals consuming hay than for animals fed concentrate (132 vs 75 liters/d; Figure 5.5). However, a level x treatment interaction was significant ($P<.02$). Likewise, liquid outflow tended to increase as level of feed intake increased in both diets although observed significant levels differed ($P<.001$ and $P<.17$) for the hay and concentrate diets, respectively. Changes in fluid passage rate and outflow were consistent with previous reports (Poutiainen, 1968; Grovum and Williams, 1977; Hartnell and Satter, 1979; Evans, 1981a; Zinn and Owens, 1983; Jacques et al., 1989). Evans (1981a), and Owens et al. (1984)

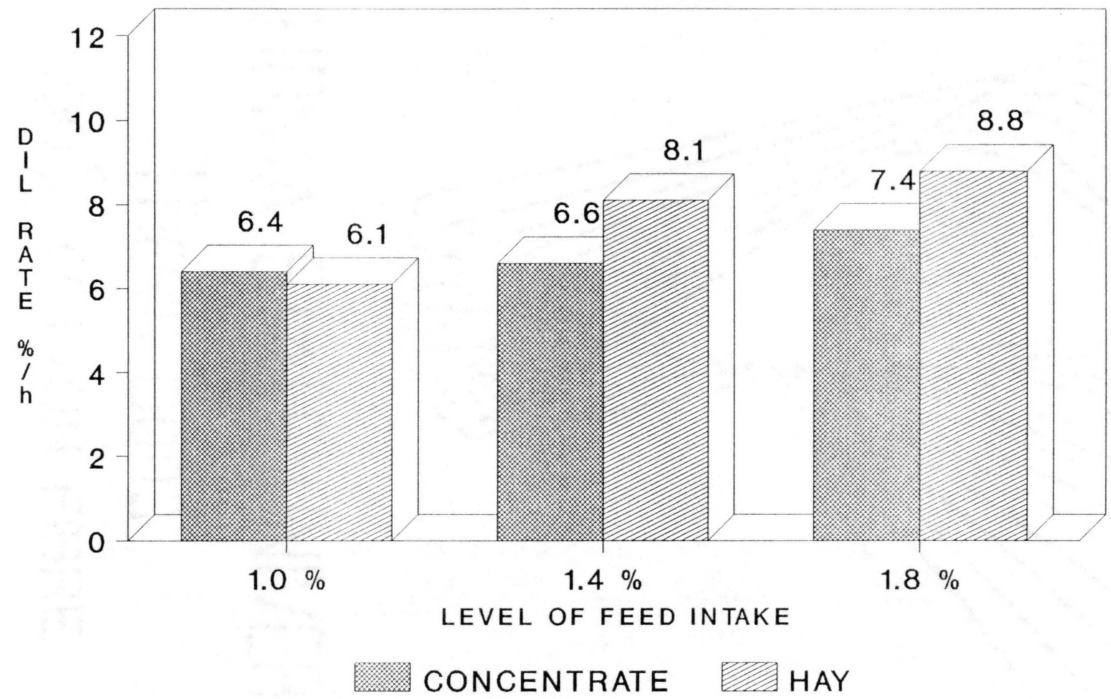

Figure 5.4 Rumen liquid dilution rate (%/h)

suggested that fluid passage rate increases as the level of roughage in the diet increases, and that concentrate diets tended to yield lower liquid passage rate, probably due to the low saliva input with concentrate diets. High levels of roughage in the diet will increase mastication and rumination with a subsequent raise in saliva production. According to Owens et al. (1984), the bulkiness of roughage may reduce fluid space in rumen; this factor together with the enhanced salivary production, may accentuate liquid passage rates with roughage diets. Increased feed intake, also may increase size of particles found in feces, suggestive of an increased exit rate of large particles from the rumen (Van Soest, 1982). This also may enhance fluid passage rate.

Owens et al. (1984), and Owens and Goetsch (1986) indicated that ruminal fluid volume often is related negatively to liquid rate of passage. The capacity of the rumen to expand, when it is subject to different intake levels, may directly affect liquid passage rate.

In this study, the correlation between liquid ruminal volume and liquid outflow (liters/d) was reasonably high ($r=.81$; $P<.001$) overall but higher for the concentrate diet ($r=.82$; $P<.001$) than for the roughage diet ($r=.49$; $P<.03$). Within or across diets, the negative relationship of ruminal volume to liquid passage rate was lower ($r=-.03$, $r=-.46$; $P>.10$, $P<=.05$) for the roughage and concentrate diets,

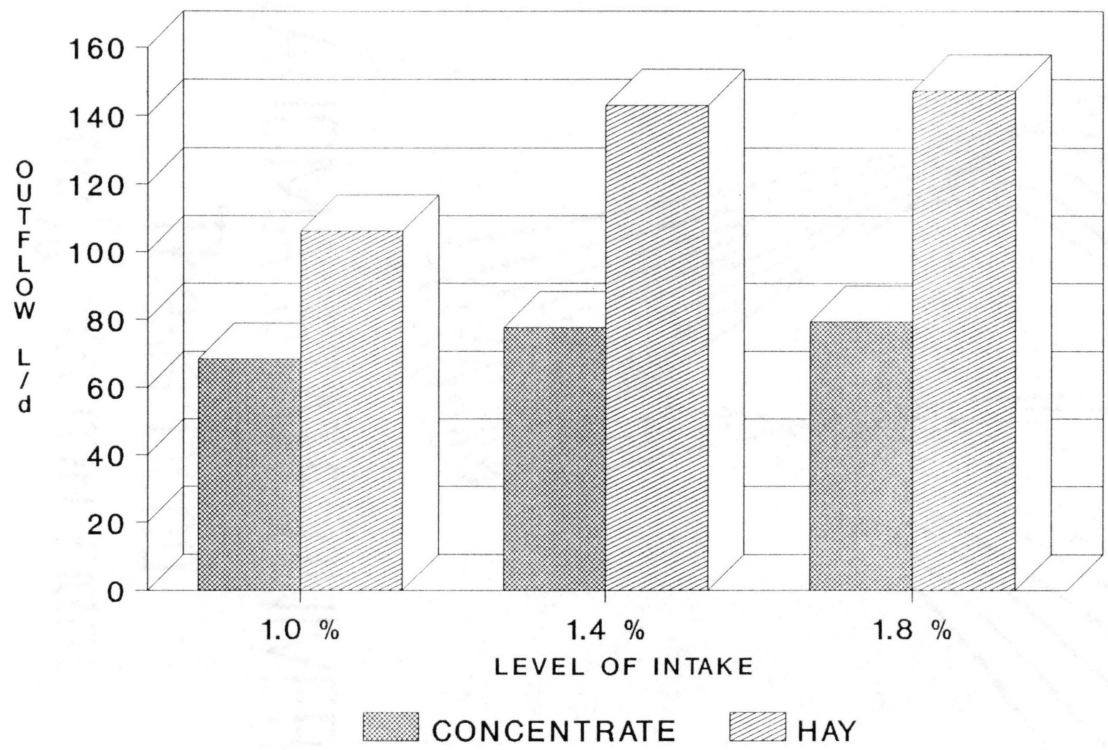

Figure 5.5 Rumen water outflow (liters per day)

respectively; however, the overall correlation was r=.10; P>.10.

Weston (1988) suggested that ruminal volumes, dilution rate and ruminal outflow were altered by water intake. Correlations within and across diets in this study were r=.18, r=-.15 and r=.20 for water intake versus ruminal volume, and r=.63, r=.39 and r=.54 for water intake versus dilution rate, and r=.65, r=.08, and r=.46 for water intake versus ruminal outflow.

Ruminal liquid pH was lower (P<.01) with the concentrate diet (6.0) than with the hay diet (6.5; Figure 5.6). Differences between levels of feed intake were only detected (P<.01) for the high vs low levels of concentrate. No significant interaction between diet x level was detected (P=.20). Values for ruminal liquid pH taken 2 hours postprandially with the same type of diets were similar in a previous study (Chapter IV). Ruminal pH usually is lowest between 1 to 4 h after a meal (Owens and Goetsch, 1986). Diet composition, and grain processing influence ruminal pH and fermentation patterns. With forage diets, ruminal pH typically varies between 6-6.5 (Beever and Siddons, 1986), whereas values with concentrate diets usually are lower and more variable (Counotte et al., 1979). Horn et al. (1979) reported that ruminal pH in dairy steers fed an 85% ground, ensiled high-moisture diet was consistently lower (5.4 to 5.5) at 4 to 8 h than at 2 h postprandially. Goetsch and Owens (1985a) found lower ruminal pH values (5.99) 2 h than

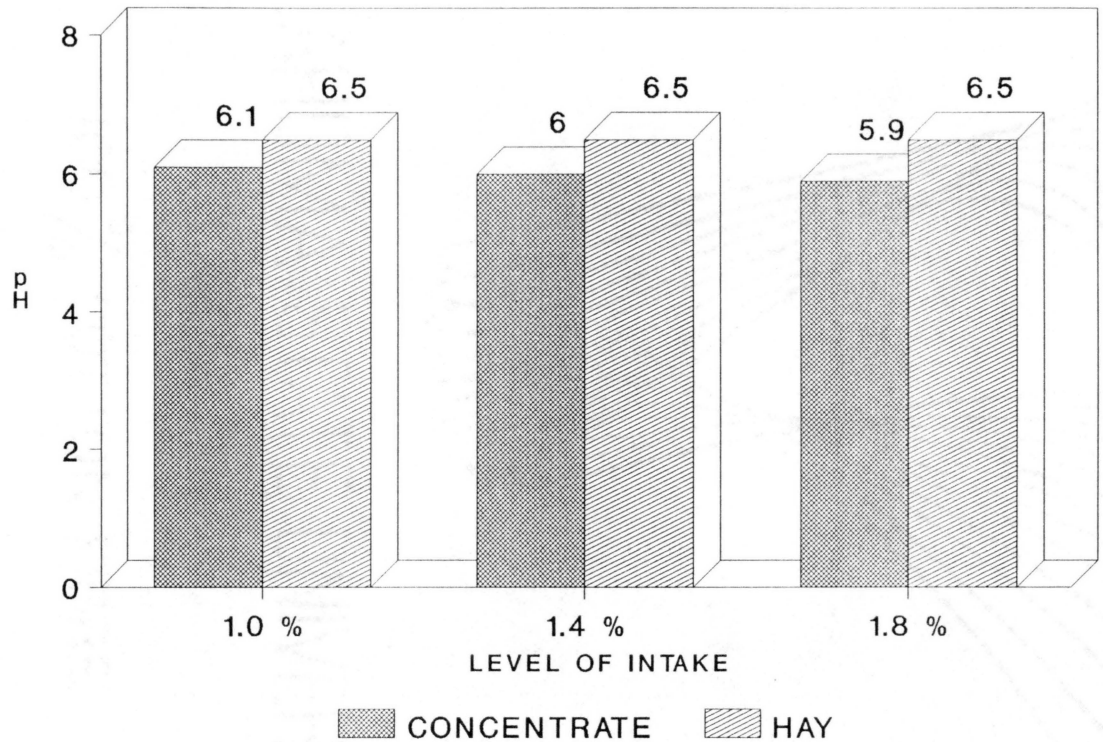

Figure 5.6 Ruminal liquid pH in heifers fed concentrate vs hay diets

6 h post-feeding in dairy steers consuming a high concentrate diet. Differences in pH between the high and low level of concentrate in our study, can be ascribed to decreased saliva production which both reduced input of buffer (Counotte et al., 1979) and decreased dilution of acids. Also, more extensive ruminal fermentation with the concentrate diet often increases total VFA concentration and thereby reduces pH values. Lactate production also will reduce ruminal pH though levels will be expected to be low in this study.

