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Influence of Prepartum Nutrition on the Periparturient Dairy Cow – Impact on Rumen Development.

By

Jeffrey Robert Kaufmann 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

In

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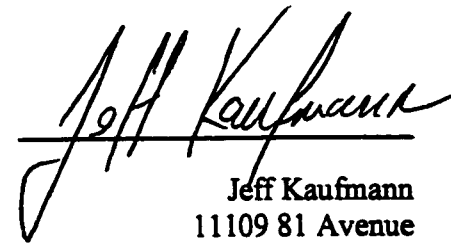

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Influence of Prepartum Nutrition in the Periparturient Dairy Cow – Impact on Rumen Development submitted by Jeffrey Robert Kaufmann in partial fulfillment of the requirements for the degree of Master of Science in Animal Science.

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Abstract

This study examined the influence of prepartum nutrition on rumen papillae development in dairy cows. Twenty-four multiparous Holstein cows (12 ruminally cannulated and 12 intact) were blocked according to expected calving date and assigned to one of four treatments. Cows received either a high or low concentrate diet for the entire dry period (61 d) or were fed a low concentrate diet beginning the day of dry off (day 61) and changed to a medium or high concentrate diet 32 d before expected calving date. After parturition, cows were fed the same lactation diet. Average rumen papillae surface area decreased during the dry period regardless of treatment. Prepartum diets did not influence total volatile fatty acid concentration in the rumen. However, by the fifth week of lactation rumen papillae tended to increase in surface area. Average prepartum dry matter intake, energy intake, and energy balance were not influenced by treatment. During the dry period, serum non-esterified fatty acids were significantly lower in cows fed the high concentrate diet however, blood glucose and β–hydroxybutyrate concentration were not significantly different among treatments. Prepartum diets did not influence lactation parameters. We concluded that prepartum nutrition did not influence rumen papillae development.

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CHAPTER 1: Literature Review

Introduction

The digestive systems of mammalian species display a large degree of structural and functional diversity compared to any other organ system in the body. This diversity derives from the different dietary habits of different species. Therefore, animals are classified on the basis of their eating habits. In general, there are two types of digestive systems typified by the carnivore and herbivore. Carnivores obtain most of their food by consuming other animals and digestion is mainly enzymatic with minimal microbial fermentation. Herbivores fall into two groups: (1) those containing simple stomachs, such as the horse, in which microbial fermentation takes place in the large intestine, and (2) those with rumens (i.e. cattle, sheep, and goats), in which extensive microbial fermentation occurs in a specialized region prior to digestion by alimentary enzymes. The omnivore displays a combination of the two digestive systems thus having the ability to consume both plant and animal products. Their digestion is mainly enzymatic, like that of carnivores but microbial fermentation can play a significant role in the large intestine of these animals.

The anatomical structure of the digestive tract varies greatly in different species. Although the functions may be similar in diverse species, the nature of their diet differs markedly and thus their gastrointestinal tract also differs. Monogastrics (carnivores and omnivores) have a stomach with only a single

compartment and digestion is mainly enzymatic in nature. However, ruminants have developed modifications in the gut, which enable them to utilize cellulose and other plant polysaccharides such as hemicellulose. The rumen has developed into an organ that provides for extensive pre-gastric microbial fermentation. Since ruminants can not produce cellulolytic enzymes themselves, they have developed a variety of ways to utilize plant materials indirectly by playing host to a variety of microorganisms. Many of these microorganisms produce cellulolytic enzymes capable of hydrolyzing cellulose which in turn can be further fermented into usable end products for the host animal. This symbiotic relationship makes the ruminant a unique animal to study.

In order to understand and appreciate the unique capabilities of the ruminant, one must examine the ruminant from the "*inside-out*". Firstly, the rumen and the dietary components that may influence the mucosal lining will be described. A brief look at the microorganisms that inhabit this environment under different conditions will also be discussed. The review of ruminal fermentation will describe a variety of feeds along with the microbial breakdown of these components into absorbable end products for the host animal. Finally, all these dynamic capabilities found in the ruminant will be related to the whole animal with particular interest in the transition dairy cow.

1.0. Anatomy, Growth, and Development of the Rumen

1.1.1. The Rumen

The stomach of ruminants shows the highest stage of evolutionary development of all mammals. However, the ruminant enters life as a simple stomach animal lacking the development and function of the forestomach compartments. The first three stomachs (in functional sequence: reticulum, rumen, omasum) do not secrete any digestive juices throughout the ruminants life cycle. The rumen is the primary organ of growth in the alimentary tract of the newborn calf. At birth the rumen is small and flaccid with minimal papillae development but by four weeks of age the rumen is approximately four to eight times its initial weight. At eight weeks of age, the rumen approaches adult proportions relative to the other digestive organs and to animals body weight (Lyford, 1988). The four compartments – reticulum, rumen, omasum, and abomasum occupy nearly three-quarters of the abdominal cavity. The forestomachs, in particular the rumen, host a large number of anaerobic microbes which can extensively ferment and digest the available substrate. The majority of fermentable products are absorbed through the rumen wall.

The rumen is a multi-compartmental structure and the largest of the forestomachs. It contains ruminal pillars on the inside of the stomach, corresponding to the grooves on the outer surface, which sub-divide the rumen into a number of different compartments. Generally the ventral, dorsal, cranial, and blind sacs describe the internal compartments of the rumen, all of which are

separated by ruminal pillars. The rumen is lined with a specialized stratified squamous epithelium.

1.1.2. The Rumen Epithelium

The ruminal lining consists of an outer layer of connective tissue covering an underlying layer of muscle with a distinct separate inner epithelium. This epithelial layer serves in the absorption and metabolism of minerals and volatile fatty acids (VFA). It also protects the internal tissues from abrasions by the digesta and from microbial invasion. The ruminal lining is considered to be a partially keratinized non-glandular tissue consisting of four stratum: basale, spinosum, granulosum, and corneum. These cell layers progressively merge into one another until they are finally sloughed-off into the lumen of the rumen. The cells of the stratum basale are situated on the basement membrane and contain fully functional mitochondria, golgi vesicles, ribosomes, and large vesicles (Steven and Marshall, 1969). The majority of these cells are columnar or cuboidal in shape. The intermediate layers show increasing signs of granular material and fibril accumulation with nuclear degradation and are termed the stratum spinosum and stratum granulosum. The stratum spinosum cells are oval in shape and serrated in outline. This appearance results from the subdivision of intercellular spaces by radically arranged cytoplasmic processes which are attached by randomly placed demosomes. Demosomes are tight gap junctions found in the rumen epithelium that act as a barrier to diffusion across the rumen wall. In the stratum granulosum the cells lie parallel to the luminal surface and

the intercellular spaces are small. The transition to the stratum corneum can be seen as the tight junctions between the cells now open out and intercellular space is present. These cells consist of a central core of fine granular material surrounded by peripheral fibrils strongly resembling terminal cells. The resulting surface is a complex system of progressively aging cells that offer good protection as well as good absorptive qualities.

The mucosal lining of the rumen is covered by rumen papillae, which can be defined as the organs of absorption in the rumen (Hofmann, 1988). The epithelial lining is drastically increased by the presence of papillae protruding from the wall of the rumen. The papillae are most abundant in the cranial sac, followed by the floor of the dorsal-caudal blind sac (Hofmann, 1988). In dairy cattle, the dorsal ruminal wall and pillars are usually devoid of papillae. Absorptive ruminal papillae contain a thin epithelial coat with a central arteriol. Molecules that pass through the epithelial barrier finally reach the venules, which transport all absorbed material via the ruminal veins into the hepatic portal vein.

1.1.3. Dietary Factors Influencing the Rumen and Epithelial Lining

The maturation of the ruminal epithelium and papillae development depends primarily on the nature of the feed. Calves fed milk alone show little rumen development, but those fed milk plus solid feed show a dramatic increase in rumen volume and weight, as well as exhibiting increased papillae length and density (Warner et al., 1956). Solid feed such as concentrate or hay stimulate rumen morphological development, whereas milk diets retard the growth and

development of the rumen. Normal development of ruminal papillae is stimulated by microbial fermentation products and physical stimulation (Flatt et al., 1958; Sander et al., 1959; McGavin and Morrill, 1976). Although the forestomach develops innately, age alone has little effect on papillae development (Thomas et al., 1962; Stobo et al., 1966; Hamada et al., 1976). Growth of rumen papillae was minimal in calves fed milk only (Tamate et al., 1962). Histological examination of the rumen of calves receiving various materials, such as plastic sponges, revealed that the epithelium remained underdeveloped with expansion in muscular development (Tamate et al., 1962). Tamate et al. (1962) reported that infusion of propionate and butyrate directly into the rumen of young calves stimulated papillae and total epithelial development but did not stimulate growth of ruminal muscle tissue. These results indicated that although the presence of feed bulk is necessary for rumen growth and muscle development, chemical stimuli by VFA are also required for the morphological development of the rumen epithelium. Hofmann (1988) describes that increasing proportions of butyric and propionic acid increases ruminal blood flow, which stimulates mucosal mitosis, resulting in vascular budding and epithelial cell proliferation. Papillae size and location reflect the stratification of ingesta and the regional differences of microbial activity located throughout the rumen (McGavin and Morrill, 1976). Lane and Jesse (1997) suggested that although VFA appear to stimulate some aspects of rumen development they may not be responsible for all morphological and metabolic changes in the rumen from birth to maturity. Instead, some other

end products of fermentation may be necessary for complete development of the rumen epithelium such as ammonia or branched-chain VFA.

The thickness of the epithelium also depends to an important extent on the rate of cell division in the basal layer and transit time from the proximal to exfoliating layers. Weekes (1972) and Fell and Weekes (1974) reported that single cell death is common in rumen papillae of sheep fed high concentrate diets during the first few weeks of consumption. However, there appeared to be an adaptation process in papillae after the first few weeks on the high concentrate diet (Fell and Weekes, 1974). Cell deletion appeared to be particularly marked in the papillae and less so in the inter-papillae areas (Weekes, 1972, Fell and Weekes, 1974). However, Gaebel et al. (1987) reported a significant increase in size of rumen papillae in sheep fed 64% or 90% concentrate diets. With the increase in surface area a concomitant increase in the number of cell layers of the epithelium was observed when concentrate diets were fed. Sheep consuming the high concentrate diets had up to fifteen cell layers in the stratum corneum, while those on the hay only diet had less than four cell layers (Gaebel et al., 1987). The increased surface area and cell layers were completely reversible by 15 weeks when the animals consuming the high concentrate diets were placed back on the hay only diet. Moon and Campbell (1973) reported a peak weight of ruminal mucosa at 45 days postpartum in Blackfaced ewes. The length of the papillae varied throughout the experiment but most of the higher values occurred after parturition. Moon and Campbell (1973) suggested the most likely explanation for these findings is that the development of the ruminal epithelium of lactating ewes

fed to appetite occurred in "waves" or "bursts" of cell division. Rumen papillae regressed into small wart like "buds" with a thin epithelial layer in cows fed a high forage diet during the dry period (Dirksen et al., 1985). Initiating a change to an energy rich diet 14 days before parturition stimulated new papillae development from vascular projections in the mucosal bed or from the regressed-like buds (Dirksen et al., 1985). By approximately seven weeks after the introduction of the energy rich diet these proliferated papillae had a thicker epithelium with developed papillae bodies. The authors suggested that the increase in papillae size in cows fed high-energy diets was due to their assistance in the stabilization of rumen pH and increased capacity to absorb nutrients (Dirksen et al., 1985, 1997).

1.1.4. Summary

Normal rumen development depends upon the ingestion of solid feed and the resulting VFA arising from microbial digestion. Rumen growth is the combination of expansion of muscle, epithelial, and papillae development. Ruminal growth rate may be accelerated by the introduction of plant material or other readily fermentable substances into the rumen. However, little rumen development takes place on all liquid diets, while very rapid growth occurs once solid feed is introduced. The presence of VFA, ammonia, branch-chained VFA and bulk is required for normal growth in capacity, muscularity, and epithelial formation. In the mature ruminant, papillae development is involved in

continuous changes of proliferation to regression depending on the stage of the lactation cycle and diet consumed.

1.2. Ruminal Ecosystem

As the calf slowly adapts to solid feed, the rumen becomes colonized by a variety of different microorganisms. The microorganisms found in the rumen have a truly symbiotic relationship with the ruminant animal. The three basic types of microbes found in the rumen are bacteria, protozoa, and anaerobic fungi. In newborn ruminants, this natural phenomenon is observable 38 h after birth as bacteria proliferate in the fluid phase and colonize the tissue of the digestive tract (Cheng et al., 1991). Protozoa and fungi populations become established in the ruminal microbiota within 12 to 29 days and 8 to 10 days, respectively (Fonty et al., 1987; Stewart et al., 1988). These microorganisms depend on the host animals to provide the physiological conditions necessary for their existence. In turn, these microbes digest and ferment plant material into useful end products that the ruminant can utilize. This section describes the microbes found in the rumen and discusses their functional characteristics during different physiological conditions within the rumen.

1.2.1. Rumen Bacteria

The bacterial community consists of obligate and to a smaller extent facultative anaerobes. The bacteria, which are found in the rumen, can range from 10^{10} to 10^{11} microorganisms per milliliter of rumen fluid (Yokoyama and Johnson, 1988). They are assigned to groups according to three main shapes (cocci, rods, spirilla), sizes (generally between 0.3 to 50μm), and different cellular

structures (including the presence of a cell envelope, cytoplasmic structures and surface adherents or appendages). Classification of the rumen bacteria has largely followed a system based on the type of substrate the bacteria attack. By this method of classification eight distinct groups of rumen bacteria have been recognized based on their utilization of cellulose, hemicellulose, starch, sugars, intermediate acids, proteins, lipids or methane production (Yokoyama and Johnson, 1988). *Primary* bacteria are those that degrade the actual constituents of the diet depending on their preference for cellulose or starch and are termed cellulolytic or amylolytic, respectively. Cellulolytic bacteria can synthesize new microbial protein exclusively from ammonia (Bryant, 1973), while amylolytic bacteria require ammonia and amino acids or peptides for microbial protein synthesis (Russell et al., 1992). According to an in vitro study, approximately two thirds of the amylolytic bacteria protein is derived from peptides or amino acids, and only one third from ammonia (Russell et al., 1983). However, describing cellulolytic bacteria as only using ammonia and amylolytic bacteria as only using peptides and amino acids for microbial synthesis may be an over-simplification due to the diversity of microbes found (and yet to be discovered) in the rumen (personal communication, T. A. McAllister). The end products from *primary* bacteria are VFA, lactic acid, carbon dioxide, ammonia, and hydrogen. *Secondary* bacteria use as their substrate the end products of the *primary* bacterial degradations, and this group includes the lactate-utilizing bacteria, which produce some of the propionate, and the hydrogen-utilizing methanogenic bacteria, which produce methane gas.

1.2.2. Rumen Protozoa

The number of protozoa found in the rumen at any one-time ranges from 10^5 to 10^6 microbes per milliliter of rumen fluid (Yokoyama and Johnson, 1988). Although protozoa are far less numerous than bacteria they are much larger, therefore they may occupy a volume nearly equal to that of the bacteria. The majority of protozoa are ciliates although flagellates do exist. All of the protozoa are strictly anaerobic. Protozoa are very versatile in their ability to degrade and ferment all major plant constituents. There are three main types of ciliate protozoa: (1) the entodiniomorphs which engulf particulate matter and have enzymes that attack cellulose and hemicellulose, (2) the holotrichs which generally depend on starch and (3) specific cellulolytic species that are not entodiniomorphs. Protozoa not only ferment and engulf particulate matter; they can also attack and digest rumen bacteria. Yokoyama and Johnson (1988) described how some protozoa engulf bacteria and digest them internally while others lyse the bacteria first and then ingest their cellular components. Amino acids from ingested bacteria are used extensively for synthesis of protozoal proteins. Sugars are fermented in part by protozoa, but if a high concentration is present, then a large fraction is stored as starch within the protozoa. When sugars are no longer available, the starch within the protozoa is digested and fermented. Thus, the amount of energy available may be extended if a large population of protozoa exist in the rumen. Similar to the *primary* bacteria, the end-products of fermentation by protozoa include the various organic acids, carbon dioxide,

ammonia, and hydrogen. Some functional attributes of the protozoa are (1) their assistance in fiber digestion, (2) their ability to provide a reservoir of microbial protein at times of intermittent food supply, and (3) they help prevent over-proliferation of bacteria at times of starch loading by engulfing starch particles.