Diet type did not affect hematocrit values, but a linear ($P<.01$) depression in this blood parameter was observed as hay intake increased (Figure 5.7). Similar trends were noted with the concentrate diet ($P<.002$) no interaction between diet type and intake level was detected ($P=.58$). Hematocrit values for heifers fed hay at high levels of intake were similar to values found previously (Chapter IV). However, hematocrit values for cattle fed the concentrate diet were higher than those reported previously. Warner and Stacy (1965) indicated that when the rumen was hypertonic (>400 mOsm/kg) a net transfer of water from blood into the rumen would cause hemoconcentration. Unfortunately those authors did not present hematocrit values to compare with our hematocrit values. Downey (1976) reported that normal hematocrit values for cattle ranged between 24-46% with a mean of 35%. Hematocrit values in our study ranged between 37.2 to 40.4%, indicating that animals fed either

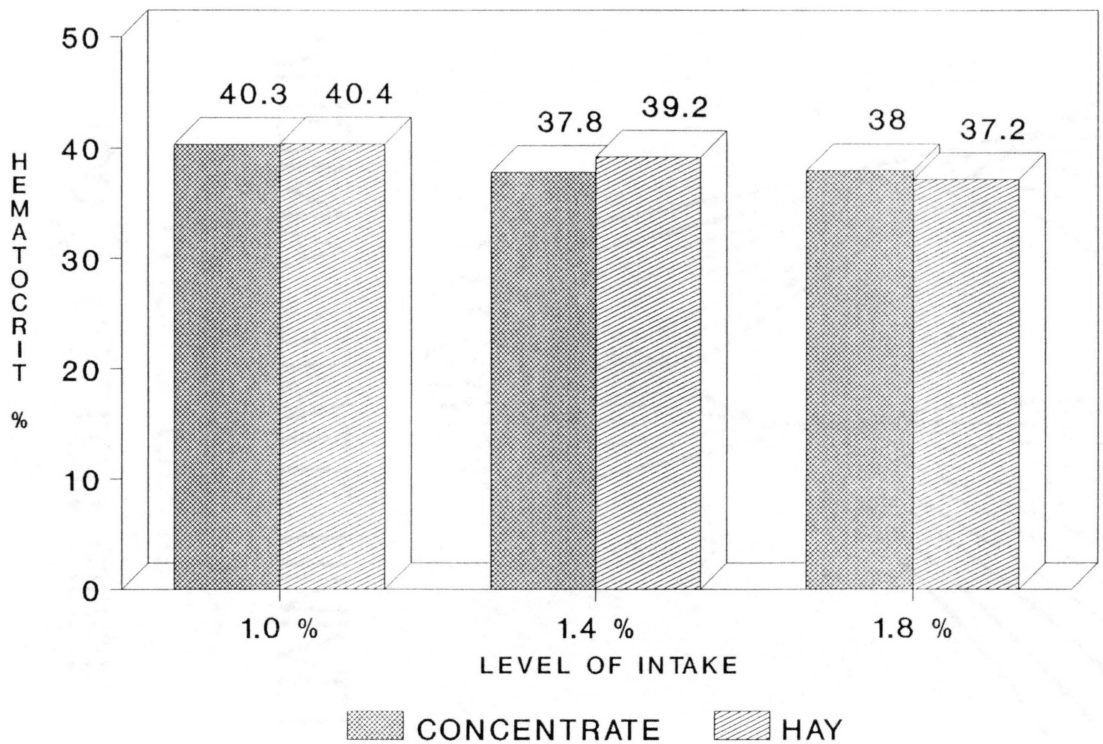

Figure 5.7 Hematocrit of heifers fed concentrate vs hay diets

diet were in the normal range. Reasons for and implications of the decrease in hematocrit when level of feed intake increased are not clear because hematocrit can be altered by input (water, absorbed fluid and minerals) and output (minerals and fluid in saliva and urine plus fluid diffusion into the rumen). Total fluid in the rumen (liters) and rumen liquid outflow (liters/d) were positively correlated with hematocrit ($r=.46$; $P<.05$ and $r=.41$; $P<.08$, respectively) for the hay diet; however, hematocrit was negatively correlated with total ruminal fluid ($r=-.63$; $P<.004$) and liquid outflow ($r=-.68$; $P<.001$) for the concentrate diet.

In summary water intake almost doubled as feed intake was increased by 80%, and was positively correlated ($r=.66$; $P<.001$) with the level of feed intake. Per kg of DM intake, water intake averaged 2.7 ± .36 liters. Changes in ruminal volume were not correlated with daily water intake ($r=.20$; $P=.24$) but ruminal solids (kg) tended to increase as intake increased ($P<.09$ for the hay diet and $P<.02$ for the concentrate diet). Although the quantity of ruminal solids was similar for the two diets, almost 50% more water was present in the rumen of heifers fed forage. Hence, solids were more concentrated (DM percentage was higher) in the rumen with the concentrate diet (18.7 vs 12.0%). Of this liquid in the rumen, an average of 67 and 26 liters (over 90% and 70%) were closely associated with the particles in rumen contents of heifers fed the roughage and concentrate

diets. Ruminal liquid passage rate estimated with Co-EDTA was faster at high levels of feed intake for both diets. Hematocrit decreased as hay intake increased, as observed by the negative relationship across diets ($r=-.34$; ; $P<.04$); this could reflect a direct escape of drinking water, fluid absorption through the ruminal epithelium or an increased saliva production. How flux of water in and out of the rumen changes with level of feed intake needs more study as salivary flow and influx through the rumen wall would be expected to be altered by ruminal conditions which affect rumination and osmolality.

Implications for these findings are discussed in detail in Chapter VI.

CHAPTER VI

QUANTITATIVE ORIGIN OF RUMINAL LIQUID WITH VARIOUS DIETS AND FEED INTAKES

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ABSTRACT

Two isonitrogenous diets composed of either an 80% concentrate or hay (60% alfalfa hay; 40% prairie hay) were fed once daily at three different levels of intake (1.0, 1.4 and 1.8% of BW/d DM basis) to twelve Hereford x Angus heifers fitted with large rumen cannulas to estimate quantitatively the origin of ruminal water. Polyethylene glycol was dosed in the drinking water for 3 consecutive days to follow the fate of drinking water through the rumen. Twenty one h previous to ruminal evacuation, Co-EDTA was pulse dosed intraruminally to estimate ruminal liquid dilution rate. Heifers fed hay diets drank more ($P<.01$) water (25.6 liters/d) than heifers fed concentrate (21.5 liters/d). Total water intake and daily DM intake were positively correlated ($r=71$; $P<.001$). As level of feed intake increased, daily water consumption increased ($P<.001$)

linearly. The amount of drinking water which entered the rumen was higher (P<.002)for the hay than the concentrate diet (9.88 vs 4.04 liters/d). Drinking water evasion from the rumen expressed as a percentage of the consumed water was higher (81%; P<.002) for the concentrate diet than for the hay diet (62%). Type of diet and level of intake did not alter ruminal osmolality, but it influenced (P<.05) serum osmolality. High levels of evasion from the rumen, may be an ideal vehicle to enhance flow of selected nutrients to the small intestine for digestion and absorption.

(Key Words: Water Intake, Ruminal Water Origin, Evasion, Beef Cattle.)

Introduction

Fluid can enter the rumen from several sources including feed, drinking water, saliva and diffusion through the ruminal wall. Feeding forages will increase the amount of time spent masticating and ruminating, both of which increase saliva production. Reports by Bailey (1961) estimated that saliva will supply between 70 to 90% of the total fluid entering the rumen of mature cows fed hay diets. The amount of water ingested with feedstuffs may vary widely depending on the type of diet. Concentrate diets and low moisture mixed diets may introduce up to 2 to 3 liters/d into the rumen, grasses or silages may add 40 to 50 liters/d of water into the rumen (Poutiainen, 1968). Fermentation,

by definition, will not produce metabolic water. Indeed, hydrolysis of polysaccharides may consume up to 1 liter/d of ruminal water.

Products of ruminal fermentation such as volatile fatty acids (VFA) as well as salts create osmotic gradients that can affect water transfer between the rumen and blood plasma. When rumen contents are hypertonic, water diffuses from blood through the rumen wall into the rumen; diffusion is reversed when the rumen is hypotonic. Such fluxes of water through the rumen wall are difficult to quantify because of the turnover of ruminal digesta. Techniques to determine liquid passage rate from the rumen have evolved, but how water moves in and out of the rumen remains to be defined clearly. Digesta flows rapidly from the rumen to the abomasum following or during either eating or drinking (Ash, 1962). Measurements of higher ruminal liquid marker concentrations immediately after drinking in sheep, suggested that an appreciable (100-300 ml) amount of consumed water either was absorbed through the rumen wall or flowed directly to the omasum without mixing with rumen contents (Warner and Stacy, 1968b). Woodford et al. (1984) reported that when drinking water was provided to lactating cows after either 4.5 or 9 h of water deprivation, a sizeable (18% and 5%) amount of the ingested water bypassed the rumen. The objective of our study was to assess quantitatively the origin of ruminal water using water

soluble markers, and to measure ruminal liquid and serum osmolalities.

Material and Methods

Animals, diets and methods were described in the previous Chapter (V). To quantitate the percentage of drinking water that evaded the rumen, polyethylene glycol (PEG MW 3350)[1] was included in drinking water at a rate of 2.6 g/liter of water offered during the last 3 d of each experimental period. This period should be adequate to achieve over 90% of steady state marker concentrations in the rumen if dilution rate exceeded 4%/h. Immediately after mixing the PEG with the drinking water, water was sampled for PEG analysis. Ruminal liquid samples, and blood were collected 2 h after feeding to determine osmolalities by the freezing point depressing procedure as outlined in Chapter IV. To estimate ruminal liquid dilution rate, 250 ml of Co-EDTA containing 1 g of Co were pulse dosed intraruminally 21 h prior to each total ruminal evacuation (day 14 of each experimental period).

The PEG contents of water samples collected during the 3 consecutive days and of ruminal samples collected at the time of evacuation were analyzed turbidimetrically (Smith, 1959). The method of Smith (1959), was modified to allow turbidity in the water and ruminal samples to develop for 30 min instead of 5 min after trichloroacetic acid was added.

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[1]Sigma Chemical Co., St. Louis, Mo.

Data from these analyses were used to calculate the total PEG intake (g/d). Amount of PEG leaving the rumen (g/d) or PEG outflow was calculated from ruminal PEG concentration and ruminal dilution rate estimated from Co-EDTA concentrations as presented in the Appendix A.

An aliquot of ruminal liquid taken at the time of ruminal evacuation, was strained through two layers of cheese cloth and centrifuged for 15 min at 3000 x g to determine cobalt concentration by atomic absorption spectrophotometry (Hart and Polan, 1984). Data from this analysis was used to calculate cobalt dilution rate as shown in the Appendix A. Sampling procedures and analyses of samples from the ruminal evacuations were described in Chapter III; calculations are illustrated in the Appendix A.

Statistical analysis was conducted using a general linear models procedure (GLM), for a split plot experiment (Steel and Torrie, 1980). Animal within treatment was used as the main plot error term to test treatment (concentrate vs roughage) effects. The interaction between period x level of feed within treatment x period x treatment was the error term to calculate level of feed intake effects. When feed intake level effects were detected ($P<.05$), linear and quadratic components were examinded.

Results and Discussion

Results are presented in Table 6.1. Water intake, rumen volume, particle-associated liquid and liquid outflow,

Table 6.1 Origin of ruminal liquid in beef heifers fed hay and concentrate diets.

	Diets										
	Hay			Concentrate				Hay		Conc	
	Low	Med	Hi	Low	Med	Hi	Diet	L	Q	L	Q
Water Intake, l/d	19.6	26.3	30.5	15.0	20.5	29.0	.10	.001	NS	.001	.001
Rumen Outflow, l/d	106.1	143.0	147.3	68.3	77.3	79.3	.001	.001	NS	NS	NS
Rumen Outflow, %/h	6.1	8.1	8.8	6.4	6.6	7.4	NS	.001	NS	.06	
Rumen Volume, l	70.5	74.0	69.5	44.6	49.1	45.7	.001	NS	NS	NS	NS
Free Liquid, l	45.3	40.6	36.3	34.2	36.8	32.0	NS	.008	NS	NS	NS
Bound Liquid, l	25.0	32.7	32.9	10.3	12.0	13.4	.001	.003	.02	NS	NS
Rumen Water from drinking water:											
Drinking water entering the rumen, l/d	9.0	9.3	11.4	3.0	4.0	5.0	.002	NS	NS	NS	NS
% from drinking	8.4	6.3	8.0	4.5	5.0	6.3	.08	NS	NS	NS	NS
Evasion of drinking water, l/d	10.6	17.0	19.1	12.0	16.5	24.0	NS	.001	NS	.001	NS
% of consumed	54.1	64.6	62.6	80.0	80.5	82.7	NS	NS	NS	NS	NS

L = Linear effect of level (treatment)

Q = Quadratic effect of level (treatment)

NS = No significant

were greater (P<.001) with the hay than the concentrate diet as discussed previously (Chapter V). Likewise, water intake and ruminal liquid outflow increased linearly (P<.001) as the intake of DM increased. A positive correlation (r=.84; P<.001) between water intake and DM intake were detected. However, ruminal liquid volume was not affected as feed and water intake increased; ruminal liquid volume was greater (P<.001) for the hay than the concentrate diet.

The amount of consumed water which entered the rumen was more than twice as great (P<.01) for the hay than the concentrate diet. Based on markers analysis, only 4.5 to 8% of ruminal liquid was obtained from consumed water. The remaining 90% presumably was derived from saliva and diffusion from blood. Diffusion from blood would be expected when ruminal osmolality exceeds blood serum osmolality.

Ruminal and blood plasma osmolalities are presented in Figure 6.1. The difference between osmotic pressure in blood serum and in ruminal fluid were quite small suggesting that net influx of liquid from blood should be insignificant. Engelhardt (1970) indicated that net flux of water between blood and rumen was very small when ruminal osmolality remained between 260 to 340 mOsm/kg in goats with a temporarily isolated rumen. In our study, ruminal and serum osmotic pressure ranged from 318 to 360 mOsm/kg, and from 289 to 296 mOsm/kg, respectively. Ruminal fluid osmolality were consistently (11 and 14%) higher than plasma

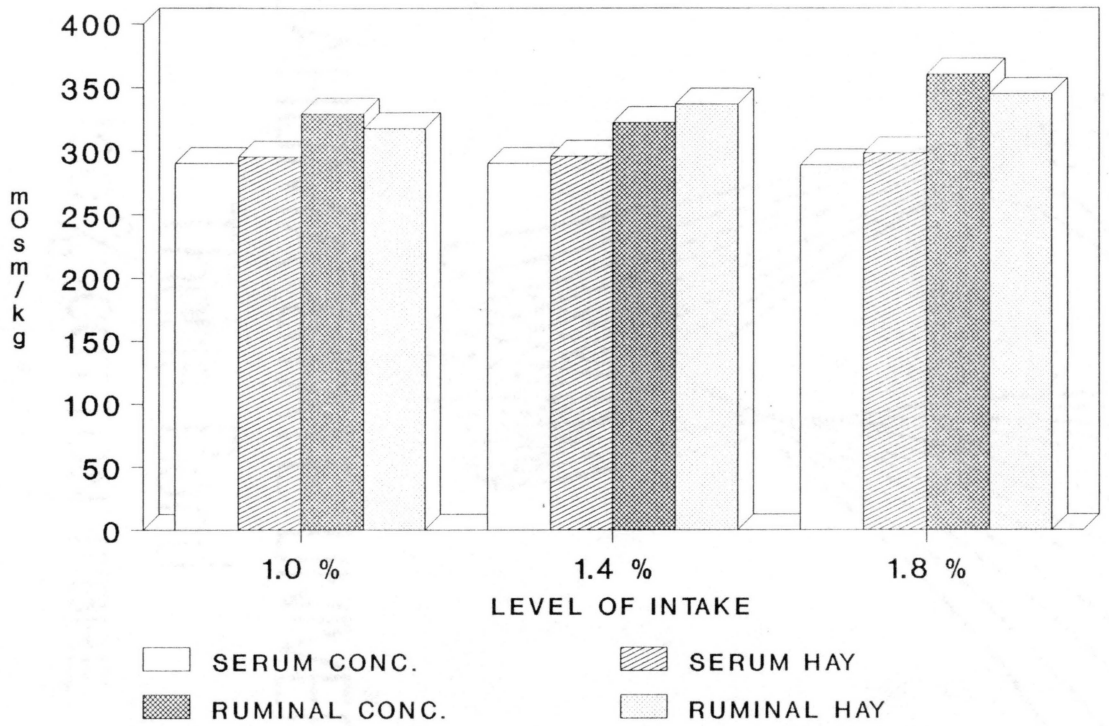

Figure 6.1 Ruminal liquid and serum osmolality changes in heifers fed concentrate vs hay diets

osmolality. Warner and Stacy (1965) suggested that shortly after feeding, the osmolality of ruminal contents can be 20% higher than of blood plasma; these values are in agreement with our findings. The osmolality of ruminal liquid was about 14% and 11.5% higher than serum osmolalities for the hay and concentrate diets, respectively. Because water may disappear from the rumen either by absorption or by passage, net differences in ruminal and serum osmolalities were low which should indicate only slight movement of water into the rumen, hence bypass of drinking water could be more important than absorption under the conditions of the present experiment.

As shown in Table 6.1, the amount of drinking water which did not mix with the ruminal contents linearly increased ($P<.001$) as the level of feed intake increased for both diets. Of drinking water consumed, 81% evaded the rumen with the concentrate diets; ruminal water evasion was lower ($P<.002$) being 62% with the hay diet. Presumably this water was flushed through the rumen due to the proximity of entry and exit points.

The nature of drinking water movement through the rumen of mature ruminants is unclear. Some workers (Ash, 1962; Warner and Stacy, 1968 a,b; Rogers et al., 1979; Rogers et al., 1982; Huber et al., 1982; Woodford et al., 1984) have suggested that some of the water ingested may flow directly to the omasum. However, little information concerning the amount of water that evades the rumen is available. Values

for water evasion in sheep calculated from Warner and Stacy (1968b data; Table 5, pp 399) showed that 32 to 43% of the consumed water evaded the rumen. Similarly, Woodford et al. (1984) calculated that water evasion was 5 and 18% for dairy cows receiving PEG in the drinking water after 4.5 or 9 h of water deprivation. Percentages of water evasion from the rumen in our study were much higher for both diets than previously reported in the literature. Our measurements are based on the assumption that PEG worked satisfactory as a liquid marker when dosed in the drinking water, that markers were in steady state in the rumen and that pools of water sampled were representative of ruminal contents. Problems with marker equilibrium within and sampling from the rumen could alter values.

Usefulness of PEG as a liquid marker for estimation of rumen volume and outflow with certain feedstuffs has been questioned (Czerkawski and Brekenridge, 1969; Alexander et al., 1969; Teeter, 1981). The latter study indicated conclusively that PEG complexes with some component(s) of cottonseed hulls and does not equilibrate with water inside shelled corn. These were minor components of our diets. Other workers have speculated that certain fluid pools in the rumen exclude PEG; their data were refuted by Teeter (1981). Nevertheless questionable behavior of PEG in the rumen led us to test other water soluble markers in the drinking water as described in three more experiments

(Chapter VIII). In essence, those studies generally validated findings of this experiment.

If a high percentages of water evades mixing with ruminal contents, this observation has a number of implications. First, if all drinking water entered the rumen, it could cause drastic shifts in volume, osmolality and, in some cases, temperature. Such changes would be detrimental to the microbial population and disrupt the fermentation process. Thus, extensive evasion would be biologically beneficial to prevent perturbation of ruminal function.

Secondly, high evasion means that drinking water consumed will not dramatically alter ruminal volume, turnover or dilution rate of ruminal contents. Hence, feeding salt or compounds to increase water intake would not be expected to improve efficiency of microbial growth in the rumen. Spears (1987) supplemented high concentrate diets with .25% salt to improve efficiency of utilization of energy. Efficiency was not improved.

Third, high bypass of drinking water can provide a vehicle to increase flow of selected nutrients to the intestines for digestion and absorption. For shipping stressed cattle, electrolytes and amino acids in water should escape fermentation and potentially could improve health status. Soluble protein or amino acids might be supplemented via water avoiding the need to select or treat these compounds to resist bacterial attack in the rumen.

High evasion of drinking water estimated by the inflow and outflow of the water soluble marker PEG might be explained by several potential mechanisms. First closure of the reticular groove as occurs in suckling calves (Orskov and Benzie, 1969) could shunt fluid directly to the omasum. Such closure is thought to be non-functional in adult animals (Huber et al., 1982). Secondly, presence of small and rapid outflow liquid turnover in the rumen or the presence of non-mixing fluid pools within the rumen could cause rapid outflow of consumed fluids. Nycterohemeral effects and non steady state conditions within the rumen could alter kinetics and thereby alter interpretation of marker data. Whether a single factor or a combination of these mechanisms are responsible for the observation that a high percentages of water evades the rumen remains to be determined.

Implications

The rumen is a very dynamic organ, and an appreciable amount of water evades the rumen. Despite increases in water consumption, ruminal liquid volume did not change with different levels of feed or water intake.