1.2.3. Rumen Fungi

The third group of microorganisms are the anaerobic fungi. These fungal zoospores were once thought to be flagellate protozoa. Their numbers range from 10^4 to 10^6 cells per milliliter of rumen fluid (Yokoyama and Johnson, 1988). The rumen fungi are completely dependent on fermentation processes for energy and survival. The zoospores attach to plant fragments and form rhizoids that penetrate plant tissues. Rhizoids invade plant cell walls to obtain fermentable carbohydrates and develop sporangia, which, on maturity, release zoospores to establish another cycle. The ability of fungi to penetrate and colonize highly fibrous plant material has been shown to stem from their ability to produce a wide range of enzymes (Steward et al., 1995).

1.2.4. Characteristics of the Rumen Microbes

Attachment

The attachment of microbes to surfaces is an important factor especially in a system where materials are continuously being introduced and removed. In order for the microbes to maintain their population in the rumen, it is necessary

that their turnover rate be greater than the passage rate of rumen digesta. If this were not the case, the microbes would eventually be washed out of the rumen.

Microbes are generally found in three distinct locations in the rumen. Though some microbes adhere tightly to the wall of the rumen, most microbes are associated with particles in the rumen or float freely in the ruminal liquid. The majority of microbes found in the rumen are attached to particulate matter and can be functionally described as two distinct subpopulations: (1) those loosely attached to feed particles, and (2) those firmly attached to feed particles (Czerkawski and Cheng, 1988). Microbes present in the liquid phase of the rumen must continuously recolonize new sources of substrate or else they will pass out of the rumen and be digested as a protein source by the host animal. The microbes that are loosely attached to feed can be easily removed from the rumen by gentle washing, whereas tightly associated bacteria remain attached. These two populations of microbes account for 70% to 80% of the microbial matter in the rumen (Craig et al., 1987). Generally, microorganisms that attach to feed particles have a slow rate of passage and prolonged residence time within the rumen (McAllister et al., 1994). High forage diets with a reduced rate of passage are beneficial for slower growing microorganisms such as protozoa and fungi. If these microbial populations are unable to attach to feed particles, their population will rapidly decline. Microbial attachment is based on a capsular carbohydrate coat or glycocalyx and cellulose binding domains (McAllister et al., 1994). The wall adherent bacteria are facultative anaerobes, which remove oxygen from entering the rumen to protect the oxygen sensitive anaerobes. They also digest

dead epithelial cells along the rumen wall and hydrolyze urea (Cheng and Costerton, 1980).

General attachment effects the population and type of microorganisms found in the rumen. Microbial attachment is advantageous to the survival and competitiveness of certain species and also creates a problem of distinguishing between microbial protein and dietary protein passing out of the rumen.

Penetration

Attachment is the initial step in microbial digestion of feed. The microbes must penetrate the surface of the feed particles or find an opening to access their preferred substrates. McAllister et al. (1994) described how forages and cereal grains are protected by a cuticle that is almost completely resistant to attachment. Even after the protective layer of plant material is breached, internal barriers are still encountered. For example, in forages the structures composing the cell wall present another obstruction for microbes and are in part responsible for the indigestible nature of plant material. In some cereal grains, starch granules are encased within a protein matrix surrounded by a ß-glucan-rich cell wall. These internal structures in wheat and barley are easily penetrated, and consequently rapidly fermented in the rumen (Orskov, 1986). In contrast, corn and sorghum protein matrix is extremely resistant to attachment and penetration. Therefore, a greater proportion of starch reaches the small intestine in cattle fed corn and sorghum as compared to those fed barley (Spicer et al., 1986). Ruminal bacteria can also strategically circumvent these physical barriers and gain access to readily

digestible material through stomata, lenticels, or damaged areas, and digestion essentially proceeds from the inside out (McAllister et al., 1994).

Mastication and feed processing can also promote microbial feed digestion by disrupting the primary barriers to microbial attachment and penetration. Mastication subjects the feed to a mixture of shearing and grinding actions, which increases the surface area for microbial attachment and digestion. Mechanically processed feed (i.e. rolled, chopped, ground) also increases the surface area of feed and augments mastication damage. However, over-processing of feeds often causes feed particles to pass from the rumen before microbial attachment, penetration, and digestion occur. The extent of mechanical processing required to facilitate microbial attachment often depends on the extent of ingestive and ruminative mastication (McAllister et al., 1994). Selection of an optimal degree of mechanical processing for microbial attachment, penetration, and digestion requires not only consideration of the properties of the feed, but also the negative effects of over-processing, and the extent of mastication.

Influence of Diet

Microbial yield in the rumen increases with increasing amounts of organic matter fermented in the rumen. For example, National Research Council (NRC, 1989) and NRC (2001) predict microbial yield as a linear function of total digestible nutrients. Thus, the flow of bacterial nitrogen from the rumen usually increases as the proportion of concentrate in the diet increases and as the level of feed intake increases. However, efficiency of microbial yield, measured as grams

of bacterial nitrogen per kilogram of organic matter apparently or truly (i.e. after correcting for organic matter contributed by microbial cells) fermented in the rumen, may decrease when microbial yield is maximized. Sniffen and Robinson (1987) and Febel and Fekete (1996) described several studies in which increasing the forage content of the diet results in a higher efficiency of microbial yield. Oldham et al. (1979) reported that increasing the forage level of the diet from 10% to 40% in dairy cows increased the efficiency of microbial yield. The NRC (1989) also supported the observation that diets containing less than 40% forage, or less than 20% neutral detergent fiber (NDF), result in decreased efficiency of microbial growth. Russell et al. (1992) reported that diets containing less than 20% NDF on a dry matter basis resulted in a 2.5% reduction in microbial efficiency for every 1% decrease in NDF. The reduced efficiency with higher concentrate levels in the diet is explained by an uncoupled fermentation. Namely, at a higher forage level the slower degradation of organic matter allows more energy to be trapped by the microbes.

The level of feed intake may markedly modify the effect of forage to concentrate ratio. Merchen et al. (1986) reported no differences in the efficiency of microbial protein synthesis between animals consuming a diet containing 25% or 75% concentrate, when feed intake was low. However, at a higher feed intake, cows consuming the diet containing 75% concentrate resulted in a higher microbial nitrogen outflow and increased microbial yield but decreased efficiency of microbial protein synthesis. Merchen et al. (1986) explained the increased duodenal flow of bacterial nitrogen was due to the increased amounts of starch or

easily fermentable carbohydrates present in the high concentrated diet. This reasoning was supported by the in vitro study of Stern et al. (1978) who reported that the microbial yield increases when the non-structural carbohydrate content of the diet was increased.

Effect of Ruminal pH and Osmolarity on Microbes

Microbes have different ruminal pH optima at which they express their maximum enzymatic activity. Ruminal pH can vary according to which substrates are present in the rumen, but generally ranges from 5.5 to 7.0. Lower ruminal pH values are usually found in animals fed a higher concentrate diet. Whereas forage-based diets promote higher ruminal pH. The protozoa, *primary* cellulolytic bacteria, and most *secondary* bacteria require a pH of 6.2 or greater for growth. The amylolytic bacteria are more active in acidic conditions around a pH of 5.8 or lower in the rumen (Leek, 1996).

Owens and Goetch, (1988) described an osmotic pressure near 260 mOsm as the most favorable for bacteria and protozoa populations. However, osmotic pressures above 350 mOms in the rumen may be inhibit digestion of starch and fibre through a direct affect upon microbial metabolism. These osmotic pressures may be observed in cattle fed high grain diets.

1.2.5. Summary

In summary, the ruminal ecosystem is a highly diversified colony of microbial organisms. Their survival is dependent on a variety of factors including

attachment to particles in the rumen, specific substrates provided by the diet, ruminal pH, and osmotic pressure in the rumen. The microbes enzymatically break down the constituents of the diet into products the ruminant can utilize. This leads us into the next section on ruminal fermentation.

1.3. Ruminal Fermentation

Once a well-developed rumen, epithelium lining, and densely populated microbial environment exist ruminal fermentation can proceed. Fermentation in the rumen is the result of physical and microbial activities which convert components of the diet to products which are useful (VFA, saturated fats, ammonia, B-vitamins, and microbial protein), useless (methane and carbon dioxide), or even harmful when excessive (ammonia, nitrate) to the host animal. Figure 1.5 provides an overview of the fermentation and fate of the main dietary components in the ruminant diet. This section will address the importance of balancing ratio of concentrates and forages in the ruminant diet, followed by an overview of the microbial breakdown of these dietary constituents to end-products that can be readily absorbed through the rumen epithelium.

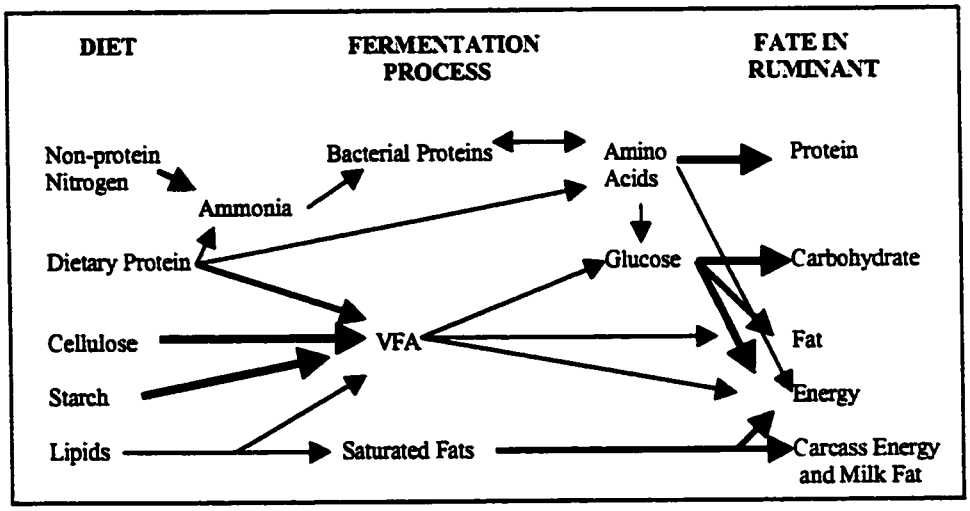

Figure 1.1. An overview of the fermentation and fate of the main dietary components in ruminants.

1.3.1. Balancing Concentrates and Forages

Carbohydrates can be categorized into two main groups: structural carbohydrates (pectins, cellulose, and hemicellulose) or non-structural carbohydrates (NSC; sugars and starches). Approximately 70% of the ruminant's diet is composed of structural and non-structural carbohydrate. Therefore, compatible combinations of carbohydrates, while supplying adequate amounts of fiber in the diet are important to promote strong ruminal digestion, support high yields of microbial growth, and maintain a stable fermentation. Ultimately, total contribution from both the structural and nonstructural carbohydrates must be considered when feeding carbohydrates to cattle.

Grains differ in the amount and fermentability of starch and therefore respond differently to mechanical and physical processing. For example, on a dry matter basis, wheat contains 77% starch, corn and sorghum around 72% and oats and barley at 58%, respectively (Huntington, 1997). Barley is digested more rapidly and completely than corn in the rumen (Orskov et al., 1971) and corn is digested more completely than sorghum (Spicer et al., 1986). In a study by Miller et al. (1986), cattle fed complete pelleted diets containing either 85.5% corn, wheat, orts, barley or sorghum grain had apparent ruminal digestibilities of 48.1%, 52.9%, 47.8%, 61.6%, and 22.2%, respectively. If whole grain is not mechanically processed or physically damaged by mastication, digestion appears to be severely limited particularly in the case of barley, wheat, and oats. Beauchemin et al. (1994) reported whole corn was substantially damaged after ingestive mastication. The rate of in sacco disappearance of dry matter from

chewed corn was greater than the corn processed into quarters or halves. In contrast, chewed barley and wheat kernels showed signs of dentition, but most kernels were not extensively damaged by mastication during eating. Therefore, the rate of in sacco dry matter disappearance of barley and wheat was lower for masticated kernels than for halved or quartered kernels. The effects of reducing particle size on ruminal digestion are complicated and subject to particle density, specific gravity, and passage rate. In general, small particles sink and are passed out of the rumen, while larger particles tend to rise within the ruminal strata and become further exposed to additional mastication and microbial attack.

In addition to mechanical treatments, heat and moisture processing methods (steam flaking, micronization, high moisture ensiling) appear to improve feed efficiency in cattle fed high concentrate diets by increasing the proportion of starch digested in the rumen (Poore et al., 1993; Zinn, 1993; Chen et al., 1995). Owens et al. (1986) calculated that starch digestion in the rumen is approximately 70% as efficient as starch digested in the small intestine. This would support the suggestion of an advantage to increasing the availability of starch present in the small intestine. However, this may be off set by reduced microbial synthesis in the rumen and a decreased efficiency of starch digestion throughout the gastrointestinal tract.

High producing ruminants (i.e. the dairy cow) also require sufficient fiber to maintain an efficient and healthy rumen. Dietary fiber contains both chemical and physical properties, which can affect the ruminal characteristics. Chemical fiber is measured by NDF and supplies fermentable carbohydrates to support

microbial growth. Physical fiber is measured by particle size and promotes chewing, resulting in the production of saliva. The NRC (1989) recommends a minimum of 25% to 28% NDF of which 75% should be supplied by forage, in order to minimize digestive disorders and maintain a 3.5% milk fat in dairy cows. Beauchemin et al. (1994b) reported the minimal amount of fiber necessary in dairy cattle diets in order to maintain healthy rumen function, for barley-based diets, is less than that recommended by NRC (1989) for maintaining a milk fat content of 3.5%. Table 1.1 provides a list of common feeds with average ruminal NDF digestibilities. Inclusion of these ingredients can result in diets with low actual forage levels (< 40% of the diet) but levels of NDF exceeding 35% (Weidner and Grant, 1994, Beauchemin et al., 1994b). Generally, diets should be balanced to maximize ruminal-fermented carbohydrates, energy intake, and microbial protein synthesis. Dietary factors to consider when balancing carbohydrate components include the level of forage, fiber source, and forage particles size. Goetsh et al. (1987) reported that long forages increase regurgitation and remastication of grain and possibly entrap grain particles in the fermentation mat, subjecting the grain to further ruminal digestion. However, the larger particles may be less suitable than smaller particles for bacterial attachment due to fewer open edges for microbial entry, thereby reducing the rate of digestion in the rumen. Lykos and Varga (1995) described carbohydrate digestion as the driving factor for microbial fermentation and discussed the importance of including a variety of carbohydrate sources ranging from fast to moderate rates of digestion to support a continuous supply of energy in the rumen.

Table 1.1. Comparative ruminal NDF digestibilites of various forages and byproducts. Adapted from Nocek and Russell (1988)

Feedstuff	NDF Content[1]	Average ruminal NDF digestibility, %
Alfalfa hay	39.6	33 - 63
Alfalfa silage	45.7	34 - 41
Corn silage	45	25 - 35
Brewers grain, dry	47.4	50
Soybean hulls	60.3	86 - 95
Corn Grain	9.5	73
Barley Grain	20.8	28

[1] NDF Content taken from NRC (2001).

1.3.2. Microbial Breakdown

The major substrates for fermentation in ruminant diets are complex carbohydrates originating from plant cells and grains. As previously mentioned, carbohydrates mostly consist of cellulose, hemicellulose, pectins, starches, dextrins, and soluble carbohydrates (mono- and disaccharides). A simplified scheme of the major carbohydrate fermentation pathways was described by Leek (1996). The description categorized the microbial fermentation process into three stages, with an additional fourth stage describing the synthesis of microbial compounds from the end products of the preliminary three stages. The initial stage involves the hydrolysis of the major plant polysaccharides to their constituent monosaccharides. Cellulolytic bacteria produce extracellular cellulases and other enzymes that degrade the cellulose and hemicellulose to oligosaccharides and finally to glucose, glucose 6-phospate, fructose 6-phospate, and triophosphate. Pectins and hemicellulose are largely degraded to xylose and

other pentoses. Amylolytic bacteria degrade starches and dextrans by amylases to produce maltose, then maltases further ferment maltose to glucose 1-phosphate. The second stage involves rapid transformation of these intermediates to pyruvate through the Embden-Meyerhof pathway. Pyruvate, in turn, is then rapidly converted to acetate, propionate, and butyrate. Lactate can also be produced from pyruvate. It is important to note that the acidity of lactate is ten times stronger then that of acetate, propionate, or butyrate, so its presence in the rumen has a greater effect on rumen pH than the primary VFA.