CHAPTER VII

EFFECTS OF MONENSIN AND LASALOCID ON WATER INTAKE, RUMEN VOLUME AND LIQUID PASSAGE RATE IN FEEDLOT HEIFERS

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ABSTRACT

Nine beef heifers (544 kg) equipped with permanent rumen cannulas were used in a triplicated 3 x 3 latin square design, to study the effect of monensin (300 mg/d) and lasalocid (220 mg/d) on water intake, rumen volume and liquid passage rate. Water intakes were not different (24.8 vs 24.7 and 24.1 liters per day) for the monensin, the lasalocid and the control diet, respectively. Kinetics of drinking water in the rumen, as estimated by inflow and outflow of PEG, showed that water evasion from the rumen was higher in heifers fed the monensin diet (22.4 vs 20.0 and 19.8 liters per day). Although ruminal liquid volume determined by evacuation 3 h postfeeding and fluid dilution rate determined by Co-EDTA dosing were not altered by either ionophore. Total solids in the rumen was 20 and 12% higher ($P<.05$) for the monensin diet and lasalocid diets than for

the control diet. Data from this study suggest that monensin increases ruminal solids fill and ruminal DM retention time without altering water intake or ruminal liquid volume.

(Key Words: Monensin, Lasalocid, Water Intake, Ruminal Dry Matter)

Introduction

The carboxylic polyether ionophores, monensin and lasalocid, are routinely fed to feedlot and grazing ruminants. As indicated by Galyean and Owens (1988) and Owens et al. (1989) some 80 to 90% of the feedlot cattle in the US currently are fed these compounds. Research with ionophores has been centered on the mode of ionophore action and effects on rate and efficiency of gain. The precise mechanisms by which ionophores improve efficiency remain uncertain (Owens et al., 1989). Ionophores improve feed efficiency partly through regulating ruminal fermentation and site of digestion, so that more propionic acid and less acetic acid are produced by the ruminal bacteria. Microbial production of propionic acid energetically is more efficient (Owens and Goetsch, 1988). The effect of monensin or lasalocid on water intake, rumen volume and liquid kinetics, has received less attention. Results from Dinius et al. (1976) shown that liquid turnover rates decline linearly as the level of monensin was increased from 0 to 200 mg/hd in the daily diet of Holstein steers fed a forage-based diet.

Similar findings were reported by Lemenager et al. (1979) with mature beef steers. Data from the latter study indicate that addition of 200 mg/hd of monensin daily depressed turnover of ruminal liquid and solid by 30.8 and 43.6%, respectively. Likewise, Owens et al. (1979) found a reduction in ruminal turnover rate for both solid and liquid fractions in steers fed a high concentrate or ad libitum prairie hay diets supplemented with monensin. Other studies with sheep (Ricke et al., 1984) have demonstrated that additions of monensin or lasalocid (33 mg/kg DM) to alfalfa hay diets, also depressed dilution rates of both ruminal liquid and solids. However, either ionophore altered ruminal liquid volume as calculated by Cr-EDTA passage.

Although monensin and lasalocid appear to behave biochemically in a similar fashion, and their effect are intimately associated to feed intake and digestibility, they may have different effects on ruminal dynamics and fermentation. The objectives of this trial were to assess the effect of feeding monensin and lasalocid on ruminal liquid passage rates, daily water intake and ruminal volume of both solids and liquids in beef heifers fed a high concentrate diet.

Materials and Methods

Nine Hereford x Angus heifers (544 kg) equipped with large rumen cannulas (10 cm i.d.) were used in a triplicated 3 x 3 latin square design to investigate the effect of two

commercial ionophores, (monensin and lasalocid), upon water intake, rumen volume and liquid dilution rate. Heifers were confined to individual pens, water was available at all times and daily water consumption was monitored during the 42-d study.

Three treatments were used, a concentrate diet without the ionophores (control diet), the control diet + monensin (300 mg), or control + lasalocid (220 mg; Table 7.1). Diets were fed twice daily (0830 and 1530) at 1.6% of body weight (DM basis).

Previous to the experiment each ionophore was mixed separately with 3 kg of finely ground corn. Subsequently, 20 g of this mixture containing either ionophore was included in the morning feeding and hand mixed with the concentrate.

Experimental periods lasted 14 days; on days 12 through 14 of each period polyethylene glycol (PEG molecular weight 3,350) was included in the drinking water (3.0 g/liter) as an external liquid marker. Water samples were collected to determine PEG concentration (Smith, 1959). On day 13 of each experimental period, 245 ml of Co-EDTA solution (Udén et al., 1980) containing 880 mg of Cobalt, was pulse dosed into the rumen of each animal to be used for calculation of liquid dilution rate (Teeter, 1981).

Approximately 21-h after Co-EDTA was dosed, total ruminal contents were removed as described by Garza and Owens, 1989. Ruminal digesta obtained by this procedure was

Table 7.1 Composition of concentrate fed (DM Basis)

Ingredient	%
Corn, dry rolled (IFN 4-02-931)	63.10
Cottonseed hulls (IFN 1-00-599)	14.10
Soybean meal, (IFN 5-04-600)	10.05
Alfalfa pellets, dehydrated (IFN 1-00-023)	6.00
Cane molasses (IFN 4-04-696)	5.00
Salt (trace mineralized)[a]	.50
Ground limestone (IFN 6-02-632)	.50
Dicalcium phosphate (IFN 6-01-080)	.50
Aurofac-50[b]	.15
Urea (42% N)	.10
Ionophore[c]	+ -
TOTAL	100.00

[a]Trace min, Carey Salt, Mission Kansas, contained: NaCl, 92-97%; Mn, .250%; Fe, .200%; Cu, .033%; I, .700%; Zn, .005%; Co, .0025%; white mineral oil.

[b]Aurofac-50, CADCO, Inc., DesMoines, Iowa. Contained: 50 g of chlortetracycline per 454 g.

[c]Rumensin-60, Elanco, Inc., Lilly Research Laboratories, Greenfield, IN. Contains: 60 g monensin/ 454 g. Bobatec-68, Hoffman LaRoche, Nutley, N. J. Contains: 68 g lasalocid/454 g.

+- With or without addition of 300 mg monensin/hd/d or 220 mg lasalocid/hd/d.

subsampled as indicated in Chapter III. The remainder of ruminal contents were returned promptly into the rumen. Ruminal solid and liquid samples were used for DM determinations. Immediately after samples were collected, ruminal fluid pH was measured and 1 kg of solid digesta and 1 liter of ruminal fluid were weighed separately and dried at 60°C for 48 h in an air forced oven, air equilibrated and ground through a 2 mm screen in a Wiley mill. The remaining portion of each individual ruminal liquid sample was centrifuged at 3,500 x g for 15 min and the supernatant fluid was divided into two aliquots and saved for later analysis. One aliquot was used to determine PEG concentration by the turbidometric method of Smith (1959), modified to allow 30 min for turbidity to develop. The other aliquot was used to measure Co concentration by atomic absorption spectrophotometry with an air acetylene flame at 240.7 nm. Standards were prepared in liquid from ruminal samples taken prior to marker infusion. Cobalt concentration also was measured in the dose solution. Dilutions were adjusted to ensure that marker concentration remained in the detection range of the atomic absorption spectrophotometer.

Rumen liquid and solid volumes were estimated directly from ruminal evacuation, after correcting for fluid density and DM in the solid and liquid phase as described in the Appendix A. Liquid dilution rate for cobalt was calculated using the ln of Co concentration at two times (dose time,

being dosed/evacuated volume and 21 h later) divided by the time difference between these two sampling times as outlined by Teeter (1981). Calculations for this procedure are presented in the Appendix A.

Data were analyzed for a triplicated latin square design as specified by Snedecor and Cochran (1980) using a general linear models procedure (SAS, 1985ab). Classes included square, animal, period and treatment. On day 14 of the first period, one animal lost some rumen fluid through the rumen cannula, so that rumen volume was not measured. As a result one missing value was generated using the general lineal model. Means were separated using a least significant difference procedure (Steel and Torrie, 1960) when significant treatment effects were detected.

Results and Discussion

Water intake and ruminal liquid volume estimated by direct ruminal evacuation, were not affected by added ionophores (Table 7.2). These findings are in agreement with data presented by Lemenager et al. (1978), Adams et al. (1980) and Ricke et al. (1984). Addition of ionophores to the diet of steers fed high concentrate or alfalfa hay-based diets also did not influence ruminal liquid volume, but, when low quality hay supplemented with monensin was fed to beef steers, ruminal volume decreased (Lemenager et al., 1978). Ruminal fluid pH (6.0) was not different between these three diets (Table 7.2) though values tended to be

Table 7.2 Effect of monensin and lasalocid on water intake, ruminal liquid distribution, volume and passage

Item	Diets			SE
	Control	Lasalocid	Monensin	
Water intake, liters/d	24.1	24.7	24.8	.63
Ruminal pH	6.0	6.1	6.1	.08
Rumen volume, liters	47.5	49.4	49.3	1.56
Free liquid, liters	40.0	40.4	39.3	1.61
Bound liquid, liters	7.5[a]	9.0[ab]	10.0[b]	.59
Rumen dilution rate, %/h	4.7	4.7	4.6	.003
Feed intake, kg/d	10.6	10.6	10.6	.03
Total solids, kg DM	7.1[a]	7.8[a]	9.0[b]	.47
Ruminal DM, %	13.2[c]	13.8[cd]	15.2[d]	.63
Filtered solids DM, %	37.6	37.0	37.1	
Total contents, kg	53.2	54.6	57.7	
Ratio of bound liquid: DM	1.0	1.1	1.0	.05
Water evasion, liters/d	19.8[e]	20.0[ef]	22.4[f]	.97
Percent of consumed	84.1[c]	81.2[c]	90.2[d]	2.68

[a,b]Means in the same row with different superscript differ (P<.01).

[c,d]Means in the same row with different superscript differ (P<.05).

[e,f]Means in the same row with different superscript differ (P<.10).

increased slightly with ionophore feeding as is noted in many experiments even though differences are seldom significant. Dinius et al. (1976) found that pH values (6.4) for steers fed forage diets were not altered with monensin feeding. They indicated that monensin in the diet had no effect on ruminal fluid pH from prefeeding through 8 h postfeeding times. Distribution of liquid associated to the solid fraction, expressed as bound liquid per kg of DM, showed no appreciable differences between the three treatments and rumen liquid outflow rate remained constant (4.7%/h) in all treatments. These results differ from those presented by Owens et al. (1979), in which liquid dilution rate decreased when monensin was added to a high concentrate diet. Though they observed changes in dilution rate, their value for the monensin supplemented diet was higher (5.59%/h) than obtained in our study. Likewise, reports from Ricke et al. (1984) indicated a reduction in liquid dilution rate in sheep fed hay diets supplemented with monensin or lasalocid. Their liquid dilution rate values for the treated animals (4.6 and 4.8%/h for monensin and lasalocid, respectively) were similar to our values (4.7%/h), but their control animals had higher dilution rates. Although daily dry matter intake was held constant (10.6 kg) between treatments, total solids in the rumen was larger ($P<.02$) for heifers fed monensin than for animals fed lasalocid diets or concentrate (9.0 vs 7.8 and 7.1 kg DM; Table 7.2). Hence, assuming extent of digestion was not

altered dry matter residence time in the rumen was increased a mean of 10% and 27% by lasalocid and monensin. Lemenager et al. (1978) observed a reduction in particle passage rate based on in grazing cattle fed monensin. Whether, this increase in ruminal dry matter fill in the animals fed monensin, with no alteration in ruminal liquid volume was due to an increased water adsorption to the solids and altered chewing (Deswysen et al., 1989), or by enhanced evasion of fluids to the lower tract is not clear. DM content of filtered solids would be expected to decrease if extent of rumination or chewing was altered. No differences in this measurement was detected. Nevertheless, an increased rumen solids volume would be expected to decrease rate of passage (%/h) of ruminal solids as reported by Lemenager et al. (1978) and Owens et al. (1979), and could explain why feeding monensin often reduces feed intake of cattle. Effect of these ionophores on ruminal water evasion is presented in Table 7.2. Evasion of drinking water, as estimated by PEG inflow and outflow was higher (90%; $P<.01$) for heifers fed monensin, but no differences ($P>.05$) were detected between the control diet and lasalocid diets. Evasion values generally agree with or exceed observations by Garza and Owens (1989) for in beef heifers fed a similar concentrate diet.

Data concerning water evasion from the rumen is scarce. No explanation is apparent for the high percentage of water evasion observed with the animals fed monensin. If ruminal

motility and mixing were depressed by monensin, water evasion would be expected to be higher as the time for outflow prior to rumen mixing would be enhanced. This also could explain an increased solids content.

In conclusion, monensin increased ruminal solid fill, but do not alter water intake, ruminal liquid volume, distribution of the fluids in the rumen or ruminal fluid pH. Increased residence time of dry matter in the rumen of heifers fed monensin should expose feed longer to ruminal microorganisms, and consequently may influence extent of ruminal digestion for less rapidly fermented feed components. High ruminal evasion of drinking water in the animals fed the monensin diet deserves more study, as it may prove useful to enhance flow of small particles and soluble nutrients to the intestines.

CHAPTER VIII

RUMINAL WATER EVASION AND STEADY STATE: ESTIMATION BY TWO DIFFERENT METHODS USING WATER SOLUBLE MARKERS

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ABSTRACT

Three experiments were conducted to investigate the fate of drinking water in the rumen of mature beef cattle. In Experiment 1, four rumen cannulated heifers (590 kg) fed a hay or an 80% concentrate diet (1.8% BW/d; DM basis) were used in a crossover experiment. Water intake was recorded every 3 h during the last 4 d of each period. To quantify entry of consumed water into the rumen, Cr-EDTA was added to the drinking water whereas cobalt-EDTA was pulse dosed intraruminally 18 h before total ruminal contents were evacuated (d 15 of each period). Compared with heifers fed concentrate, animals fed hay, tended to drink more ($P>.2$) water (33 vs 29 liters), had more ($P<.05$) liquid in the rumen (65.3 vs 53.4 liters), particularly ($P<.05$) that associated with solids (26 vs 18 liters; Table 8.4).

Despite a higher R^2 (.96 vs .50), ruminal volumes based on evacuation did not match Co-EDTA estimates because the regression differed in both slope and intercept for animals fed concentrate; values for those fed hay were more accurate but less precice. Liquid outflow (liters/d) and dilution rate were 36.1 and 19.4% greater ($P<.05$) for heifers consuming hay than for heifers consuming concentrate. Water evasion from the rumen calculated by either PEG outflow (44 vs 79%) or Cr-EDTA kinetics (52 vs 66%) was higher ($P<.05$) for the concentrate vs the hay diet.

In Experiment 2, five mature cattle (450 kg) were fed a concentrate diet and dosed with Cr-EDTA in the drinking water. Cobalt-EDTA was infused into the rumen at 8 h intervals during an 80 h period. Water evasion calculated from the Cr/Co ratio in the rumen averaged 41%. Though evasion estimated by the ratio approach was lower, values still indicated that a sizable percentage of imbibed water evaded the rumen. In Experiment 3, eight Angus x Hereford cattle fed as outlined for Experiment 1 were dosed intraruminally with Cr-EDTA and Co-EDTA at different hours of the feeding cycle. Estimates of liquid passage rate were similar for Co-EDTA and Cr-EDTA, but dilution rate and ruminal volume estimates differed at different times of the day suggesting that ruminal volume may change from within a feeding cycle or across days.

(Key Words: Drinking Water, Beef Cattle, Evasion, Steady State, Markers)

Introduction

Ruminal passage rate of liquids and solids can influence digestibility, feed intake and efficiency of microbial growth (Owens and Isaacson, 1977). In general, as dilution rate of liquids increases, efficiency of bacterial growth increases. Consequently, changes in rate of flow can have a direct effect on animal performance. The rate at which water passes through the gastrointestinal tract of the ruminant is influenced partially by the amount of liquid ingested or by the level of ruminal fill (Woodford et al., 1984); however, how much of the water ingested enters the rumen has not been clearly defined. According to Balch (1958) saliva and drinking water enter the rumen in approximately equal amounts. In contrast, Bailey (1961) reported that the sum of water in feed and drinking water accounted for only 13-24% of the total volume of fluids entering the rumen. These values may vary greatly with the type of feed consumed. Grasses and silages with higher moisture contents than concentrates or hays, may supply appreciable water to the rumen (Pountiainen, 1968) and increase mastication, rumination and saliva input. Passage of liquid directly to the abomasum, has been observed in adult cattle, sheep or goats (as reported by Watson, 1944), but the quantity of water that evades the rumen has not been clearly specified.

Estimates of drinking water evasion in sheep, calculated from Warner and Stacy's (1968b) data indicated

that 32-43% of the ingested water evaded the rumen. Rogers et al. (1979) reported that 43% of liquids leaving the reticulo-rumen of Holstein steers could be accounted for by water intake if all liquid consumed enters the rumen. Woodford et al. (1984) calculated that small amounts (5% and 18%) of drinking water evaded from the rumen of lactating cows when PEG was dosed in the drinking water. In contrast, estimates of drinking water evasion by inflow and outflow of PEG in the rumen of beef heifers fed high concentrate or hay diets, have been as high as 80% (Garza and Owens, 1989; Chapter VI).

The objectives of this research were 1) to compare the behavior of two markers, Cr-EDTA and PEG, in the rumen when dosed in the drinking water, and 2) to verify previous evasion estimates based on PEG. An alternative approach to calculate water evasion was devised based on Cr/Co ratios in the rumen. Rumen liquid volume and passage of fluids during a 48-h period were estimated with liquid markers.

Materials and Methods

Experiment 1.

Four ruminally cannulated Hereford heifers (590 kg) in individual pens were fed either a prairie hay or a high concentrate diet (Table 8.1) at 1.8% of BW DM basis, twice daily (0830, 1600) in a crossover experiment (diets were switched) with 2 wk periods.

Table 8.1 Composition of concentrate fed (DM Basis)

Ingredient	%
Corn, dry rolled (IFN 4-02-931)	63.10
Cottonseed hulls (IFN 1-00-599)	14.10
Soybean meal, (IFN 5-04-600)	10.05
Alfalfa pellets, dehydrated (IFN 1-00-023)	6.00
Cane molasses (IFN 4-04-696)	5.00
Salt (trace mineralized)[a]	.50
Ground limestone (IFN 6-02-632)	.50
Dicalcium phosphate (IFN 6-01-080)	.50
Aurofac-50[b]	.15
Urea (42% N)	.10
TOTAL	100.00

[a]Trace min, Carey Salt, Mission Kansas, contained: NaCl 92-97%, Mn .250%, Fe .200%, Cu .033%, I .700%, Zn .005%, Co .0025%, white mineral oil.

[b]Aurofac-50, CADCO, Inc., DesMoines, Iowa. Contained: 50 g of chlortetracycline per 454 g.

All heifers were adapted to their diets during the initial 11 days of each period. Water was available at all times and water intake was recorded daily. On day 12 through 15 of each period, water soluble markers (PEG, 2.64 g/liter and Cr-EDTA 2.36 g Cr/liter) were included in the drinking water; Co-EDTA (1.2 g Co in 250 ml solution) was pulse dosed into the rumen 18 h prior to total ruminal evacuation on d 15 of each period.

Sampling procedures during the ruminal evacuation and analytical methods were outlined in Chapter VI. Calculations and formulas are shown in the Appendix A.

Mean water intake, rumen volume, marker concentration in ruminal fluid and dilution rate were used to simulate ruminal "steady state" conditions to compare this condition with the observed behavior of markers in the rumen.

Data were analyzed using a general linear models procedure, for a crossover experiment as outlined by Cochran and Cox (1957). Classes were represented by animal, period and treatment. Means were separated using a least squares method when significant effects were detected.

Experiment 2.

Five mature beef heifers (450 kg) were used to investigate a new method for estimating ruminal evasion of drinking water. This method is based on comparing ruminal marker concentrations when one marker is dosed in the rumen and the other is provided in the drinking water.

Composition of the concentrate diet (Table 8.1), frequency of feeding, water intake records, markers and analyses were similar to those described for Experiment 1 except that only one period was used. In this experiment, animals were dosed via ruminal cannula with Co-EDTA (60 ml containing 150 mg Co) every 8 h during an 80 h period while Cr-EDTA (3.2 g/liter) was included in the drinking water.

Water and ruminal samples were collected immediately after dosing began for 3 consecutive days at 0800, 1200, 1400, 1600, 2000 and 2400 h. Data from marker concentration in the drinking water and ruminal liquid were used to calculate water evasion every 2 h during the 80 h period, based on the following ratio:

$$\frac{\text{[Cr-EDTA] in ruminal fluid / imbibed Cr-EDTA}}{\text{[Co-EDTA] in ruminal fluid / ruminally dosed Co-EDTA}}$$

This ratio will represent directly the proportion of drinking water mixing with rumen liquid; consequently, 1 minus this value, equals the proportion of water that evaded the rumen. No attempt was made to correct for any marker absorption from the rumen.

Data were analyzed as repeated measurements using an split plot design. Classes were animal, day and hour; the interaction of day x animal was used as the error term for the main plot (day) whereas the animal x hour x d interaction was the error term to test for hour effects.

Experiment 3.

Eight Angus x Hereford heifers (558 kg) fitted with ruminal cannulas were used in a 48-h trial. Animals were fed as outlined for Experiments 1 and 2. All heifers were dosed in the rumen at 0400, 1200 and 0400 of day 2 with 120 ml of Co-EDTA (2.12 g Co/liter) and Cr-EDTA (1.35 g Cr/liter) solutions according to the schedule presented in Table 8.2. Ruminal samples were collected at approximately 2 h intervals for 40 h after dosing. Water intake was recorded for the intervals between times of rumen sampling. Ruminal samples were centrifuged 3000 x g for 20 min and the supernatant fraction was used to determine Cr and Co concentrations, as described in Chapter V. Liquid rate of passage was estimated for each marker within and between infusion times by regressing the natural logarithm of the marker concentration in ruminal fluid against time (Grovum and Williams, 1973). Rumen volume was calculated by dividing marker dose by the antilogarithm of the intercept of the slope line (marker concentration at time zero).