1.3.3. Ruminal VFA Production

The concentration of VFA in the rumen is highly variable, although the total amount present is usually between 60 to 160 mM (Gaebel and Sehested, 1997). The pattern of VFA production in the rumen is dependent on both the composition of the diet and the intake pattern. Acetate predominates in the rumen when ruminants are fed high forage diets, but amounts of propionate and butyrate are always present. The proportion of VFA derived from the fermentation of cellulose is generally in the range of 70:15:10 for acetate, propionate, and butyrate, respectively (Leek, 1996). These forage-based diets typically have a ruminal pH value of 6.2 or greater, which is the optimum pH range for cellulolytic bacteria. Diets rich in starch, such as high concentrate diets, favor propionate production and generally gives rise to less acetate. Molar ratios of acetate, to propionate to butyrate in the rumen for dairy cows fed a high grain diets were 65:20:12, respectively (Murphy et al., 2000). The pH of the rumen fluid usually

declines below values of 6.3, but a rapid fermentation can potentially lower the pH to less then 5.5. Lower pH seems to encourage the growth of organisms that primarily produce propionate and lactate. If the proper *secondary* lactate-utilizing bacteria are not present in the rumen, or population numbers are low, the pH will continue to drop causing may of the cellulolytic bacteria and protozoa to become inactivated or killed. This type of situation can lead to rumen acidosis.

Bergmen (1990) indicated that the three primary VFA could account for 65% to 75% of the total metabolizable energy plus an additional 5% from the five to seven carbon VFA. Therefore, the total available VFA could easily account for 80% of the ruminant's daily energy requirements. The concentration of each particular VFA in the rumen represents a balance between the rate of production and the rate of removal from the rumen.

1.3.4. Ruminal Absorption of Organic Acids

The vast majority of the metabolic acids produced in the rumen are absorbed directly through the ruminal epithelium with less than 10% passing to the small intestines (Harfoot, 1978). However, it should be pointed out that the amounts of acids appearing in the portal circulation are not equal to the amount produced in the rumen. This is because each of the individual metabolic acids is metabolized to different extents by the rumen mucosa during the process of absorption. Continuous absorption of acids by the rumen is not only important to meet the animal's energy needs but also to help maintain a stable rumen pH.

Dijkstra et al. (1993) explained that organic acid absorption models are primarily based on diffusion, the ruminal pH, cell contents and intracellular metabolism of VFA. Organic acids are absorbed across the rumen epithelium in both the dissociated but primarily undissociated form (SCFA = Short Chain Fatty Acid; Gaebel and Sehested, 1997).

$$\underset{\text{(undissociated)}}{HSCFA} \longleftrightarrow H^{+} + \underset{\text{(dissociated)}}{SCFA^{-}}$$

The ratio of undissociated compared to dissociated SCFA is dependent on the ruminal pH and the pKa values of the individual VFA and lactate. The VFA are relatively weak acids with an approximate pKa value of 4.8 (propionate = 4.87, acetate = 4.87, and butyrate = 4.82) compared to lactic acid (pKa = 3.86). Generally because the rumen pH is above 6.0, most of the organic acids are present in the dissociated state. However, absorption of VFA and lactate from the rumen is enhanced by a decrease in the pH, indicating greater permeability of the epithelium to the undissociated form. Passive transport is the favorable mode of transfer for undissociated acids (Figure 1.2).

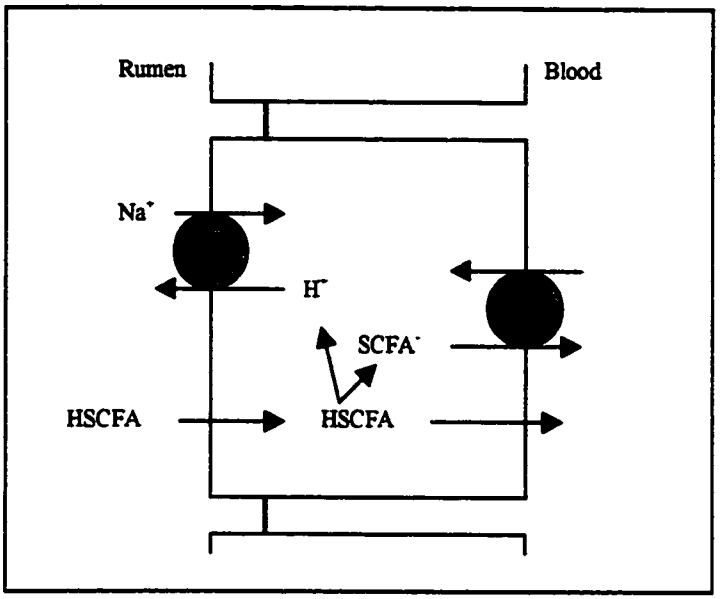

Figure 1.2. Tentative model explaining the stimulatory effect of SCFA on Na^+ transport by the transmembrane permeation of undissociated SCFA. Adapted from Gaebel and Sehested, (1997).

For individual acids, the rate of absorption from the rumen increases with increasing chain length of the acid: lactate < acetate < propionate < butyrate, and this is in keeping with their relative lipid solubilities. However, dissociated acids can also be actively transported across the ruminal epithelium and appears to be coupled to the operation of a non-selective, electroneutral anion exchanger (Gaebel and Sehested, 1997). This exchange is linked with chloride absorption and bicarbonate secretion (Figure 1.3).

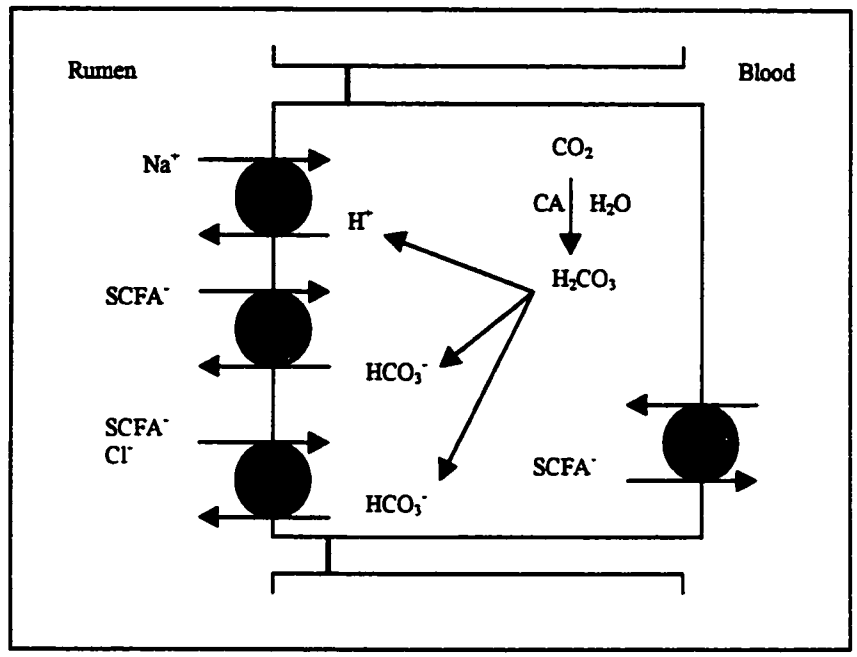

Figure 1.3. A tentative model for the transport of dissociated SCFA and for possible interrelationship with the transport of chloride, bicarbonate and sodium. CA = Carbonic Anydrase. Adapted from Gaebel and Sehested, (1997).

In contrast to the organic acids absorbed through the ruminal epithelium, the amount transported or appearing in the venous effluent is reversed: butyrate < propionate < acetate < lactate. This is because each of the individual VFA is metabolized to different extents by the rumen epithelium and the lower pKa value of lactate. The proportion of VFA metabolized markedly increases with chain length. Approximately 90% of butyrate is metabolized by the rumen epithelium (Bergman, 1990). Most of the butyrate metabolized by the rumen epithelium results in the formation of ketone bodies or oxidation to carbon dioxide. This process of ruminal epithelial metabolism of butyrate to ketone bodies has been

termed alimentary ketogenesis to distinguish it from the more usually recognized hepatic ketogenesis. Ruminal propionate metabolism is approximately 50% of the total propionate produced in the rumen (Bergman, 1990). Propionate metabolism by ruminal epithelium gives rise to lactate and carbon dioxide but may also give rise to alanine and other amino acids (Bergman, 1990). However, most of the data on propionate metabolism in the ruminal epithelium has come from studies in sheep. Studies on cattle do not give such high estimates of propionate loss during rumen absorption. Cook et al. (1969) reported that 3% to 15% of rumen propionate in cattle was converted to lactate in the rumen epithelium. Thus, the metabolic production of lactate from propionate undoubtedly occurs, but species differences in its quantitative importance exist. Acetate is the least metabolized VFA in the epithelial lining of the rumen at 30% (Bergman, 1990). It is likely that acetate metabolism is limited by the very low activity of acetyl-CoA synthetase in the rumen epithelium (Cook et al., 1969). Also, the rumen epithelium may metabolize butyrate and propionate more extensively than acetate because of energy efficiencies. For example, one mole of butyrate and propionate can produce 28-adenosine triphosphote (ATP) and 18 ATP, whereas one mole of acetate can only produce 10 ATP.

In addition to its role in energy metabolism, VFA absorption plays a major role in pH regulation since it alkalizes the rumen contents. Volatile fatty acid absorption causes an alkalizing effect due to the disappearance of the undissociated VFA and the exchange between the dissociated VFA absorbed and bicarbonate molecule secreted as shown in Figure 1.7 and 1.8.

1.3.5. Summary

Balancing the dietary source of carbohydrates continues to be a challenge in ruminant nutrition. Diets containing excessive levels of NSC tend to be rapidly fermented in the rumen causing the pH to drop resulting in unstable fermentation patterns and possible health problems if sufficient fiber is not available. Microbial digestion of feed can either be a slow or fast process depending on the dietary components. Regardless, substantial amounts of acetate, propionate, and butyrate are produced in the rumen and readily absorbed through the rumen epithelium and used as an energy source for the host animal. However, the rate of absorption depends on the rumen pH and the pKa value of the organic acid. The rate of organic acid absorption does not equal the amount transported or appearing in the venous effluent because individual acids are metabolized to different extents in the rumen epithelium.

1.4. The Host Animal: The Transition Cow

Now to complete the story, all the previously presented factors shall be put into context of the whole animal with additional nutritional and physiological factors facing the transition cow. The transition cow throws a twist into the story due to the fact that the animal is faced with a variety of hormonal, metabolic, and possible dietary changes from the onset of parturition and the initiation of milk production. The transition period usually refers to the final three-weeks prior to calving and the first three weeks of lactation. Traditionally, the transition period has been used to slowly adapt dairy cows to high-energy diets that are commonly fed during early lactation. The gradual introduction of these close-up dry cow diets (i.e. increased amounts of concentrate) may help reduce the risk and possibility of cows going off-feed after parturition. These are some of the unique aspects of managing the transition cow. Thus, it is important to describe some of the nutritional and metabolic challenges surrounding the transition period along with different management techniques that may help reduce the incidence of stress and metabolic disorders commonly associated with this period.

1.4.1. Nutritional Status

The National Research Council recommendations (NRC, 1989) lists only one set of nutrient requirements for the dry cow. This is definitely an oversimplification. It is important to understand the overall picture that the dairy cow is faced with as it approaches parturition. The nutrient requirements for the

growing fetus increase approximately 23% during the last trimester of pregnancy (Moe and Tyrrell, 1972). To make matters worse, dairy cows tend to go off-feed during the final three-weeks of gestation, particularly during the final week prior to calving. Bertics et al. (1992) reported that dry matter intake (DMI) decreased approximately 30% during the final week before calving. Similar decreases in prepartum DMI have been reported in dry cows (Kunz et al., 1985; 1992; Emery, 1993; Vazquez-Anon et al., 1994). Data from 65 cows in the University of Alberta dairy herd demonstrated that DMI drops 30% between day nine and day one before calving (Figure 1.4; Doepel et al., 2000).

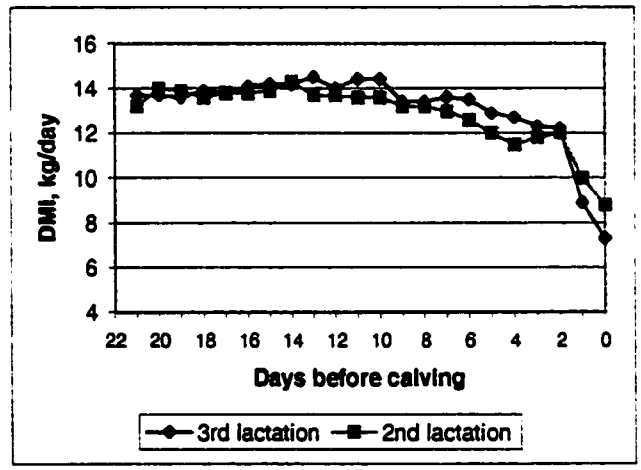

Figure 1.4. Dry matter intake of 2nd and 3rd lactation cows from the University of Alberta dairy herd.

In the weeks prior to calving nutrient demands are increasing at a time when feed intake is decreasing. Therefore, the dairy cow usually enters into a period of negative energy balance prior to calving. Bertics et al. (1992) calculated the energy and protein requirements for the transition cow based on the NRC

(1989) recommendation for intake. The magnitude of energy and protein deficiency was much greater during the early lactation period relative to the prepartum transition period (Bertics et al., 1992). However, some cows still enter into a period of negative energy balance well before parturition (Bertics et al., 1992). Van Saun et al. (1993) compared several models for determining net protein requirements for gestation and concluded that the crude protein recommendation of NRC (1989) may be too low. However, the majority of experiments in which dry cows have been fed extra protein, including rumen undegradable sources, have not yet yielded clear-cut positive responses in production or health during early lactation (Putnam and Varga, 1997). This is especially true when protein supplementation has been confined to the close up period (21 days before parturition). Crude protein requirements during the late stages of gestation are still under question, and it is clear that they are more poorly defined than energy requirements.

In general, cows that avoid severe DMI depression before calving have a favorable nutritional balance before and after calving and those that go almost completely off-feed experience extended periods of negative energy and protein balance during the transitional period (Grummer, 1995).

1.4.2. Metabolic Status

The late pregnancy period is one of metabolic transition. The processes associated with parturition do not occur abruptly but gradually over a period of several weeks. The transition cow is faced with exceedingly high turnover rates

of adipose tissue, liver, and skeletal muscle, especially during the first three weeks of lactation. Several hormonal changes are also involved in the initiation of parturition and maintenance of milk production.

As parturition approaches, plasma insulin decreases and growth hormone increases, with surges in concentrations of both hormones at parturition (Kunz et al., 1985). Progesterone concentrations during this period are elevated for maintenance of pregnancy but decline rapidly approximately 2 days before calving (Chew et al., 1979). Estrogen, primarily of placental origin, increases in plasma during late gestation with a rapid increase just before calving and then decreases at calving (Chew et al., 1979). Grummer et al. (1990) hypothesized that the peak of estrogen may promote fatty acid mobilization from adipose tissue during late pregnancy independently of any changes in feed intake and energy balance. Additional to the peak estrogen levels, low progesterone levels may further enhance esterification of fat in the liver. The drop in progesterone at calving may also have an influence on fatty liver development.

During late gestation the changes in the endocrine status and decrease in DMI influence metabolism and leads to the mobilization of fat from the adipose tissue and glycogen from the liver (Grummer, 1995). Lipolysis results in the release of nonesterified fatty acids (NEFA) into the bloodstream. Nonesterified fatty acids increase during late gestation and generally peak at or around the time of calving. The NEFA can be taken up by organs such as the mammary gland, skeletal muscle, or liver and be incorporated in to milk fat or used as an energy source. The liver is a major site for fatty acid metabolism. When the NEFA are

extracted by the liver they can be oxidized to completion, partially oxidized to ketone bodies or esterified to form triglycerides. Many researchers have found that ruminants do not efficiently export fatty acid as very low-density lipoproteins (Rukkwamsuk et al., 1999; Minor et al., 1998; Bertics et al., 1992; Kleppe et al., 1988; Pullen et al., 1988, 1989). Therefore, a significant amount are esterified and stored in the liver, leading to varied levels of fatty liver. Fatty liver appears to be a predisposing factor for the development of ketosis. Plasma ketone concentrations mirror hepatic triglyceride concentrations and may increase during the postpartum transition period until clinical ketosis is experienced (Grummer, 1995). Thus, liver triglyceride:glycogen ratio at parturition may be an indicator of a cow's susceptibility to ketosis (Veenhuizen et al., 1991).

Plasma glucose concentrations remain relatively constant during the prepartum period, increase dramatically at parturition and then decrease immediately postpartum (Kunz et al., 1985; Vazguez-Anon et al., 1994). The demand for glucose increases substantially after calving and is approximately 2.5 times greater at 21 days postpartum compared with that during the three weeks preceding calving (Overton et al., 1998). Propionate derived from ruminal fermentation is the principal substrate used to make glucose by the liver, followed under normal conditions by amino acids, lactate, and glycerol (Table 1.2; Seal and Reynolds, 1993). The capacity of the liver to make glucose from propionate appears to be substrate dependent, especially during the first 21 days of lactation (Overton et al., 1998). Cows also metabolize body protein and utilize amino acids from muscle to synthesize some of the additional glucose needed during early

lactation (Bell, 1995). Recent data indicated that liver capacity to synthesize glucose from amino acids increases substantially during the first 21 days of lactation (Overton et al., 1998). Additionally, the rate of skeletal muscle degradation seems to increase during the first 21 days of lactation (Overton et al., 1998). However, using amino acids to make glucose is not energetically efficient. This may partly explain why feeding diets containing more than the NRC (1989) requirements of 12% CP in the diet during the dry period and upwards of 18% in the diet of early lactation cows may prove to be effective in practice.

Table 1.2. Maximal contributions of substrates to glucose synthesis in liver. Adapted from Seal and Reynolds, (1993).

Substrate	Maximal Contribution (%)
Propionate	32 - 73
Amino Acids	10 - 30
Lactate	15
Glycerol	small amounts

1.4.3. Nutritional Strategies During the Transition Period: Prevention of Metabolic Disorders

The challenge for the dairy producer is to manage the dairy cow through the transitional phase and minimize the amount of stress and metabolic disorders associated with this period. Important goals should be to maximize energy intake, reduce fatty acid mobilization from adipose tissue, and ensure smooth dietary changes from the dry to lactating period. The following section outlines some key nutritional management strategies that should help reduce the amount of stress

and incidence of metabolic disorders commonly associated with the transition cow during early lactation.

Maximize Feed Intake

A priority in feeding the transition cow should be to maximize feed intake prepartum. Although the decrease in prepartum intake seems unavoidable during the final weeks of gestation, it is important to recognize and identify the factors that influence prepartum intake. Increasing feed intake during the weeks of gestation appears critical for the prevention of metabolic disorders, increased production parameters, and improved reproductive status. However, dry matter intake depends on a variety of nutritional, hormonal, and physiological conditions. Factors influencing DMI include (1) environment, (2) cow, and (3) diet. Table 1.3 summarizes these variables.

Table 1.3. Variables that Influence Dry Matter Intake of Dairy Cows.

Environment	Cow	Diet
Temperature	Milk Production	Physical Texture
Ventilation	Body Size	Palatability
Humidity	Hormonal Status	Fiber Content
Feeding Frequency	Breed	Nutrient Balance
Water	Body Condition	Moisture Content
Sprinkler, fans etc.	State of Health	Forage Quality

Dietary composition and nutrient content may influence prepartum DMI. Increasing the amount of concentrate in prepartum diets has been shown to increase DMI during days 7 to 28 prepartum (Emery, 1993; Coppock et al., 1972; Johnson and Otterby, 1981; Vandehaar et al., 1999). The possible benefits of

increasing the energy density of the diet will be discussed later. However, some studies show that cows on these high concentrate diets have greater decrease in DMI during the final week before parturition. Bertics et al. (1992) evaluated the effects of prepartum intake depression on the development of fatty liver. Eleven cows were fed ad libitum intakes prior to calving (control), and eleven cows were maintained at the same level of DMI as recorded during days 21 to 17 prepartum by force feeding refusals via rumen cannulas. Dry matter intake declined 28% over the final 17 days prepartum in the control cows. Liver triglycerides increased 227% and 75% for control and force-fed cows (Bertics et al., 1992). Cows that were force-fed prepartum produced milk with a higher fat content and tended to produce more 3.5% fat corrected milk over the first 28 day postpartum (Bertics et al., 1992).

Identification of factors that influence intake is important. Davenport and Rakes (1969) revealed no effect of prepartum body condition on peripartum DMI, whereas Garnsworthy and Jones (1987) reported that over-conditioned cows had poor appetites postpartum. Holter et al. (1990) confirmed that over-conditioned cows are more likely to have lower intakes in early lactation. Because postpartum feed intake is related to prepartum feed intake, over-conditioned cows may also consume less feed prepartum. Emery (1993) examined the feed intake of 20 cows during the dry period and reported that cows with the higher body condition score consumed 1.5% of their body weight on a dry matter basis compared to the lower body conditioned cows which consuming 2% of their body weight. The over-conditioned cows also had a higher incidence of health disorders postpartum. It is

generally accepted that cows entering the dry period should be maintained at a body condition score of 3.25 to 3.5, using a 5-point scale (1=thin, 5=obese).

Increasing dietary energy density

One way to offset reduced nutrient intake associated with feed intake depression during the prepartum period is to increase the nutrient density of the diet. Generally, increasing the nutrient density of the diet for the last three weeks prepartum increases energy intake because there are minimal declines in feed intake (Doepel et al., 2000). Vandehaar et al. (1999) fed cows one of four diets for the last 28 days of gestation: 1) 1.3 Mcal/kg NE_L and 12.2% CP (LL), 2) 1.49 Mcal/kg NE_L and 14.2% CP (MM), 3) 1.61 Mcal/kg NE_L and 15.9% CP (HH), 4) 1.48 Mcal/kg NE_L and 16.2% CP (MH) (NE_L= net energy for lactation). The cows fed the HH diet consumed 14% more dry matter and 40% more energy than cows fed the LL diet. In addition, the HH cows tended to have lower NEFA concentrations over the last 2 weeks before calving compared to the LL cows. Minor et al. (1998) reported that increasing non-fiber carbohydrates (NFC) beyond 40% in dairy cows from 19 days prepartum through 40 weeks postpartum increased DMI and energy balance, tended to increase plasma glucose, and decreased plasma NEFA and ketone bodies compared to cows consuming 23.5% NFC.

Bauman et al. (1971) reported that increasing NE_L intake by increasing the level of concentrate in the diet could substantially increase propionate production in the rumen (Table 1.4). Although, these diets are not representative of typical

diets fed to dairy cows during the transition period, the point is that supply of propionate and other gluconeogenic precursors can be increased effectively by dietary manipulation and feeding management. Holtenius et al. (1993) increased concentrate intake while forage intake remained the same during the final 4 weeks prepartum. Cows fed the increased concentrate levels showed a greater increase in serum insulin (100 to 300%) however, the prepartum concentrate feeding did not influence the serum glucagon concentration. The increased insulin levels reduced the extent of adipose catabolism, thus decreasing the amount of NEFA in the circulating system (Holtenius et al., 1993).

Table 1.4. Propionate Production in the Rumen of Lactating Cows. Adapted from Bauman et al. (1971).

Forage:Concentrate	DMI kg/d	DE Intake Mcal/d	Propionate Production g/d
55:45	16.2	49.7	985
15:85	14.3	48.5	2296

Feeding supplemental fat to dry cows could also be another method of increasing dietary energy intake. Grum et al. (1996) showed that adding supplement fat into the diet might actually decrease the accumulation of fat in the liver during the transition phase. However, the work was not conclusive because feeding the high fat diet decreased DMI and therefore confounded effects on energy balance of the close up cow. Grummer (1995) stated that cows seem to respond best to fat at approximately the time they reach positive energy balance. Further research is required to determine the optimal strategy for fat supplementation of dairy cows in the periparturient period.

Rumen Adaptation

Increasing the proportion of fermentable carbohydrates in the prepartum diet may benefit the transition cow beyond the provision of calories and increased DMI. Dirksen et al. (1985) indicated that increasing the intake of fermentable carbohydrates during the prepartum and early lactational period promoted the development of rumen papillae size. Dirksen et al. (1985) replaced a low energy diet with a high-energy diet 14 d prior to calving. They reported substantial papillae development with an increased capacity to absorb VFA in the cows with increased papillae size. The proliferation of rumen papillae were considered to be an adaptive process attained by increased levels of concentrate in the diet resulting in elevated levels of ruminal propionate and butyrate. The authors suggested the increase in papillae development was a function of stabilizing ruminal pH and extra requirement for energy during peak lactation. Dirksen et al. (1985) also reported that as much as 50% of the absorptive area during the first seven weeks of the dry period might be lost because of reduction in the length of the rumen papillae. The reduction in length and absorptive capacity of the rumen mucosa was attributed to a lower energy diet. Dirksen et al. (1985) suggested that it was necessary to feed a high energy diet for a period of seven weeks to maximize rumen papillae development. This leads to an important question of the length of time required during the transition period to maximize the development of the epithelia lining of the rumen.

The microbial population also needs to be acclimatized to changes in the diet. The *primary* lactate-producing bacteria can respond rapidly to high starch diets and in turn produce large amounts of lactate in the rumen. However, the *secondary* lactate-utilizing bacteria respond more slowly to changes in the diet. Huntington et al. (1981) and Goff and Horst, (1997) described that these secondary bacteria required approximately three to four weeks to reach sufficient levels to effectively prevent lactate from building up in the rumen. More importantly, the poorly developed rumen epithelium of the unadapted animal will be unable to absorb the VFA quickly enough to prevent a build-up of organic acids in the rumen. As a result, the rumen pH may fall to a point where the protozoa and many of the *secondary* bacteria in the rumen may die or become inactivated.

Slowly changing the close-up dry cow diet to mimic an early lactation diet may be beneficial for the development of the epithelium lining of the rumen (i.e. papillae) and microbial population. However, the traditional three-week preparation period may not be long enough based on the suggested time required to adapt the rumen papillae and microbial environment. The present research evaluated the duration and level of grain fed during the prepartum period required to influence papillae development.

Increasing Protein Density of the Diet

Increasing the protein content of the prepartum diet beyond current NRC (1989) recommendations may be beneficial. Two theories have been presented to

explain why increasing protein content of the transition cow diet may affect performance (Grummer, 1995). Firstly, protein requirements listed by NRC (1989) may be under estimated. As a result, underfeeding protein may cause maternal reserves to be depleted, leading to compromised lactation, health, and reproduction (Van Saun et al., 1993). Second, improving the amino acid status of the prepartum cow may beneficially influence endocrine profile and enhance lactation performance (Chew et al., 1984). Curtis et al. (1985) reported that feeding protein above NRC recommendations during the final three weeks prepartum decreased the risk of retained placenta and primary ketosis. Compared to NRC (1989), Van Saun et al. (1993) fed a higher level of crude protein (15.3%) during late gestation by increasing the amount of undegradable protein in the diet. The authors speculated that the additional undegradable protein was used to meet the fetal and maternal growth requirement during the prepartum period, thus reducing mobilization of the maternal protein pools. This resulted in improved body condition postpartum and increased milk protein percentage (Van Saun et al., 1993). Doepel et al. (2000) suggested that feeding high protein diets to dairy cows provided additional amino acids that maybe used by the fetus as an energy source, thus sparing glucose and increasing the maternal glucose concentration. Moorby et al. (1996) reported that increasing undegradatable protein supply to cows in late gestation improved milk production and milk protein yield. However, Putnam and Varga, (1998) found no differences in milk production or milk protein content when cows were fed prepartum diets containing 10.6, 12.7, or 14.5% crude protein. Bell (1995) and Overton et al. (1998) suggested that the

supply of glucogenic amino acids could be increased both in the prepartum and postpartum diet. However, research has yet to evaluate the implications of feeding high protein diets during the transition period on gluconeogenesis from amino acids.

1.5. Summary

The NRC (1989) does not adequately define the nutrient requirements of the non-lactating cow, particularly during the transition period. Due to the variety of hormonal, metabolic and dietary changes that may occur around the time of calving, an understanding of the nutritional and physiological conditions is necessary to provide the least stressful transition. The current research attempts to provide new information on the development of the rumen papillae during the dry period and during early lactation.

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CHAPTER 2: Influence of Prepartum Nutrition on the Periparturient Dairy Cow – Impact on Rumen Development.

2.1. Introduction

During the dry period as much as 50% of the absorptive area in the rumen may be lost because of a reduction in the length of the rumen papillae in dairy cows fed a low energy diet (Dirksen et al., 1985). Dirksen et al. (1985) indicated that increasing the intake of fermentable carbohydrates during the prepartum and early lactation period may promote the development of rumen papillae. Therefore, increasing the intake of fermentable carbohydrates during the dry period may benefit the dairy cow by increasing the surface area of rumen papillae, which in turn could enhance the animal's capacity to absorb volatile fatty acids (VFA). Moon and Campbell (1973) reported that the length of rumen papillae were larger during the postpartum period (50 d to 70 d after parturition) compared to those found in the prepartum period in sheep. Gaebel et al. (1987) observed substantial papillae development in sheep fed a high concentrate diet. Dirksen et al. (1985) suggested that dairy cows required approximately seven weeks on a high-energy diet to achieve maximum papillae surface area. These results have caused researchers recommend the inclusion of fermentable carbohydrate in the diet during the dry period to optimize papillae development.

Our objectives were to compare the duration and level of concentrate fed to Holstein dairy cows and to determine the effects of experimental diets on papillae

surface area, dry matter intake, body weight and condition score, ruminal fermentation characteristics, blood metabolites, and lactational performance.

2.2. Materials and Methods

2.2.1. Animals and Experimental Design

Twenty-four multiparious Holstein cows were housed at the University of Alberta Dairy Research and Technology Centre. Animals were blocked by expected calving date and randomly assigned within blocks to one of four dietary treatments before entering the dry period. Twelve of the twenty-four cows were fitted with a large rumen cannula (#1C- rumen cannula with rolled inner flange, 10 cm centre diameter, Bar Diamond Inc. Parma, ID) and twelve remained intact. The cows were cannulated during late lactation providing each treatment with three cannulated cows. All surgeries occurred at a minimum of four weeks prior to individual dry off dates. Cows were housed in tie stalls bedded with wood shavings. Animals were turned out for approximately 2 h for daily exercise and assessment of health. Cows were treated for any detectable illness and continued on the experiment. All procedures were approved by the University of Alberta Animal Care Committee.

2.2.2. Treatments

Treatment diets were fed as a total mixed ration (TMR) for *ad libitum* intake from the day of dry off. The nutrient composition of the prepartum diets is shown in Table 2.1. All diets were formulated to meet or exceed NRC (1989)

requirements. Treatments were 1) high concentrate diet (**High**) for the entire dry period (61 d), 2) low concentrate diet (**Low**) for the entire dry period (61 d), 3) low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**), and 4) low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**). The moisture content of barley silage, rolled barley, and grass hay were determined once a week and used to maintain appropriate proportions of diet ingredients on a dry matter (DM) basis. The **LM** and **LH** treatments were to simulate extended (greater than 21 d prepartum) transitional cow diets. Feed intake was recorded daily and adjusted to maintain 5 to 10% orts. Refusal amounts were recorded prior to each morning feeding. After calving, all cows were fed the same early lactation TMR for *ad libitum* intake. Water was available for consumption at all times.

Table 2.1. Ingredients and nutrient composition of diets.

	Diets			
	Low	Medium	High	Lactation
Ingredients	% DM Basis			
Barley Silage	67.8	63.4	51.4	20.0
Alfalfa Silage	...	...	...	15.0
Grass Hay	31.7	15.8	7.8	...
Alfalfa Hay	...	...	...	10.0
Rolled Barley	...	19.7	39.3	...
Dairy Supplement[1]	...	...	...	55.0
Calcium Carbonate	...	0.6	1.0	...
Dry Cow Mineral-Vitamin Mix[2]	0.5	0.5	0.5	...
Nutrient Composition	mean ± SD	mean ± SD	mean ± SD	mean ± SD
DM, %	48.8 ± 4.8	50.7 ± 5.8	53.5 ± 4.8	57.9 ± 3.9
CP, %	11.8 ± 1.0	12.5 ± 0.6	12.9 ± 0.6	18.7 ± 1.5
NDF, %	58.9 ± 1.5	56.3 ± 2.5	53.4 ± 1.3	45.0 ± 1.6
ADF, %	35.5 ± 1.9	33.6 ± 1.3	32.1 ± 2.4	27.0 ± 1.4
NFC[3], %	18.7 ± 1.5	21.7 ± 2.6	25.3 ± 0.8	30.2 ± 0.7
NE_L,[4] Mcal/kg	1.39	1.50	1.60	1.69

[1] Included 49.2% rolled barley, 14% corn, 12% canola meal, 4.4% corn gluten, 4.0% soybean meal, 3.6% tallow, 2.4% distillers grain, 1.2% molasses, 0.9% Megalac (Church and Dwight Co., Inc.), 0.89% sodium bicarbonate, 0.72% phosphate, 0.62% limestone, 0.54% magnesium oxide, 0.38% fishmeal, 36% iodine salt, 0.32% fortified salt, 1 000 IU/kg vitamin E, 7 000 IU/kg vitamin D, 5 000 IU/kg vitamin A.