Data were analyzed using a general linear models procedure for repeated measurements on the same animal. Classes in the statistical model were animal, infusion and hour. The slopes (liquid dilution rate) were compared between markers for the same infusion time; in addition, the overall slopes also were compared between the 3 different

Table 8.2 Sequence of markers used for intraruminal dosing (Experiment 3)

	Infusions[a]		
Animal	1	2	3
1	Cr,Co	-	Cr,Co
2	Co	Cr	Co
3	Cr,Co	-	Cr,Co
4	Cr	Co	Cr
5	Cr	Co	Cr
6	Co	Cr	Co
7	Cr	Co	Cr
8	Co	Cr	Co

[a]Infusion 1 = markers were dosed at 0400 h on day 1; Infusion 2 = markers were dosed at 1200 h on day 1; Infusion 3 = markers were dosed at 0400 h on day 2.

infusion periods. When significant effects were detected, means were separated using a least squares method.

Results and Discussion

Consumption and frequency of drinking water across the three experiments typically was higher during the late afternoon (Figure 8.1) following the evening meal. Although in the majority of the cases, animals drank in the morning, water intake of drinking tended to be lower in the morning than in the afternoon and evening (Figure 8.1). A report by Ray and Roubicek (1969) indicated that feedlot cattle drank more water in the late afternoon and at night during warm weather. Drinking patterns vary greatly from animal to animal and are altered by physiological conditions such as gender, age and pregnancy (NRC, 1981). Sekine et al. (1989) indicated that Holstein steers drank most of their daily water during a 3 h postfeeding period. Recent observations by Schutte et al. (1990) with yearling beef steers fed a 60% concentrate diet twice daily (1800 and 2000) indicated that 80% of water was consumed within 4 h postprandially. These results contrast with our findings but differences may be in part due to differences in age, feeding or housing of the animals.

Compared to the hay diet, estimates of drinking water evasion from the rumen were higher for the concentrate diet (79 vs 44% for PEG; $P<.02$ and 66 vs 51% for Cr; $P<.08$). Marker comparison within diets showed a higher estimate of

Table 8.3 Water evasion estimates using two different approaches

Method	Evasion, % of Water Intake		
	Concentrate	Hay	SE[a]
Experiment 1			
Inflow/outflow, PEG	79[bf]	44[c]	3.6
Inflow/outflow, Cr-EDTA	66[dg]	51[e]	3.2
Experiment 2			
Marker ratio[h]	41	-	9.9

[a]Standard error.

[b,c]Means in the same row with different superscripts differ ($P<.05$).

[d,e]Means in the same row with different superscripts differ ($P<.10$).

[f,g]Means in the same column with different superscripts differ ($P<.02$).

[e] $\frac{\text{Cr-EDTA in rumen/drinking water dosed Cr}}{\text{Co-EDTA in rumen/intraruminally dosed Co}}$

drinking water evasion fro PEG as compared to Cr (79 vs 66%, $P<.02$) for the concentrate diet. No difference among markers was observed for the hay diet (Table 8.3, Experiment 1). These values are slightly lower than our previous (Chapter VI) estimates of water evasion for concentrate (81%) and hay (62%) diets based upon PEG as an external liquid marker. However, our values for water evasion are much higher than those reported (5% and 18%) for dairy cattle by Woodford et al. (1984). They indicated that a higher ruminal evasion (18%) of drinking water was observed in their cows with higher ruminal fill. Our results, with evasion higher with concentrate than roughage diets, suggests that ruminal volume was not related directly to ruminal water evasion. Ruminal volumes of animals consuming hay always were larger than for those fed concentrate, yet evasion of drinking water was higher for the concentrate diets (Table 8.3). Thus, diet type also may influence water evasion.

We did not correct for marker absorption; the consistently lower water evasion observed with Cr-EDTA than for PEG for the concentrate diet, could be attributed partly to Cr-EDTA absorption through the ruminal wall. Reports by Udén et al. (1980), and Teeter and Owens (1983) indicated that following intraruminal infusions of Co-EDTA, small amounts (1-5%) of marker were detected in urine. Dobson et al. (1976) suggested that absorption of Cr-EDTA in the rumen was enhanced when ruminal liquid osmolality exceeded plasma

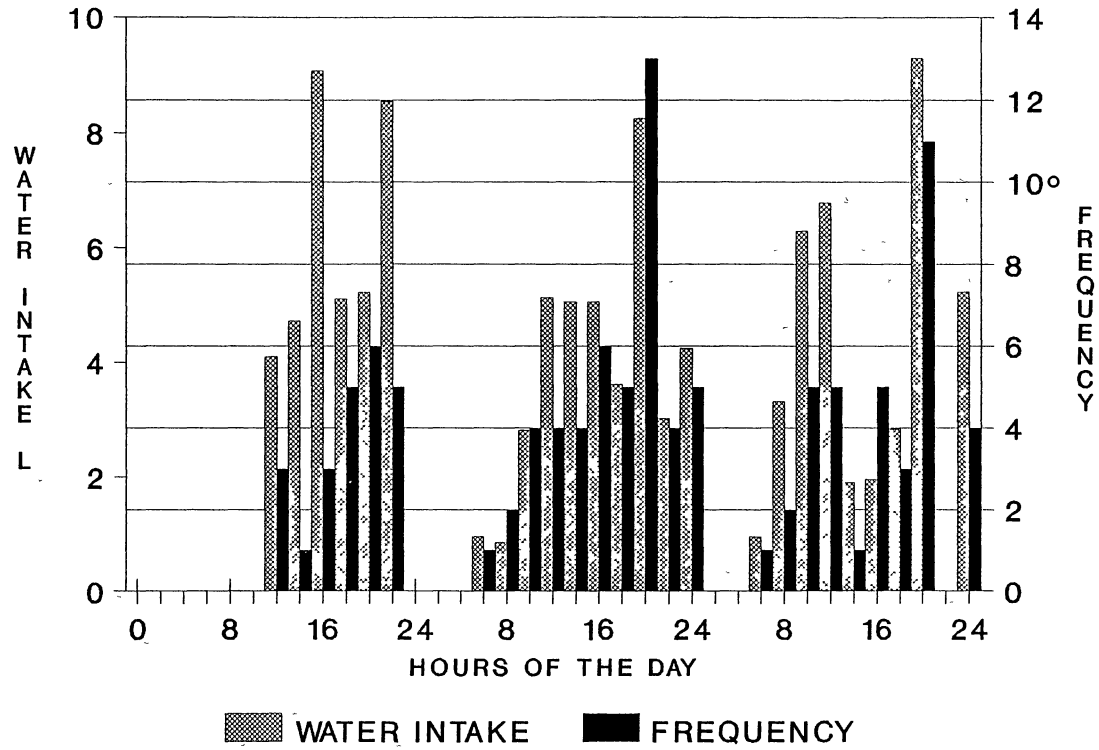

Figure 8.1 Water intake of beef heifers fed concentrate diets

osmolality by 30 to 40 mOsm/kg; thus overestimation in ruminal water inflow or outflow might be as high as 10%. Similar claims were made by Canadian workers (Petit et al., 1987) when they compared Cr-EDTA vs PEG for correction of ileal digesta flow and digestibility in Holstein calves. But they suggested that high urinary recovery of Cr-EDTA (17% to 9%) could have been due to changes in osmolality in the abomasum of the calves. In our study, ruminal and serum osmolalities were not measured; however, results from previous experiments (Chapter VI) using this same concentrate diet indicated that ruminal liquid osmolality exceeded plasma osmolality by 47 mOsm/kg similar to values reported by Dobson et al. (1976) suggesting in part that the differences in water evasion estimated with Cr-EDTA vs PEG values for the concentrate diet could be attributed partially to absorption of Cr-EDTA through the ruminal wall.

In contrast to results with the concentrate diet, water evasion for the hay diet was estimated to be greated based on for Cr-EDTA than on PEG (51.5% vs 44%). Absorption of Cr-EDTA with the hay diet may also have occurred but probably to a lesser extent than with the concentrate diet. In previous work (Chapter VI), ruminal osmolality for similar animals exceeded serum osmolality less with the hay than the concentrate diet (38 vs 47 mOsm/kg). Dobson et al. (1976) suggested that the extent of postprandial ruminal hypertonicity may affect the rate of absorption of Cr-EDTA from the rumen.

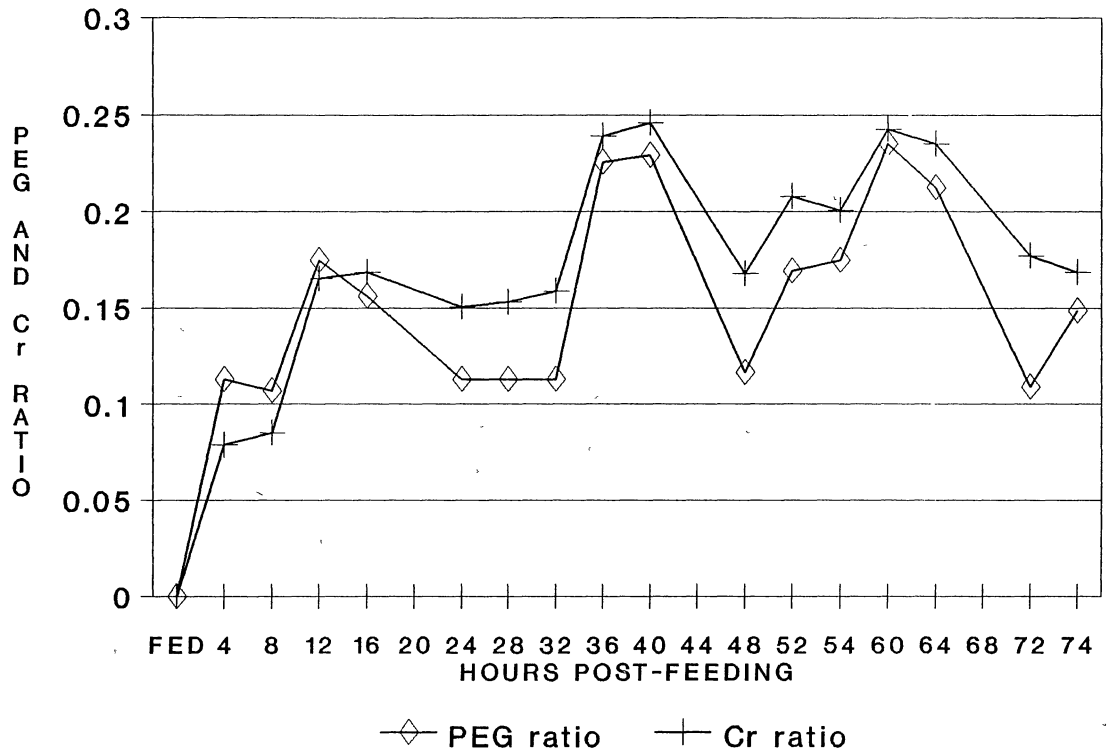

Figure 8.2 Marker (PEG and Cr) concentration ratios in ruminal fluid of beef heifers fed hay or concentrate diets (Experiment 1 Chapter VIII)

Higher calculated percentages of water evasion with Cr-EDTA than with PEG for the hay diet also may be attributed to the possibility that Cr-EDTA occupied a larger proportion fluid space in the rumen than PEG did (Downes and McDonald, 1964). Teeter and Owens (1983) found that delayed marker mixing would be influenced by rumen size, diet, type and quantity of ruminal solids. Moreover, they indicated that the rate to which marker mixed with liquids was positively correlated (r=.67) with the liquid associated to the ruminal solids. Concentration of PEG or Cr-EDTA in the ruminal fluid expressed as a ratio to the amount dosed (Figure 8.2) followed a similar diurnal patterns within the rumen during a 72 h period, indicating that when PEG or Cr-EDTA were consumed with drinking water, they behaved similarly in the rumen. These results support previous (Chapter VI) ruminal water evasion values estimated with PEG alone. However, marker concentration in the rumen, never reached the "steady state" conditions (Figure 8.2) that were expected. This suggests that ruminal liquid volume or liquid outflow were not constant but changed within and between days as feeding and water intake behaviors change.

Ruminal liquid volumes estimated with Co-EDTA compared closely (R^2=.96) with ruminal liquid volumes estimated measured by total evacuation in animals fed a concentrate diet though the slope was less than unity (Figure 8.3). Rumen volumes with animals fed hay were estimated less precisely (R^2=.60) by the slope did not differ from unity

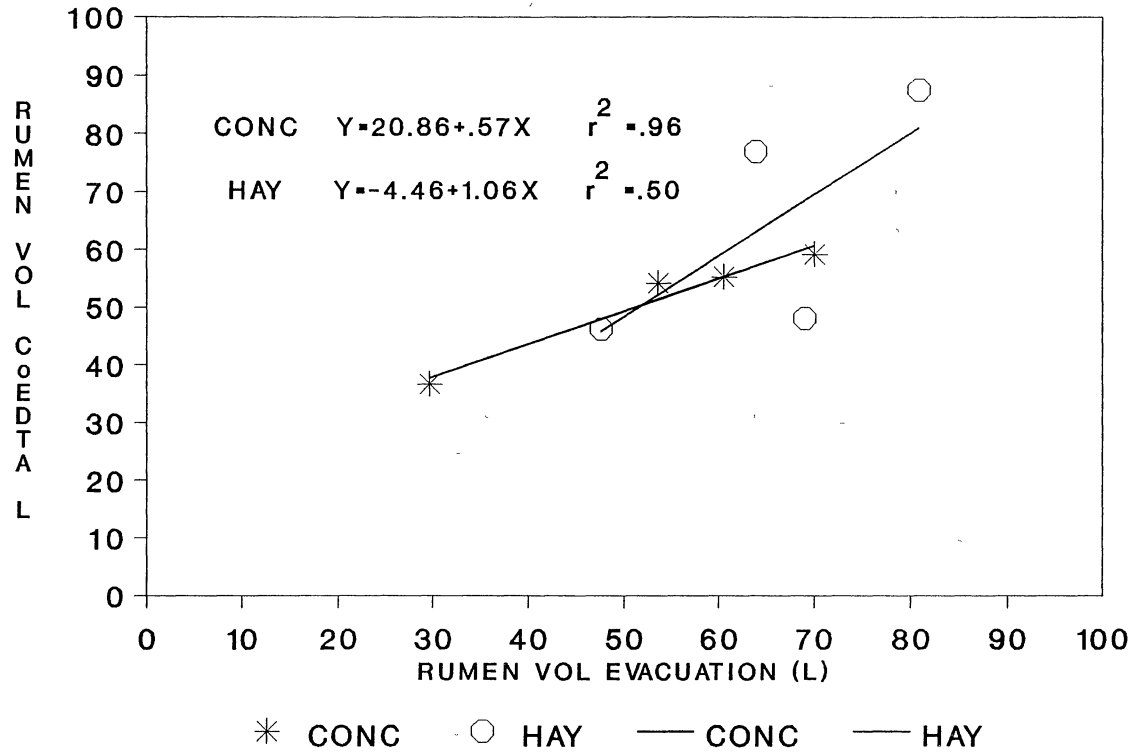

Figure 8.3 Rumen volume estimates in beef heifers fed hay or concentrate diets

and the intercept did not differ from zero indicating that accuracy was good. Overall, rumen volumes measured by total evacuation of ruminal contents, were slightly higher than estimates of liquid volume with Co-EDTA (1.1% and 4.1% for the animals fed hay and concentrate, respectively). Similar underestimations were reported by Teeter and Owens (1983) with animals fed similar hay and concentrate diets. When discussing reasons for these discrepancies, they indicated that less than instantaneous marker mixing, binding or exclusion of markers by ruminal solids, marker absorption, or errors associated with estimating rumen volume by evacuation may be responsible for the difference.

Kansas workers (Del Curto et al., 1990) reported that ruminal volumes were always (10 to 20%) greater when based on Cr-EDTA than when based on evacuated volumes. Some of the factors mentioned above may be involved. In addition, the time to reach marker equilibration in the rumen may be influenced by animal and diet type (Jacques et al., 1989) and time postfeeding will alter estimates. Kansas workers tipically have evacuated rumen contents 12 to 24 h after a meal whereas our estimates have usually been taken 2 to 8 h after a meal. Evacuated volumes have been consistently higher 4 h after than 24 h after a meal (Del Curto et al., 1990).

Compared with heifers fed the concentrate diet, heifers fed the hay diet had greater ($P<.05$) ruminal volume (65.3 vs 53.4 liters) increased ($P<.05$) liquid associated to the

Table 8.4 Liquid kinetics in beef heifers fed hay or concentrate (Experiment 1)

Item	Diets		SE
	Hay	Concentrate	
Heifers	4	4	
Water intake, liters/d	32.8	28.5	1.96
Rumen volume evacuated, liters	65.3[a]	52.0[b]	2.04
Free liquid, liters	38.7	33.3	1.86
Bound liquid, liters	26.6[a]	18.6[b]	.97
Rumen dilution rate %/h	6.2[a]	5.0[b]	.18
Rumen outflow, liters/d	94.6[a]	60.4[b]	4.30
Drinking water entering the rumen, liters/d			
PEG	18.4[a]	5.7[b]	1.85
Cr-EDTA	14.4	10.8	1.83
Evasion of drinking water, liters/d			
PEG	14.4[a]	22.8[b]	1.32
Cr-EDTA	18.4	17.6	1.48
Percent of consumed			
PEG	44.4[a]	79.1[b]	3.57
Cr-EDTA	51.4[c]	65.7[d]	3.15

[a,b]Means in the same row with different superscripts differ ($P<.05$).

[c,d]Means in the same row with different superscripts differ ($P<.10$)

ruminal solids (26 vs 18 liters) and have a higher ($P<.05$) ruminal liquid dilution rate (6.2 vs 5%/h) (Table 8.4). This agrees with observations by other workers as reviewed by Evans (1981a,b), Teeter and Owens (1983) and Owens and Goetsch (1986). Likewise, values from this study are in agreement with those values in Chapter V where these factors were discussed.

In summary, data from this experiment indicated that when dosed in the drinkin water PEG or Cr-EDTA gave reasonably similar estimates of ruminal kinetics. Water evasion estimates with Cr-EDTA were similar to previous estimates of evasion based on PEG (Chapter VI) supporting those findings.

Experiment 2.

Drinking water evasion from the rumen calculated based on the percentage difference between Cr-EDTA and Co-EDTA ratio averaged 41% (Table 8.3). This percentage of water evasion is slightly lower than those values reported in Experiment 1, Chapter VI and Chapter VII. As was demonstrated in Experiment 1, these two water soluble markers behaved similarly in the rumen so they should reliably estimate evasion. Yet, concentrations in the rumen never reached a "steady state condition" (Figure 8.4). This suggests that ruminal liquid volume and outflow may vary diurnally, postprandially or nycterohemerally. Our previous estimates of water evasion with the inflow outflow method

were made under the assumption that providing a water soluble marker in the drinking water for 4 consecutive days would result in a steady concentration of the marker in the rumen. Further, the intraruminal pulse dose of Co-EDTA was assumed to be subjected to a steady dilution rate so that outflow of the marker from drinking water could be calculated to quantifying the amount of drinking water evading the rumen. Variation over time questions the veracity of the "steady state" assumption. The inflow-outflow method gave consistent evasion estimates in three different studies (Chapter VI, VII and Experiment 1 of this Chapter). In each case, the percentage of water evasion was estimated to be quite high. In contrast, the water soluble marker method relyed on more continuous (8 h apart) intraruminal doses of Co-EDTA during an 80 h period to maintain a more constant concentration of the marker in the rumen. The rationale for using this approach, was that ingested water with a known marker concentration, if completely entered and mixed with the ruminal fluid, would yield a concentration similar or equal to that of the marker infused intraruminally. Hence, the Cr-EDTA/Co-EDTA ratio estimates entrance of labeled water into the rumen; the difference between this value and 1.00 gives an estimate of the fraction of water that did not equilibrate with ruminal fluid.

Both methods partially assume "steady state" conditions in the rumen; however, as shown in Figure 8.4, ratio

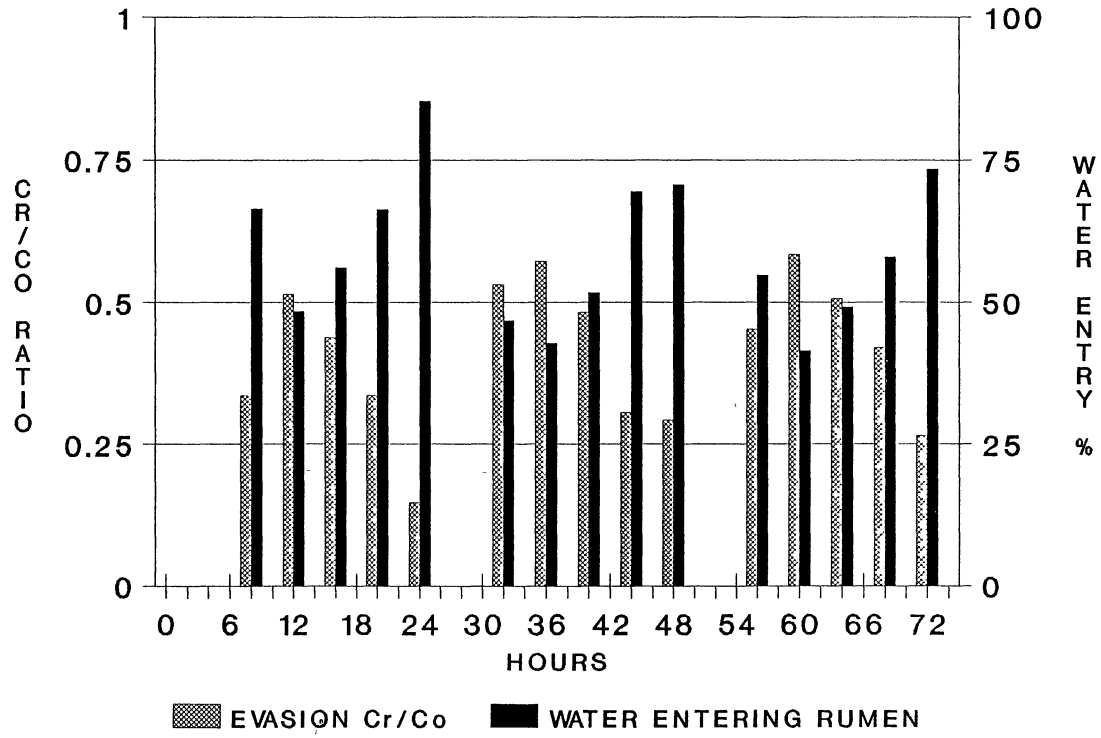

Figure 8.4 Ruminal water entry calculated with water soluble markers ratios

estimates of ruminal water evasion can be estimated at different hours of the day. Surprisingly these differed ($P<.001$). The difference between days was not significant ($P>.05$). The hourly variations may be the results of nycterohemeral changes in liquid outflow, as outlined by Jacques et al. (1989) or simply due to greated intake of water containing marker as certain times of the day. Jacques et al. (1989) indicated that nycterohemeral variations in liquid outflow were observed only when two pulse doses/d of Cr-EDTA were given to Jersey cows, as compared to continuous intraruminal infusions of Co-EDTA to Holstein steers. Data from Experiment 1 (Figure 8.2) support the idea that ruminal marker concentrations vary diurnally; similar diurnal changes were observed when water evasion was estimated with the ratio method (Figure 8.4).