[2] Included 15% phosphorus, 1.75% magnesium, 0.03% potassium, 0.04% sodium, 0.79% sulphur, 12 000 mg/kg iron, 14 500 mg/kg zinc, 12 700 mg/kg manganese, 5 000 mg/kg copper, 100 mg/kg cobalt, 318 mg/kg iodine, 120 mg/kg selenium, 120 000 mg/kg niacin, 2 800 000 IU/kg vitamin A, 600 000 IU/kg vitamin D, 20 000 IU/kg vitamin E.

[3] NFC was calculated based on NRC (2001) equation: NDF = 100 - (NDF% + CP% + fat% + ash%). Fat % and ash % were estimated based on NRC (2001) values.

[4] Estimates of NE_L content were based on NRC (1989) values for rolled barley. Forages were analyized by Central Testing Laboratory Ltd. (Nisku, Alberta, Canada) for NE_L value (Barley silage= 1.44 Mcal/kg, CP (%)=12.98, NDF (%)=57.70, Alfalfa silage=1.35 Mcal/kg, CP (%)=18.57, NDF (%)=43.48, Grass hay=1.30 Mcal/kg, CP (%)=11.95, NDF (%)=58.71, and Alfalfa hay=1.32 Mcal/kg, CP (%)=17.41, NDF (%)=44.16).

2.2.3. Feed Sampling and Analysis

Feed ingredients, TMR, and orts were collected weekly during the experiment. Orts were collected prior to the morning feeding and weighed within 3 h after collection. The samples were immediately dried at 60°C for 72 h and stored until they were compositied by period for each cow. Cows in the **High** and **Low** treatments had weekly samples pooled for the entire dry period (61 d). Cows on the **LH** or **LM** treatments had weekly samples pooled into two periods

depending on which diet they were consuming during the dry period. The low concentrate diet was considered period 1 and when they were consuming the higher concentrate diet (the medium or high concentrate diet) was designated as period 2. Weekly samples taken during the lactation period were also pooled and averaged for each cow. Pooled samples were ground to pass a 1-mm screen (Thomas-Wiley Laboratory Mill, Model 4, Arthur H. Thomas Co., Philadelphia, PA, USA) prior to analysis. All of the samples were analyzed in duplicate. Composited samples were analyzed for DM, crude protein (Leco FP-428 Nitrogen Determinator, Leco® Corporation, St Joseph MI, USA), neutral detergent fibre (NDF) determined by amylase and sodium sulfate, and acid detergent fibre (ADF) (Van Soest et al., 1991). The analytical DM was determined by drying at 110°C to a constant weight and the true DM was determined using the following calculation:

True DM = (60°C DM sample (%) x 110°C DM sample (%))/100.

2.2.4. Body Weight and Body Condition Score

Cows were weighed on two consecutive days each week at ~1300 h for the entire 15 week experimental period. Body condition score (BCS) was assessed weekly, to the nearest 0.25 unit, using a 5 point scale (1 = thin, 5 = obese) by two experienced individuals (Wildman et al., 1982). Body weight (BW) and BCS for each animal were averaged by week relative to parturition. Similar to Vandehaar et al. (1999), body weight was used to calculate the NE_L

requirement prepartum according to NRC equations (NE_L required = $0.104*BW^{0.75}$, where NE_L is megacalories per day) and energy balance was calculated as the difference between daily intake and requirement of NE_L.

2.2.5. Rumen Papillae Measurements

Rumen papillae samples were collected from the twelve ruminally cannulated cows. Six biopsies per animal were taken over the course of the experiment (dry off day, -32 d, and -14 d, within 24 h after calving, 14 d, and 35 d). On the day of sampling, biopsies were taken from three different locations in the rumen: below the rumen fistula (approximately 20 cm below the cannula), bottom of the ventral sac, and approximately 10 cm over the dorsal coronary pillar in the caudodorsal blind sac. The rumen was completely evacuated immediately prior to biopsy sampling. Rumen contents were placed in a preheated insulated container, flushed with carbon dioxide gas, and sealed in order to minimize any environmental changes. The rumen wall was gently pulled through the rumen fistula and curved surgical scissors were used to remove the samples at the base of the basement membrane (approximately 1 to 2 cm^2 in size). Samples were washed in distilled water and placed in individual containers containing 10% buffered formalin phosphate. At the time of analysis, ruminal tissue samples were removed from the buffered formalin and manually trimmed. A minimum of 20 intact, well-orientated papillae were measured from each site and an average was determined for each location. Also, an overall average was taken from the three different locations. The rumen papillae were digitally photographed and analyzed

for surface area using an image analysis software program (Northern Exposure 1996, Expix Imaging, Inc., Missisauga, Ontario, Canada).

2.2.6. Rumen Fermentation

Rumen fluid samples were collected from the twelve cannulated cows. Samples were collected the week after dry off and every two weeks until 42 d after parturition. Rumen fluid was sampled using a stainless steel strainer attached to a plastic tube, which was inserted into the rumen. Rumen liquid was extracted by applying a vacuum to the end of the tube with a syringe. Approximately 250 ml of fluid was collected at 1, 3, 6, 12, and 24 h after feeding. After collection, rumen pH was recorded immediately using a calibrated pH meter. Approximately 4 ml of fluid was allocated to three 10-mm polypropylene tubes and immediately frozen. Analysis for VFA in rumen fluid was according to Khorasani et al. (1996) with modifications outlined in Appendix (2.2.1). Lactic acid analysis was according to the procedures outlined by Khorasani et al. (1996) with modifications outlined in Appendix (2.2.2). Ammonia nitrogen analysis was according to Fawcett and Scott (1961) and involved preparing the following reagents: sodium phenate, sodium nitropurside, sodium hypochlorite, and a standard solution (100 μl NH_3-N/ml). Appendix 2.2.3 provides details of the procedure for the analysis of ammonia nitrogen.

2.2.7. Blood Metabolites

Blood was sampled from the coccygeal vein or artery 2 h prior to feeding. Samples were taken every second week, within 24 h of calving, and on day -7 before expected calving and day 7 after parturition. Blood samples were collected into evacuated test tubes (Vacutainer®, Becton Dickson Vacutainer Systems USA, Rutherford, NJ.) containing dried sodium fluoride for plasma glucose, sodium heparin for heparinized β-hydroxybutyrate (BHBA) plasma, and no anticoagulant for serum non-esterified fatty acids (NEFA). Blood was centrifuged and stored at –20°C until analysis. The concentrations of serum NEFA (NEFA-C Kit; Wako Pure Chemicals, Inc., Osaka, Japan) adapted for use in microtiter plates (Johnson and Peters, 1993) and plasma BHBA (Gibbard and Watkins, 1968) were determined enzymatically. Plasma glucose concentration was determined by the Beckman Glucose Analyzer 2 (Beckman Instruments Inc., Fullerton, CA., USA) by means of the oxygen rate method.

2.2.8. Milk Production and Composition

After calving, cows were milked twice daily in a single side herringbone parlor. Milk yield was electronically recorded daily for the duration of the study. Milk samples were collected four times a week, preserved with potassium dichromate, and stored at 4°C until analysis. Samples were analyzed for fat, protein, and lactose using near infrared spectroscopy by the Alberta Agriculture, Food and Rural Development Milk Testing Laboratory (Edmonton, Alberta, Canada). Calculations for NE_L in milk were estimated by NRC (1989) equations.

Body weight, DMI, milk fat and yield were used to predict energy balance during the postpartum period.

2.2.9. Statistical Analysis

Data were analyzed as repeated measures using the PROC MIXED procedure of SAS® (1998). One of the cannulated cows on the **High** treatment died shortly after parturition for reasons unrelated to the treatment (due to a difficult calving). Therefore, only prepartum data was available for this cow. The statistical model used for analysis included the main plot factor of treatment. The subplot factors included time (week relative to calving) and the interaction of treatment and time. In order to provide a more representative value for the individual diets fed during the experiment based on ruminal fermentation patterns, the effect of diet was analyzed as a main factor rather than the treatment. Therefore, any cows consuming the low concentrate diet regardless of treatment was included in the analysis for the low concentrate diet. For each analyzed variable, cow nested within the interaction of treatment and block was subjected to three covariance structures; spacial power law, compound symmetric, and autoregressive order one. However, with repeated data that contained unequal spacing the autoregressive order one covariance structure was not used because it assumes equal spacing (SAS®, 1996). The covariance structure that produced the value closest to zero in Akaike's information criterion was considered to be the most desirable analysis. Least square means were separated using a Tukey test when the main effect of treatment was significant ($P < 0.05$). All data were

covariately adjusted if the covariate was significant. Significance was declared at $P < 0.05$, and trends were declared at $P < 0.10$.

2.3. Results

2.3.1. Dry Matter Intake

Feeding diets higher in concentrate did not influence prepartum feed intake however the treatment by week interaction was significant during the prepartum period (Table 2.2). Therefore, cows consuming the different treatments did not respond similarly during the course of the prepartum period. The **LH** treatment cows had higher intakes during wk -2 and -1 compared to the **Low** treatment cows (Figure 2.1). Similar results were observed when intake was adjusted for body weight. As expected, cows on all treatments exhibited a decrease in feed intake during the final weeks before parturition, with the most dramatic decline occurring on the day of calving. From 3 wk prepartum to the day of calving, DMI declined 40%, 35%, 30%, and 35% for the **High**, **Low**, **LM**, and **LH** cows, respectively. However, at calving cows on the **LH** treatment consumed over 2 kg/d more dry matter than the cows on the **High**, **Low**, and **LM** treatments, although this difference was not significant.

Postpartum DMI was measured over the first 6 wk of lactation and also the average DMI during the last 3 wk of lactation were reported in order to provide a more representative value for DMI. Prepartum treatments had no effect on DMI during the postpartum period. As expected, DMI increased by the sixth week of lactation for all animals.

Table 2.2. Dry matter intake, net energy intake and balance, body weight, and body condition score during the prepartum and postpartum period.

	Treatments[1]					Effect (P)[2]		
	High	Low	LM	LH	SE	T_i	W_j	TW_{ij}
Prepartum[3]								
DMI (kg/d)	11.95	10.86	11.81	13.75	0.54	0.01	< 0.01	0.05
NE_L Intake (Mcal/d)	19.13	15.09	17.07	20.90	0.83	< 0.01	< 0.01	< 0.01
NE_L Balance (Mcal/d)	4.18	0.24	2.44	6.42	0.92	< 0.01	< 0.01	0.01
BW (kg)	735	734	737	748	9	0.71	< 0.01	0.03
BCS	3.56	3.60	3.52	3.60	0.08	0.91	< 0.01	0.03
Postpartum[4]								
DMI[5] (kg/d)	14.11	13.40	15.37	14.65	1.12	0.63	< 0.01	0.10
DMI[6] (kg/d)	16.09	15.42	16.66	16.73	1.25	0.86	< 0.01	0.36
NE_L Intake (Mcal/d)	23.85	22.66	24.76	25.97	1.89	0.64	< 0.01	0.10
NE_L Balance (Mcal/d)	-14.56	-14.66	-12.10	-10.38	1.94	0.36	< 0.01	0.30
BW (kg)	662	635	647	652	23	0.87	< 0.01	0.53
BCS	3.07	2.61	2.50	2.68	0.22	0.32	< 0.01	0.90

[1] High = high concentrate diet for entire dry period (61 d), Low = low concentrate diet for entire dry period (61 d), LM = low changed to medium concentrated diet 32 d prepartum, and LH = low changed to high concentrated diet 32 d prepartum.

[2] T_i = treatment effect, W_j = week effect, and TW_{ij} = treatment by week interaction effect.

[3] Prepartum = entire dry period, including the day of calving.

[4] Postpartum = first 6 wk of lactation. All cows consumed the same lactation diet.

[5] DMI = dry matter intake for the first 6 wk of lactation.

[6] DMI = dry matter intake for the last 3 wk of lactation.

2.3.2. Net Energy Intake and Balance

Prepartum energy intake increased with the energy density of the diet (Figure 2.2). Similar to DMI, a significant treatment by week interaction was observed for energy intake and energy balance (Table 2.2). Therefore, treatment effects were dependent upon the specific week measured. The LH treatment cows had significantly greater energy intakes during wk -4, -3, -2, and -1 compared to the Low treatment cows. The LH treatment cows also had significantly higher energy intakes at wk -1 compared to those cows on the LM treatment. Corresponding to energy intake, the LH treatment cows had a greater

energy balance than the **Low** treatment cows during wk -4, -3, -2, and -1 (Figure 2.3).

Increasing the amount of concentrate fed prepartum did not influence postpartum energy intake or energy balance. As expected, all cows were in a state of negative balance during early lactation. However, as DMI increased the magnitude of body weight loss declined so that the extent of mobilization of BW was significantly less at six weeks postpartum compared to the day of calving.

2.3.3. Body Weight and Body Condition Score

Prepartum treatments had no affect on average BW or BCS during the dry and early lactation period (Table 2.2). However, there was a significant treatment by week interaction effect during the prepartum period for BW and BCS. The **High**, **Low**, **LM**, and **LH** treatment cows increased body weight by 8%, 5%, 8%, and 13% respectively, as they approached calving (Figure 2.4).

Postpartum body weights declined across all treatments. However, the cows on the **Low** treatment showed the greatest loss in body weight during the final four weeks of the experimental period compared to those cows on the **LM**, **LH**, and **High** treatments.

2.3.4. Rumen Papillae Measurements

Increased levels of concentrate did not influence the average surface area of rumen papillae during the dry period (Table 2.3). Although cows on the **High** and **LH** treatments had numerically higher surface areas for all rumen sites measured

(blind sac, rumen cannula wall, ventral sac, and overall average) no significant treatment differences were found. However, by the time of parturition, a significant weekly decline in papillae surface area was observed in the rumen cannula wall papillae (Figure 2.5) and overall average papillae surface area (Figure 2.6).

Prepartum treatments did not influence the surface area of rumen papillae postpartum. However, the overall average papillae surface area tended ($P = 0.06$) to increase during the five weeks of lactation regardless of prepartum treatment. The average surface area of papillae found in the postpartum period (for all sites) was clearly greater than those found in the prepartum period.

Table 2.3. Rumen papillae measurements during the prepartum and postpartum period.

	Treatments[1]					Effect (P)[2]		
	High	Low	LM	LH	SE	T_i	W_j	TW_{ij}
Prepartum[3] (mm^2)								
Blind Sac	27.61	18.04	22.61	26.61	4.42	0.46	0.37	0.26
Rumen Cannula Wall	34.45	25.15	29.81	28.56	3.47	0.44	< 0.01	0.96
Ventral Sac	36.09	26.08	32.22	33.12	4.48	0.56	0.68	0.97
Overall Average	32.08	23.74	27.80	29.76	3.26	0.38	0.01	0.61
Postpartum[4] (mm^2)								
Blind Sac	38.71	31.41	36.45	32.57	4.83	0.74	0.17	0.46
Rumen Cannula Wall	44.46	31.96	45.82	31.70	4.65	0.19	0.82	0.75
Ventral Sac	44.71	34.22	40.21	33.73	9.13	0.83	0.12	0.44
Overall Average	42.74	32.93	40.17	32.17	5.19	0.45	0.06	0.21

[1] High = high concentrate diet for entire dry period (61 d), Low = low concentrate diet for entire dry period (61 d), LM = low changed to medium concentrated diet 32 d prepartum, and LH = low changed to high concentrated diet 32 d prepartum.

[2] T_i = treatment effect, W_j = week effect, and TW_{ij} = treatment by week interaction effect.

[3] Prepartum = entire dry period, including the day of calving.

[4] Postpartum = first 6 wk of lactation. All cows were fed the same lactation diet.

2.3.5. Rumen Fermentation

Rumen fermentation characteristics are presented in Table 2.4. In order to provide a more representative value for the individual diets fed during the

experiment, the effect of diet was analyzed as a main factor rather than the treatment. Therefore, any cows consuming the low concentrate diet regardless of treatment was included in the analysis for the low concentrate diet.

The prepartum diets did not affect the average rumen pH (Figure 2.7). During the dry period, cows fed the medium and high concentrate diets had a lower ruminal pH for the first 6 h after feeding. However, by approximately 8 h after feeding the cows fed the high concentrate diet had greater rumen pH than those cows consuming the low concentrate diet. All cows fed the lactation diet showed a lower ruminal pH compared to when the cows were consuming the prepartum diets. At 3, 6, and 12 h after feeding, cows consuming the lactation diet had significantly lower rumen pH compared to when the cows were consuming the low or high concentrate diets.