In conclusion, ruminal water evasion estimated with the ratio method was only about half (41%) that estimated by the inflow-outflow method (80%) for animals fed a high concentrate diet. Despite these differences, values still indicated that a sizable percentage of the consumed water evaded the rumen. Nycterohemeral water intake or ruminal outflow patterns may be responsible for the diurnal variations in estimated drinking water evasion from the rumen. How these changes affect ruminal parameters are not clearly understood.

Experiment 3.

Calculated ruminal liquid volumes and liquid passage rate estimated with Cr-EDTA and Co-EDTA dosed three times during a 48 h period, are shown in Table 8.5. The ruminal volume estimate differed (P<.01) with infusion times and markers. Mean estimated volume from the 0400 infusion of the second day were smaller than the 1200 or 0400 infusions of the first day (24.0 vs 32.2 and 31.4 liters). Mean ruminal volumes across infusions when calculated with Co-EDTA was higher (P<.01) than for Cr-EDTA (32.4 vs 26.0 liters). Differences in dilution rate were observed (P<.01) at various times of the day (Table 8.5). A lower dilution rates was estimated (5.5%/h) for the 1200 infusion than the 0400 infusions of day one (6.3%/h) and day two (6.4%/h). Ruminal volumes estimated in this study, were smaller than those reported in the majority of the experiments conducted in this thesis. These differences may be attributed to the time in relation to feeding when they were estimated. While in the majority of the experiments in this thesis, ruminal volume was estimated by direct ruminal evacuation at approximately 2 h after feeding; in this study rumen volume was calculated 4 h before or after feeding. Data summarized by Owens and Goetsch (1986) indicated that at 80% concentrate level in the diet, mean ruminal volume is 54.7 ± 9.2 liters, this figure is higher than our values. Teeter and Owens (1983) suggested that ruminal liquid volume could

Table 8.5 Ruminal volume and liquid passage rate estimates during 48 h (Experiment 3)

Item	Marker	Infusion time[a] 0400	1200	0400	Mean
Rumen vol. (Liters)	Co	37.0[b]	32.4[b]	27.7[c]	32.4[f]
	Cr	25.7[b]	32.0[b]	20.1[c]	26.0[g]
	Mean	31.4[b]	32.2[b]	24.0[c]	
Dilution rate (% h^{-1})	Co	6.7	5.8	6.6	6.4
	Cr	6.0[d]	5.1[e]	6.2[d]	5.8
	Mean	6.3[b]	5.5[c]	6.4[b]	

[a]Infusion 1 = Markers were dosed at 0400 h on day 1.
Infusion 2 = Markers were dosed at 1200 h on day 1.
Infusion 3 = Markers were dosed at 0400 h on day 2.

[b,c]Means in the same row with different superscript differ (P<.01).

[d,e]Means in the same row with different superscript differ (P<.05).

[f,g]Means in the same column with different superscripts differ (P<.01).

be underestimated due to exclusion of the water soluble markers, when large quantities of whole corn are present in the rumen. Results from Experiment 1, shown that ruminal liquid volume estimated with Co-EDTA closely compared (R^2=.96) with direct ruminal evacuation volumes, suggesting that water soluble marker exclusion was minor.

Data from this experiment, suggested that liquid volume may change from day to day, consequently the use of only one ruminal volume estimated, to extrapolate it to the overall day, may not be entirely correct under certain conditions.

Ruminal liquid dilution rate values were similar than those summarized by Owens and Goetsch (1986), and values reported in Chapter VI, VIII and Experiment 1 in Chapter VIII. Reduced dilution rate during the 4 h postfeeding infusion (infusion 2) suggested that ruminal outflow (ml/h) was constant, since ruminal volume at this time was increased. However this results are in contrast to those of Warner and Stacy (1968b) and Teeter and Owens (1983). They reported that dilution rate of Cr-EDTA or Co-EDTA was increased immediately after feeding. But these responses were seen in animals consuming 90% alfalfa hay diets. Ruminal liquid dilution rate is usually slower with concentrate than with hay diets, basically due to salivary flow. Because saliva flow varies during or after feeding and has been estimated (Poutiainen, 1968) that about 11.5 to 13.5 liters/kg DM might enter the rumen, it is likely that fluctuations in salivary flow may directly affect ruminal

outflow and volume. Changes in liquid dilution rate, ruminal liquid outflow and ruminal volume, have been demonstrated (Jacques et al., 1989) in response to high salivary flow. Continuous infusions of Co-EDTA during a 48 h period and 6 sequential pulse doses of Cr-EDTA in the rumen of non-lactating Jersey cows to estimate liquid volume and outflow, shown no differences between markers when outflow was predicted (Jacques et al., 1989). Likewise, in our study Cr-EDTA and Co-EDTA provided similar ($P<.05$) dilution rate means.

In summary changes in rumen volume and dilution rate are likely to occur from day to day, and estimates of these parameters rely on the accuracy of determination of marker concentration.

A general summary of four different trials, where similar ruminal parameters were estimated, is presented in Appendix B. Compared to the concentrate diet, animals fed the hay diet tended to drink more water (29 vs 26 liters), had larger ruminal liquid volume (68.3 vs 43.5 liters), and total ruminal contents (72 vs 54 kg). Likewise ruminal liquid dilution rate and outflow was higher (7 vs 6 %/h and 113 vs 63.5 liters/d) for the heifers fed hay. In contrast, DM % in the rumen was higher for animals fed the concentrate diet (18%) than heifers fed hay (12%). Estimates of drinking water evasion based on water soluble markers, were higher for those animals on concentrate diets.

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APPENDIXES

APPENDIX A

DESCRIPTION OF FORMULAS

1. WATER KINETICS CALCULATIONS (RUMEN EVASION)

Description of variables:

PEGINT = PEG intake, g/d

WATIN = Intake of drinking water, l/d

PEGWA = PEG concentration in drinking water, g/l

PEGOUFL = PEG outflow, g/d

PEGRU = PEG concentration in the rumen, g/l

CODR = Cobalt dilution rate, %/h

PEGBYP = PEG evasion, g/d

DWBPR = Drinking water evading the rumen, l/d or %

DWENRU = Drinking water entering the rumen, l/d

EVASION = Drinking water evading the rumen, l/d or %

RUVOL = Rumen volume, l

T1 = Time of initial dose, h

T2 = Time of sampling, h

Calculations:	Units
PEGINT = WATIN * PEGWA, l/d*g/l	g/d
$CODR = \frac{LN\ [MARKER\ DOSE/RUVOL] - LN\ [MARKER]}{(T1 - T2)}$	%/h
PEGOUFL = PEGRU*RUVOL*CODR*24, g/l*/h*24 h/d	g/d
PEGBYP = PEGINT - PEGOUFL, g/d - g/d	g/d

Calculations:	Units
DWBPR = WATIN - DWENRU, l/d - l/d	liters
DWENRU = PEGOUFL/PEGWA, (g/d)/(g/l)	l
EVASION = (PEGBYP/PEGINT)*100, (g/d)/(g/d)	%

2. CALCULATIONS TO DETERMINE THE DISTRIBUTION OF LIQUIDS AND SOLIDS IN THE RUMEN

Description of variables:

S = Total wet solids from rumen retained on seive, g

L = Total liquid from rumen passing through seive, g

LV = Liquid volume, l

DML = Dry matter in liquid phase, fraction

DMS = Dry matter in solids phase, fraction

SWT = Solid weight, g DM

BLIQ = Bound liquid, l

FLIQ = Free liquid, l

KGFL = Weight of fluid, kg

KGSOL = Weight of solids, kg

RLDE = Rumen liquid density, g/ml

DMST = Total dry matter solids, kg

RUVOL = Rumen volume, l

Calculations:	Units
BLIQ = S(1 - DMS)/RLDE	l
FLIQ = L(1 - DML)/RLDE	l
KGFL = BLIQ + FLIQ	kg

Calculations:	Units
RUVOL = KGFL/RLDE, kg/(g/ml)	l
DMS = DMS*KGSOL, fraction*kg	kg
DML = DML*KGFL, fraction*kg	kg
DMST = DMS + DML, kg+kg	kg
RUMINAL DM % = (DMST/RUVOL)*100	%

3. DRY MATTER DIGESTIBILITY CALCULATIONS

Calculations:	Units
$\text{DM DIGESTIBILITY} = 100 - \frac{100 * (\text{DOSED MARKER, g/g DM})}{(\text{MARKER IN FECES, g/g DM})}$	%

APPENDIX B

Summary of 4 trials with cannulated beef cattle in ruminal parameters

Items	Experiments							
	1	2		3	4		AVG	
Diet	CONC	CONC	HAY	CONC	CONC	HAY	CONC	HAY
Animals	6	12		9	4		31	
Weight, kg	587.5	604.0		544.0	590.0		581.4	
Feed intake, kg	10.0	8.8		8.7	10.6		9.5	
% BW	1.7	1.4		1.6	1.8		1.6	
Liters/kg DM	3.1	2.4	2.9	2.8	2.7	3.1	2.8	3.0
Water intake, liters	30.1	21.4	25.6	24.6	28.5	32.8	26.2	29.1
Rumen volume, liters	39.5	46.5	71.3	49.0	53.4	65.3	46.5	68.3
Free liquid, liters	24.2	34.3	40.7	40.0	33.4	38.7	33.0	40.0
Bound liquid, liters	14.1	12.1	30.6	8.86	18.6	26.6	13.2	28.6
Rumen dilution rate, %/h	7.3[a]	6.8	7.7	4.7	5.0	6.2	6.0	7.0
Rumen outflow, liters/d	69.2	75.0	132.0	55.3	60.4	94.6	63.5	113.0
Total solids, kg DM	9.4	8.5	9.7	8.0	12.6	8.6	9.6	9.1
Ruminal DM %	19.5	18.7	12.0	14.1	19.2	11.9	17.9	12.0
Total contents, kg	48.1	45.4	80.3	55.2	65.5	64.0	53.5	72.1
Water evasion, liters/d	-	17.5	15.5	21.0	20.6	15.6	19.7	15.5
Percent of consumed	-	81.1	61.7	85.0	72.5	47.5	79.5	54.6
Ruminal liquid pH	5.6	6.0	6.5	6.1	-	-	5.9	6.5
Density	0.98	0.97	0.99	0.93	0.98	0.98	0.97	0.99

[a]Mean of Yb, Cr_2O_3 and Co calculated from fecal samples.

VITA

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