Average rumen ammonia N concentration were not significantly influenced by prepartum diets (Figure 2.8). A significant diet by hour interaction was observed for ruminal NH_3 N concentrations. Increased levels of NH_3 N occurred at 3 h after feeding when the cows were fed the lactation diet compared to when the cows were fed the low or high concentrate diets.

The average rumen concentration of lactate was not affected by dietary treatment however, an interaction between diet and hour after feeding was observed. After the first hour of feeding cows consuming the lactation diet had significantly higher ruminal lactate concentration compared to when cows were fed the medium concentrate diet.

There was no diet effect on acetate or propionate concentrations (mol/100mol) in cows fed the prepartum diets. However, the ruminal concentration of butyrate, isobutyrate, and isovalerate were significantly increased in cows fed the high concentrate diet compared to when the cows consuming the low concentrate diet. Cows consuming the medium concentrate diet also had significantly higher ruminal concentrate of butyrate than those cows consuming the low concentrate diet. Total VFA concentrations were increased when cows were fed the lactation diet compared to when the cows were consuming the prepartum diets however it was only significantly different from the low concentrate diet (Figure 2.9). Cows consuming the lactation diet had a significantly lower concentration of acetate and significantly higher concentration of propionate compared to when the cows consumed the prepartum diets.

Table 2.4. Effect of diets on rumen fermentation.

	Diet					Effect (P)[1]		
	Low	Medium	High	Lactation	SE	D_i	H_j	DH_{ij}
pH	6.70	6.47	6.67	6.27	0.05	<0.01	<0.01	<0.01
NH_3N, mg/dl	8.67	12.07	8.66	13.75	1.03	<0.01	<0.01	0.02
Lactate, mM	9.12	8.18	13.11	19.59	2.52	<0.01	<0.01	0.03
VFA concentration, mol/100mol								
Acetate (A)	64.98^a	64.47^a	63.75^a	59.33^b	0.83	<0.01	<0.01	0.09
Propionate (P)	20.04^b	20.89^b	19.72^b	23.78^a	0.78	<0.01	<0.01	0.21
Butyrate	10.02^b	11.41^a	11.51^a	11.80^a	0.30	<0.01	<0.01	0.20
Isobutyrate	0.90^b	0.85^b	1.04^a	0.95^{ab}	0.04	0.03	<0.01	0.13
Valerate	1.45^b	1.48^{ab}	1.68^{ab}	2.03^a	0.14	<0.01	<0.01	0.08
Isovalerate	1.27^c	1.28^{bc}	1.68^a	1.60^{abc}	0.08	<0.01	<0.01	0.13
Total VFA, mM	89.21^b	98.80^{ab}	95.79^{ab}	105.15^a	3.43	<0.01	<0.01	0.07

[1] D_i = diet effect, H_j = hour effect, and DH_{ij} = diet by hour interaction effect.

^{a,b,c} Means in a row are significantly different ($P < 0.05$).

2.3.6. Blood Metabolites

The concentrations of glucose in plasma 2 h before feeding tended to be greater in the **High** treatment cows during the prepartum period (Table 2.5).

However, the prepartum treatment by week interaction was significant and showed that cows on the **High** treatment had significantly higher levels of plasma glucose the day of calving compared to the **Low** and **LM** treatment cows (Figure 2.10).

There were no significant treatment differences in plasma glucose concentration during the first six weeks of lactation. As expected, plasma glucose concentrations slowly increased in all cows by week six of lactation.

Serum NEFA concentration was significantly lower in the **High** and **LH** treatment cows during the dry period compared to the **Low** and **LM** treatment cows (Table 2.5). As expected, cows in all dietary treatments had higher serum NEFA concentration at parturition compared to the beginning of the dry period (Figure 2.11).

Average postpartum serum NEFA concentrations were similar among cows on the prepartum treatments. Serum concentration of NEFA significantly declined during the first six weeks of lactation in all cows.

Measurement of plasma ketone body concentration provided mixed results. There were no significant effects due to the dry period treatments. The treatment by week interaction revealed that on the day of calving significantly greater amounts of plasma BHBA were found in the **LM** treatment cows versus the **LH** treatment cows (Figure 2.12).

In early lactation, the plasma concentration of BHBA varied considerably among cows, represented by the large standard error (SE = 3.81). Due to the large

animal variation, no significant differences were found in plasma BHBA during early lactation.

Table 2.5. Blood metabolites concentration during the prepartum and postpartum period.

	Treatments[1]					Effect (P)[2]		
	High	Low	LM	LH	SE	T_i	W_j	TW_{ij}
Prepartum[3]								
Glucose (mg/dl)	73.05	66.35	67.39	66.77	1.79	0.11	< 0.01	0.04
NEFA (umol/l)	220.37[a]	320.52[b]	273.19[b]	199.49[a]	21.27	0.01	< 0.01	0.13
BHBA (mg/dl)	7.80	6.72	7.61	5.83	0.54	0.08	< 0.01	0.01
Postpartum[4]								
Glucose (mg/dl)	55.22	58.63	60.28	56.17	1.87	0.27	< 0.01	0.09
NEFA (umol/l)	501.56	518.16	418.22	553.60	47.00	0.24	< 0.01	0.83
BHBA (mg/dl)	16.70	14.09	8.57	11.08	3.81	0.50	0.19	0.69

[1] High = high concentrate diet for entire dry period (61 d), Low = low concentrate diet for entire dry period (61 d), LM = low changed to medium concentrated diet 32 d prepartum, and LH = low changed to high concentrated diet 32 d prepartum.

[2] T_i = treatment effect, W_j = week effect, and TW_{ij} = treatment by week interaction effect.

[3] Prepartum = entire dry period, including the day of calving.

[4] Postpartum = first 6 wk of lactation. All cows were fed the same lactation diet.

[a,b] Means in a row are significantly different ($P < 0.05$).

2.3.7. Milk Parameters and Postpartum Health Disorders

Prepartum treatments had no significant influence on milk yield or milk composition. Milk yield and lactose concentration increased with time whereas protein and fat concentration significantly decreased by the sixth week of lactation (Table 2.6). The **High** and **LH** treatment cows had numerically higher milk yields (37.32 kg/d and 39.51 kg/d). However, there was no significant difference in daily milk yield due to treatment (Figure 2.13).

No differences in the frequency of postpartum health disorders were observed among treatments (Table 2.7).

Table 2.6. Milk parameters during the postpartum period.

	Treatments[1]					Effect (P)[2]		
	High	Low	LM	LH	SE	T_i	W_j	TW_{ij}
Yield, kg/d								
Milk	37.32	36.84	34.49	39.51	2.40	0.52	< 0.01	0.97
4% FCM	38.04	36.77	33.77	38.38	1.90	0.33	0.23	0.36
Fat	1.54	1.47	1.33	1.51	0.08	0.32	0.62	0.26
Protein	1.18	1.11	1.10	1.22	0.07	0.64	< 0.01	0.99
Lactose	1.62	1.62	1.51	1.73	0.10	0.47	< 0.01	0.97
Milk Compostion, %								
Fat	4.32	4.12	4.05	4.02	0.21	0.79	< 0.01	0.10
Protein	3.27	3.04	3.21	3.15	0.12	0.61	< 0.01	0.62
Lactose	4.24	4.36	4.33	4.37	0.07	0.72	< 0.01	0.04

[1] High = high concentrate diet for entire dry period (61 d), Low = low concentrate diet for entire dry period (61 d), LM = low changed to medium concentrated diet 32 d prepartum, and LH = low changed to high concentrated diet 32 d prepartum. All cows were fed the same lactation diet.

[2] T_i = treatment effect, W_j = week effect, and TW_{ij} = treatment by week interaction effect.

Table 2.7. Frequency of postpartum health disorders with different treatments.

	Treatments[1]			
	High	Low	LM	LH
Number of animals	5	6	6	6
Twins	0	0	1	0
C-sections	1	0	1	0
Displace Abomasum	0	1	1	0
Milk Fever	1	0	0	2
Ketosis	0	1	1	0
Retained Placenta	0	0	1	0

[1] High = high concentrate diet for entire dry period (61 d), Low = low concentrate diet for entire dry period (61 d), LM = low changed to medium concentrated diet 32 d prepartum, and LH = low changed to high concentrated diet 32 d prepartum.

2.4. Discussion

This experiment was developed to determine whether a dry cow nutritional regime influences the development of rumen papillae. An increased surface area of rumen papillae should increase the capability of the rumen to absorb the end products of fermentation. This in turn could allow the dairy cow to better cope with the transition to the high grain diets fed in early lactation. We hypothesized

that the duration and amount of concentrate fed in the dry period would influence the extent of rumen papillae development.

2.4.1. Diets

Dirksen et al. (1985) suggested rumen papillae required approximately seven weeks on a high-energy diet to reach maximum size. In view of this critical fact, the **LM** and **LH** treatments were formulated to evaluate the duration and level of concentrate required to promote maximal rumen papillae surface area. To increase the concentrate levels in the diets, rolled barley was added at the expense of forage. The National Research Council (NRC, 1989) recommends that dry cows should normally be fed diets containing 1.25 Mcal of NE_L/kg and 12% CP. The **Low** treatment used in the present experiment is an example of such a diet. The **LM** and **LH** treatments were designed to simulate extended (greater than 21 d prepartum) transition cow diets. The **High** treatment in the present study was used as an extended energy dense diet that exceeds NRC (1989) recommendations. The **Low** and **High** treatments were fed to maximize the likelihood of detecting treatment differences. Thus, rather than provide diets that meet NRC (1989) recommendations, prepartum treatments were composed to provide varied levels of concentrate for different lengths of time.

2.4.2. Dry Matter Intake

The potential advantage of feeding a high concentrate diet to dairy cows in late gestation would be to counter balance the depression in feed intake that

normally occurs as parturition approaches. However, there were no differences among treatments for average DMI during the prepartum period, although the **LH** treatment cows had numerically higher intakes than those cows on the **High, LM,** and **Low** treatments. The effects of prepartum DMI due to increased levels of available carbohydrate in the diet vary considerably. Some studies have shown an increase in prepartum intake in response to increased concentrate feeding (Johnson and Otterby, 1981, Flipot et al., 1988, Minor et al., 1998), while other studies have shown minimal changes in DMI in cows fed higher levels of concentrate during the dry and lactating period (Lykos et al., 1997, Dann et al., 1999). Aldrich et al. (1993) reported a decrease in DMI in lactating cows fed a higher level of rumen-available non-structural carbohydrate. During the final weeks of gestation DMI declined which is consistent with previous work in dairy cows (Bertics et al., 1992, Grum et al., 1996, Minor et al., 1998, Vandehaar et al., 1999). Coppock et al. (1972) reported that prepartum DMI depression increased as concentrates in the diet increased. In the present study, feed intake depression did not significantly differ among cows consuming the different treatments.

Average postpartum DMI was not affected by prepartum treatments. Similar postpartum DMI were reported in primiparous cows fed a standard prepartum diet (59.7% TDN) versus cows fed a high-energy prepartum diet (69.3% TDN) (Grummer et al., 1995). Grum et al. (1996) also showed similar postpartum intakes in cows fed a high grain prepartum diet for seven days before expected calving date compared to cows fed a low energy diet for the entire dry period. In contrast to our results, Rukkwamsuk et al. (1999b) reported a

significant reduction in postpartum DMI in cows fed a high energy diet during the prepartum period (19.8 kg/d vs. 23.1 kg/d control diet). However, cows in that study were fed energy rich diets during the dry period to induce postpartum fatty livers. In the present study, postpartum DMI increased during the first six weeks of lactation which is consistent with pervious work in dairy cattle (Bertics et al., 1992, Grum et al., 1996, Minor et al., 1998, Vandehaar et al., 1999).

2.4.3 Net Energy Intake and Balance

Feeding prepartum energy dense diets for prolonged periods of time resulted in greater energy intakes and reduced incidence of negative energy balance during the prepartum period. Our results are in agreement with other studies (Coppock et al., 1972, Johnson and Otterby, 1981, Minor et al., 1998, and Vandehaar et al., 1999), which showed that increasing the energy density of diets during late gestation results in increased energy intake. Vandehaar et al. (1999) suggested that NE_L during the close up dry period could be increased up to 1.61 Mcal/kg and NDF decreased to 30% with no decreases in feed intake. The NE_L of the high concentrate diet was similar to those suggested levels, however a 53% NDF was achieved in the present study. The increased NDF levels in the high concentrate prepartum diet could be related to the higher NDF content found in barley (19 to 25%) compared to that of corn (7%) which was fed in the Vandehaar et al. (1999) experiment. Increasing the energy density approximately 32 d before parturition improved the energy balance in the **LH** treatment cows at wk -4, -3, -2 and less so in the **LM** treatment cows at wk -3 and -2. Approximately two weeks before

calving, feed intake declined in all cows regardless of treatment, thus reducing energy intake. The reduced energy intake caused a lower energy balance in all cows however, the magnitude varied according to the level of concentrate fed and the amount of feed consumed prior to parturition. The **Low** treatment cows were in a state of negative energy balance approximately -17 d before calving. The **LM** treatment group entered into a state of negative energy balance approximately -1 wk before calving while the **High** treatment cows entered into a negative energy balance a few days prior to calving. The **LH** treatment cows maintained a positive energy balance throughout the entire prepartum period due to their increased feed intake and the higher level of concentrate fed. Minor et al. (1998) reported that cows and heifers fed a standard NFC diet (23.5%, NE_L=1.34 Mcal/kg) were in negative energy balance 6 d before calving, whereas, cows and heifers fed a higher level of NFC (43.8%, NE_L=1.63 Mcal/kg) for 19 d prepartum remained in a positive energy balance until the day of calving.

2.4.4. Body Weight and Body Condition Score

In the final weeks before calving, increased body weights among cows fed the different treatments was probably the result of increased adipose tissue deposition. However, there was no indication of a higher BCS with increasing BW. Wright and Russell (1984) estimated that tissue composition associated with body condition accumulation was 73% fat, 6% protein, 1% ash, and 19% water. In the present study, we believe that the increase in prepartum BW was a combination of fetal growth and anabolism of adipose tissue. Rukkwamsuk et al.

(1999a) reported similar prepartum BW gains in cows fed a prepartum energy dense diet. The **Low** and **LM** treatment cows grained less BW during the final weeks of gestation compared to the cows on the **LH** and **High** treatments. This may be attributed to the earlier induction into a state of negative energy balance for the **Low** and **LM** group cows.

Based on BW loss during the first six weeks of lactation, the **Low** treatment cows tended to show a more substantial loss in BW compared to the cows on the **LM**, **LH** or **High** treatments. Although our results were not significant, these postpartum findings suggest that cows fed increased levels of concentrate during the prepartum period replenished body stores to a greater extent than the **Low** treatment cows. Our postpartum results are in contrast to those of Rukkwamsuk et al. (1999a, 1999b) who reported significant postpartum BW losses in cows fed high-energy diets during the prepartum period. However, intense lipolysis was expected because those cows were fed high-energy diets to induce postpartum fatty livers.

2.4.5. Rumen Papillae Measurements

The proliferation of the ruminal papillae is considered to be an adaptive process to increase the absorptive capacity of the rumen. It has long been assumed that increasing prepartum intake of fermentable carbohydrates will promote the development of rumen papillae in dairy cows. Dirksen et al. (1985) fed an energy poor diet and replaced it with an energy rich diet 14 d prior to calving. They reported substantial papillae proliferation and suggested rumen

papillae required seven weeks on an energy rich diet to reach maximum development (Dirksen et al., 1985). In contrast to Dirksen et al. (1985) our data show no significant change in papillae surface area with increasing levels of concentrate fed during the dry period. There was no evidence of an increase in papillae surface area in any regions in the rumen (blind sac, rumen cannula wall, ventral sac, and overall average) during the dry period regardless of treatment. In fact, our prepartum results showed a significant linear reduction in papillae surface area in the rumen cannulae wall area and in the overall average papillae surface area during the prepartum period. The **High** treatment was chosen to provide the dairy cows with a substantial amount of time and substrate to ensure optimal rumen papillae development. The medium concentrate diet was introduced 32 d before expected calving date to determine if diets containing 20% concentrate (**LM**) were sufficient to promote maximum development of rumen papillae. The medium and high concentrate diets fed prepartum resulted in significantly higher ruminal concentrations of butyrate. Butyrate is the primary VFA that is metabolized in the rumen epithelium (Bergman, 1990). This may in part explain why cows on the **High** treatment had numerically larger papillae than those cows on the **Low** treatment. However, the increased ruminal concentrations of butyrate in cows fed the high and medium concentrate diets does not explain the observed linear decrease in papillae surface area during the prepartum period. Reynolds et al. (2000) measured the visceral tissue mass in transition dairy cows from six weeks prior to expected calving date to 22 d after calving. The dry cows were fed a grass-silage based TMR (control), supplemented with 800g/d of barley

concentrate or 750g/d of rumen protected soybean meal. At specific days during the transition period three cows from each treatment group were slaughtered. There were no significant differences in length, width or surface area of the rumen papillae that were digitally analyzed in cows fed the different diets (Reynolds et al., 2000). Gaebel et al. (1987) reported an increase in rumen papillae size in sheep fed a 90% concentrate diet for 15 weeks. However, these sheep were fed four consecutive diets (1) hay only, (2) 36% hay, 64% concentrate, (3) 10% hay, 90% concentrate and (4) hay only for 15 week periods. Therefore, these sheep actually consumed a high-energy diet for a total length of 30 weeks. Gaebel et al. (1987) still reported a 200% increase in papillae size after the animals were switched to the 90% concentrate diet from the 64% concentrate diet. The increase in papillae size was attributed to the increased production of VFA produced in the rumen from the diets rich in carbohydrates. Ruminal VFA are considered to be one of the physiological trophic factor for rumen epithelium development (Sakata and Yajima, 1984). However, the level of concentrate fed during the prepartum period (low, medium, or high concentrate) did not significantly influence the total VFA concentration in the rumen. Therefore, the duration or level of prepartum concentrate fed during this period did not influence papillae development.

Our postpartum results indicated that the surface area of rumen papillae increased during early lactation. The overall average surface area of rumen papillae increased by the fifth week of lactation regardless of prepartum treatment ($P = 0.06$). Moon and Campbell (1973) also observed increased lengths of rumen papillae in post parturient ewes. Although the length of the rumen papillae varied

throughout the experiment the highest mean length of papillae were observed approximately 50 d to 60 d after parturition (Moon and Campbell, 1973). Gaebel et al. (1987) reported that with the increase in papillae surface area a concomitant increase in the number of cell layers in the rumen epithelium occurs when high concentrate diets were fed to sheep. These findings support the increased amount of total deoxyribonucleic acid and ribonucleic acid content found in the ruminal mucosa of ewes 45 d postpartum (Moon and Campbell, 1973) and total ruminal tissue mass 22 d after calving (Reynolds et al., 2000).

The high concentration of VFA in cows fed the lactation diet may have contributed to the increased papillae size during the postpartum period. In the present study, cows consuming the lactation diet showed the highest concentration of butyrate in the rumen (11.80 mol/100mol). Gaebel et al. (1987) also reported high concentrations of butyric acid in rumen fluid from sheep fed either 64% or 90% concentrate diet. Dirksen et al. (1985, 1997) postulated that a well-proliferated rumen with developed papillae helps stabilize the rumen pH in dairy cows fed a high-energy diet and also assists in diminishing the energy gap at peak lactation. In relation to our results, these functional characteristics attributed to advanced papillae development were only obtained during the first five weeks of lactation.

Papillae profiles varied substantially among cows consuming the same diet in approximately the same quantities. The cause of morphological differences in papillae development is still not understood and a better understanding of papillae growth and development is required in the adult dairy cow. Endogenous effects,

hormonal, metabolic, genetic, and individual variations of VFA, as well as biopsy techniques are all possible factors that may influence papillae development and measurements.

2.4.6. Rumen Fermentation

Prepartum diets did not influence ruminal pH. This finding suggests that the buffering capacity of the rumen was adequate for cows consuming the high concentrate diet. Similar rumen pH results were obtained in cows fed either a high cracked corn diet or a high steam-flaked corn diet (Dann et al., 1999). Cows consuming the lactation diet showed the lowest rumen pH but still remained within acceptable limits. Ruminal NH_3 N concentrations were not affected by the prepartum diets. The elevated ammonia N concentrations found in the rumen of the cows fed the medium concentrate diet may be attributed to the large animal variation and lower number of cows fed this diet (n=3). In the analysis of the low and high concentrate diets we combined cows within different treatments to get a better estimate of the rumen fermentation profiles. At no time did the rumen NH_3 N concentration fall below 5mg/ml, which is considered to be a critical concentration for microbial protein synthesis and fiber digestibility (Nocek and Russell, 1988). Lykos et al. (1997) observed similar rumen NH_3 N concentrations in cows fed varied levels of nonstructural carbohydrates.

Total VFA concentrations were not affected by the prepartum diets. Similar results were obtained in lactating and dry cows fed high levels of concentrate (Lykos et al., 1997, Dann et al., 1999). Lykos et al. (1997) suggested

that total VFA concentration may not have been affected by the diet because the digestibility of organic matter was similar across diets.

2.4.7. Blood Metabolites

Nutritional regimes do not generally cause plasma glucose concentrations to vary greatly in cows. In the present study, plasma glucose concentrations were not significantly different in cows fed the different prepartum treatments. Lykos et al. (1997) and Dann et al. (1999) also found relatively constant plasma glucose levels in dry and lactating cows. As expected, plasma glucose concentrations peaked at the time of calving. This may result from increased glucagon and glucocorticoid concentrations associated with the onset of parturition (Grummer, 1995). During the first week of lactation serum glucose concentrations decreased, but by the next few weeks of lactation plasma glucose concentration started to increase. Vazquez-Anon et al. (1994) and Grum et al. (1996) reported similar results in early lactating dairy cows. The increase in plasma glucose concentration may reflect the recovery of feed intake during the initial stages of early lactation.

The prepartum decrease in serum NEFA concentration in the **High** and **LH** treatment cows was expected. Increasing prepartum DMI and energy density generally decreases serum NEFA concentration. Minor et al. (1998) reported a similar reduction in serum NEFA concentration in cows and heifers fed a 43% NFC prepartum diet. The elevated serum NEFA concentration in the **Low** and **LM** treatment cows may be explained by the earlier induction to a negative

energy balance. Entering into a negative energy balance causes cows to increase the mobilization of adipose tissue thus increasing the level of blood NEFA concentration before parturition. This could have been further enhanced by the limited amount of available carbohydrates in these lower energy diets and reduced feed intake. Serum NEFA concentration typically peaked at or near the day after calving. Increasing serum NEFA concentrations are commonly seen as cows approach parturition (Bertics et al., 1992, Vazquez-Anon et al., 1994, Minor et al., 1999). The change in hormone concentration around the time of parturition may also contribute to the increased catabolism of adipose tissue, thus increasing serum NEFA concentration (Grummer, 1995). Serum NEFA concentrations were high after calving but decreased as lactation progressed. These results are similar to other early lactation studies in dairy cows (Bertics et al., 1992, Vazquez-Anon et al., 1994, Minor et al., 1999).

As expected postpartum plasma BHBA concentrations were higher than prepartum concentrations. These results are commonly seen in transition cow studies (Veenhuizen et al., 1991, Bertics et al., 1992, Vazquez-Anon et al., 1994, Minor et al., 1999). Increased plasma BHBA concentration may be due to the higher energy demand commonly associated with the onset of lactation. Elevated ketone concentrations usually coincide with elevated blood NEFA and low blood glucose concentration. In the present study, plasma BHBA concentration were not significantly influenced by treatments. The large degree of animal variation reduced the ability to detect treatment differences. Postpartum plasma BHBA concentrations for the **High** treatment cows present somewhat of a mystery

considering none of the cows showed any symptoms of clinical ketosis (i.e. depression in feed intake or milk production). Generally, hepatic ketogenesis increases because of an enhanced rate of fatty acid oxidation and gluconeogenesis.

2.4.8. Milk Parameters and Postpartum Health Disorders

Increasing the level of concentrate during the dry period did not significantly affect milk yield or milk composition. Modifications of prepartum diets (regardless of energy source) did not significantly alter the subsequent lactation in cows (Rukkwamsuk et al., 1999a, 1999b, Doepel et al., 2000), primiparous cows (Grummer et al., 1995), or restricted or force-fed cows (Bertics et al., 1992). Generally, early lactation represents a time of hormonal and metabolic adjustment due to the onset of parturition and the initiation of milk production. Cows differ in their ability to cope with the stress of parturition and early lactation. This results in greater variability in reference to treatment, which makes it more difficult to detect treatment differences during early lactation.

As expected no differences in disease incidence were observed among treatments. In order to detect significant differences in health disorders the number of animals required would need to be substantially increased.

2.5. Conclusion

Increasing the level of concentrate for dairy cows in the prepartum period did not increase DMI, BW, BCS, or the surface area of rumen papillae during this

period. However, prepartum energy intake and balance was significantly improved in the final weeks before calving in the LH treatment cows. The increased energy intake resulted in less mobilization of adipose tissue as parturition approached and lowered serum NEFA concentration. Rumen papillae decreased in surface area during the dry period regardless of prepartum treatment. The reduction may have been associated with the physiological status of the animal considering that the dry period is a relative "down time" and energy requirement are only needed for maintenance and fetal growth. In comparison to the energy requirement during early lactation the dairy cow does not require a substantial amount of energy during the prepartum period. In the present study, prepartum nutrition did not influence rumen papillae development. Therefore, the physiological status of the dairy cow seemed to be of primary importance during the prepartum period for rumen papillae development. There is limited research that examines the development of rumen papillae in the adult dairy cow and additional studies are required to understand the adaptive process of the rumen epithelium during different physiological stages. Further research should also evaluate the development of rumen papillae in animals fed different levels of energy substrate and consequently different VFA concentrations. Rumen papillae tended to increase in surface area during the first five weeks of lactation. The increased surface area of papillae during the lactation period could be explained by the increased concentrations of butyrate produced in the rumen or the significant increase in DMI by the sixth week of lactation. Gaebel et al. (1987) showed an increase in papillae size and butyric acid in sheep fed a high level of

concentrate. Moon and Campbell (1973) reported a significant relationship between the increased length of rumen papillae with increased feed intake in sheep. The increased level of feed intake during early lactation would increase the level of non-structural carbohydrates consumed, which may further develop rumen papillae by increasing the concentration of total VFA in the rumen. However, the increase in papillae surface area may also be associated with the higher energy demand of lactation. Although no significant changes were observed in papillae surface area among cows fed different prepartum diets, the increase in papillae surface area during the experimental period warrants further investigation. We concluded that level of concentrate fed during the prepartum period did not influence papillae development.

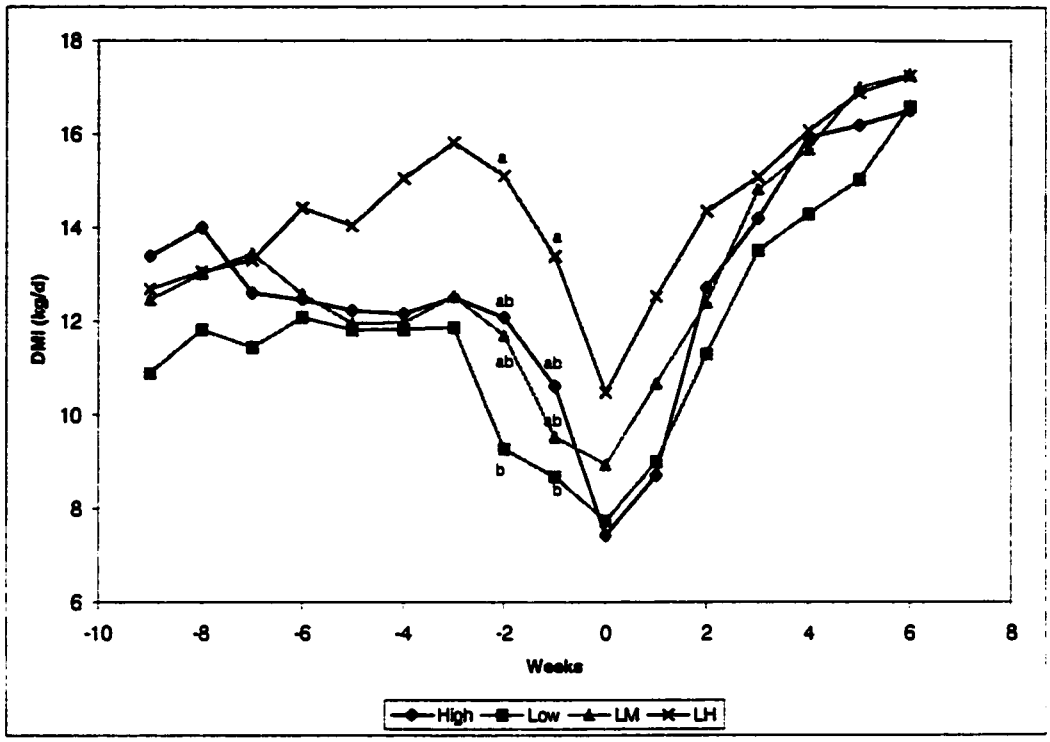

Figure 2.1. Effect of treatments on prepartum and postpartum DMI.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

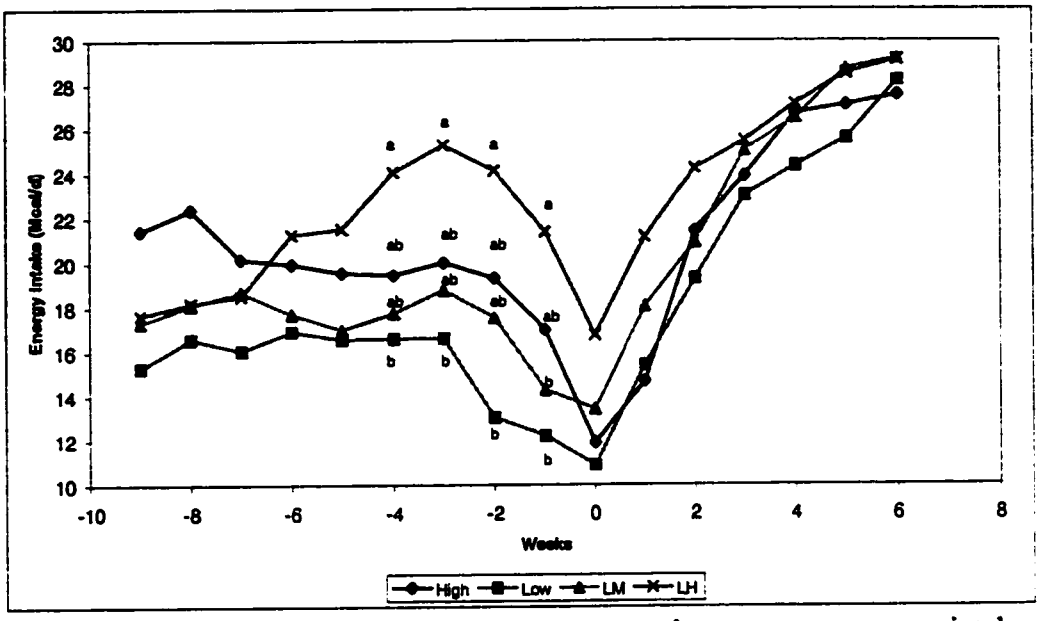

Figure 2.2. Effect of treatments on prepartum and postpartum energy intake (Mcal/d).

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

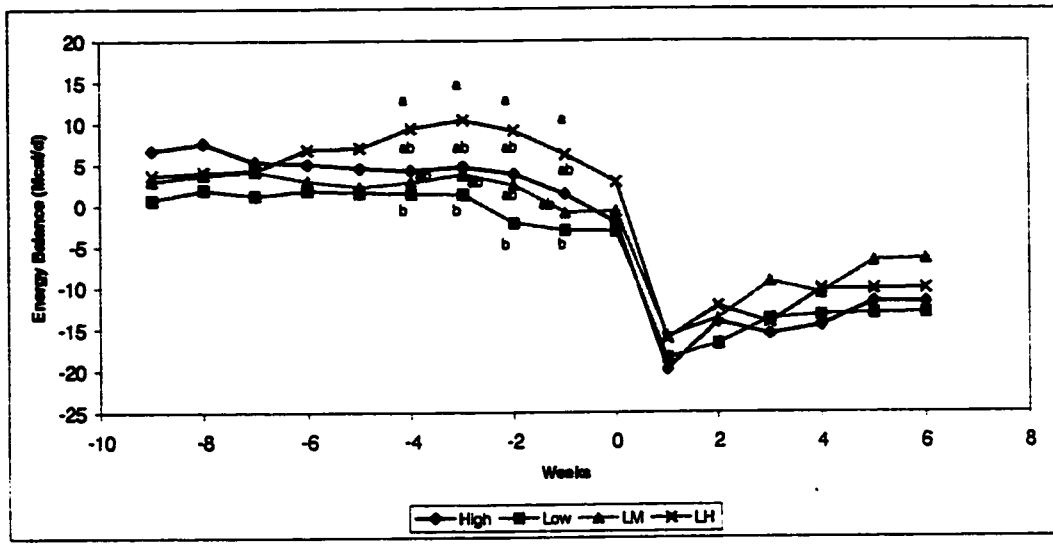

Figure 2.3. Effect of treatments of prepartum and postpartum energy balance (Mcal/d).

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

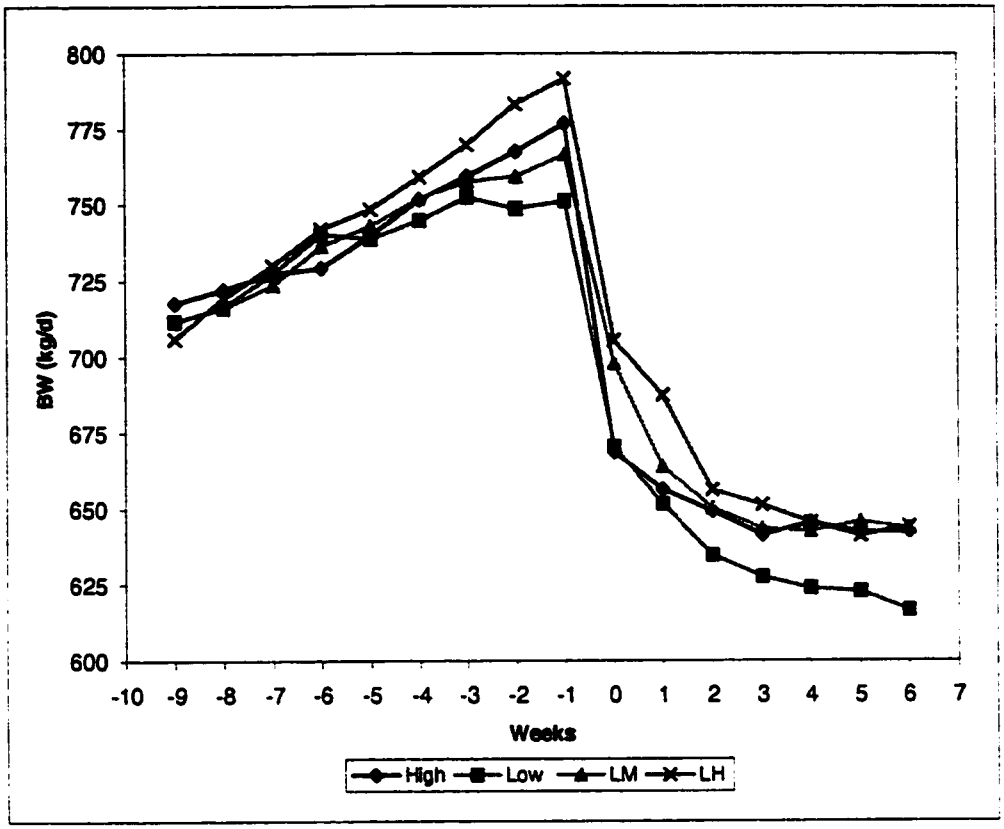

Figure 2.4. Effect of treatments on prepartum and postpartum body weights (kg/d).

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

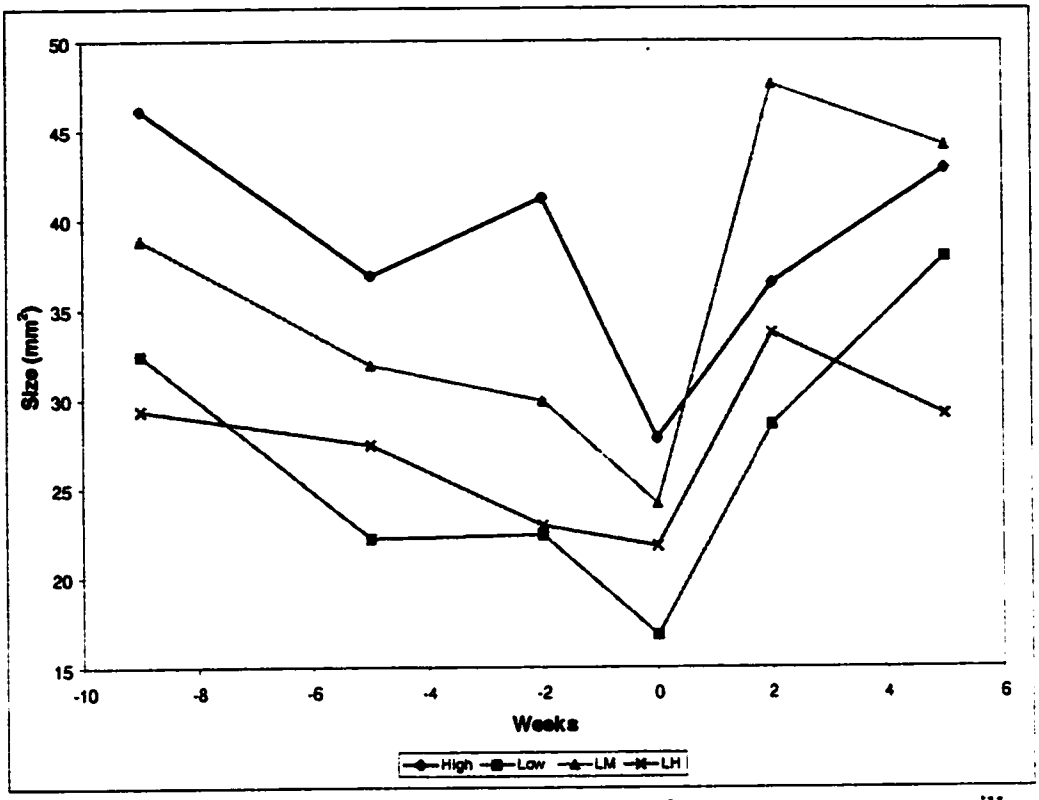

Figure 2.5. Effect of treatments on prepartum and postpartum rumen papillae located on the rumen cannula wall.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

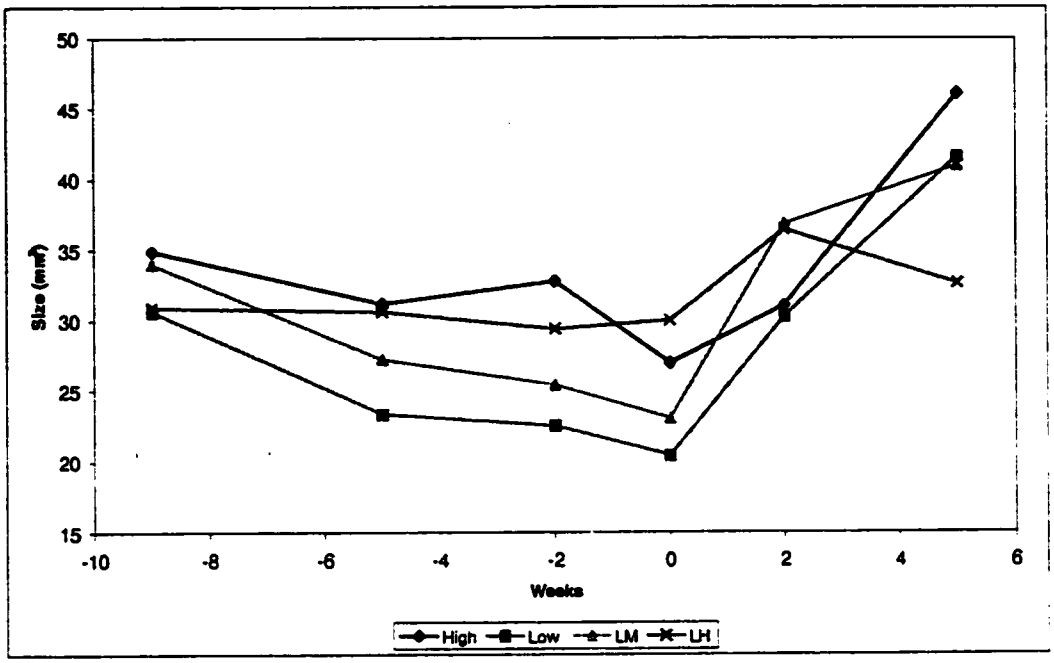

Figure 2.6. Effect of treatment on prepartum and postpartum overall average papillae surface area.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

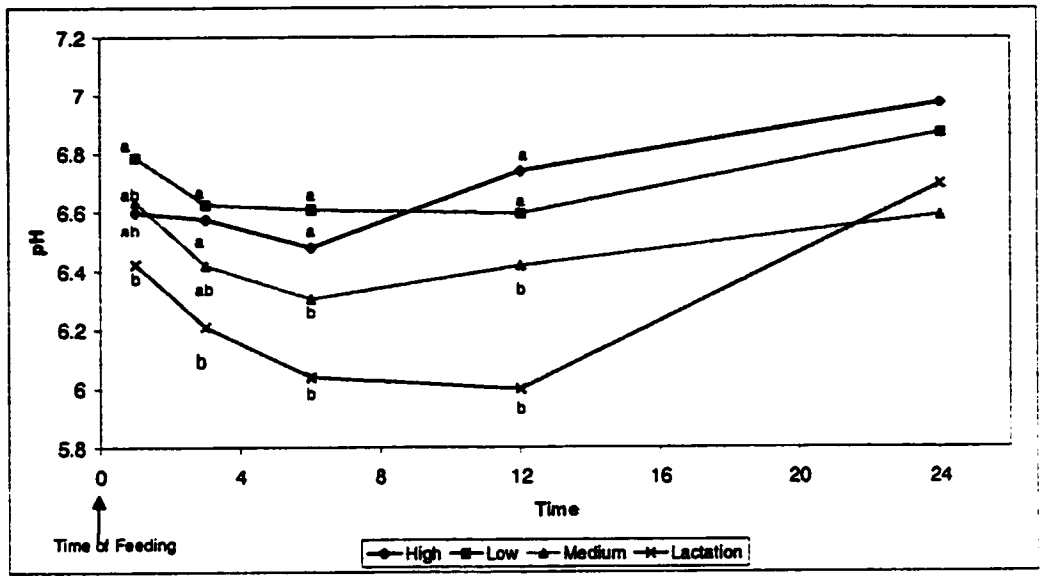

Figure 2.7. Diurnal patterns of ruminal pH as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

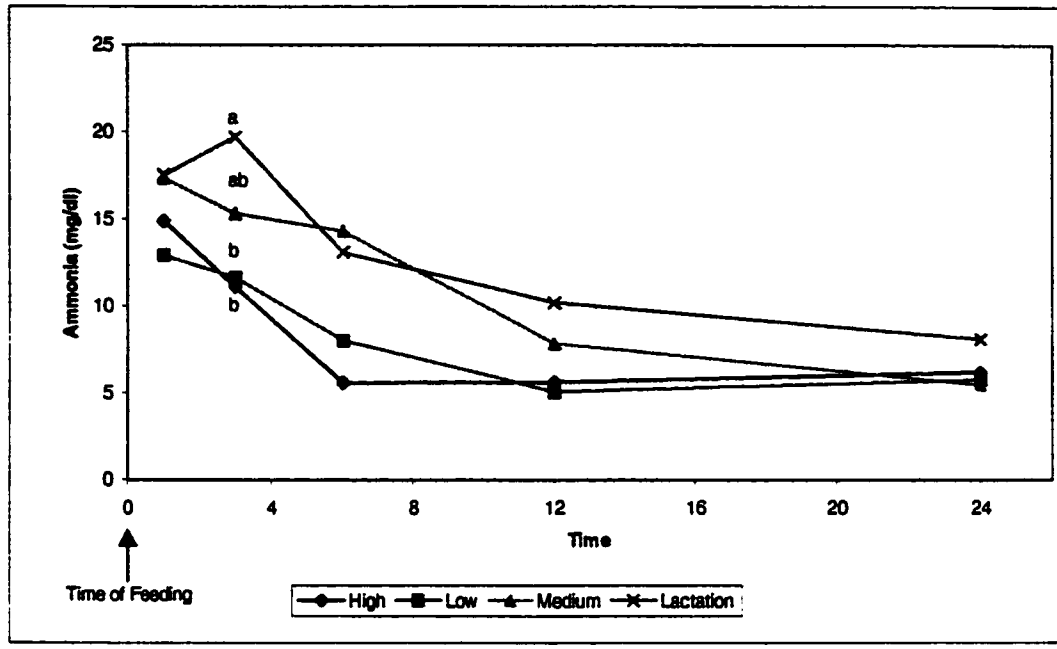

Figure 2.8. Diurnal patterns of ruminal ammonia as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

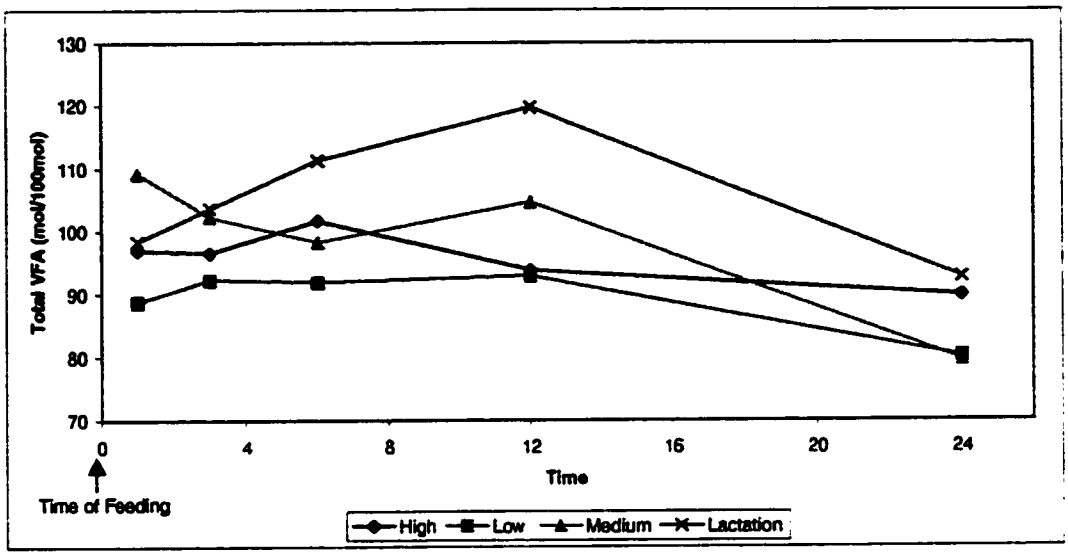

Figure 2.9. Diurnal patterns of total volatile fatty acids in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

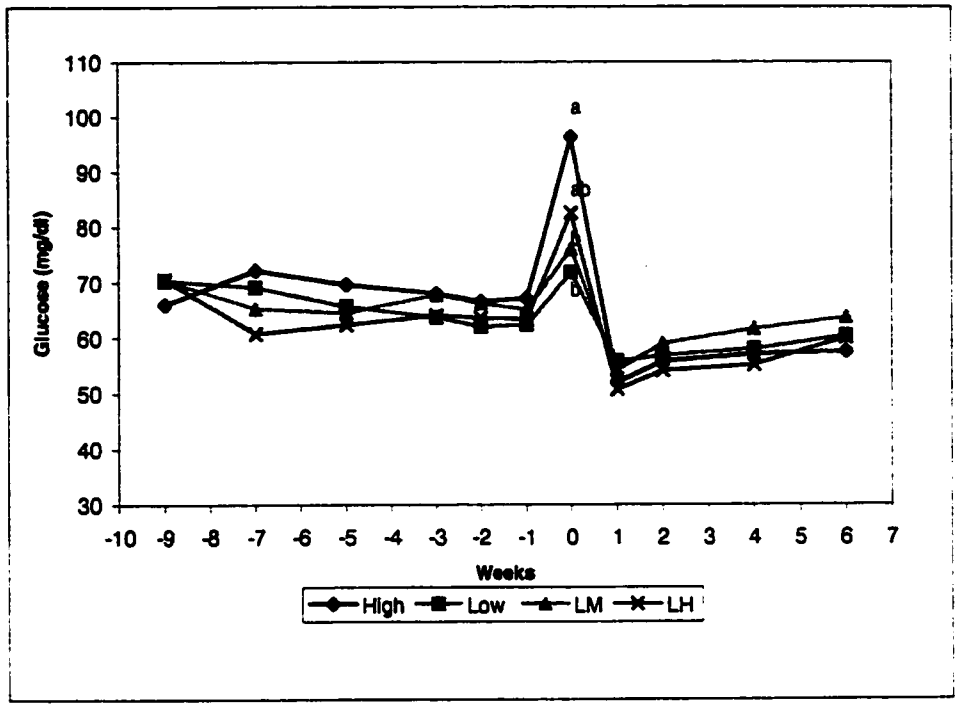

Figure 2.10. Effect of treatments on prepartum and postpartum plasma glucose concentrations.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

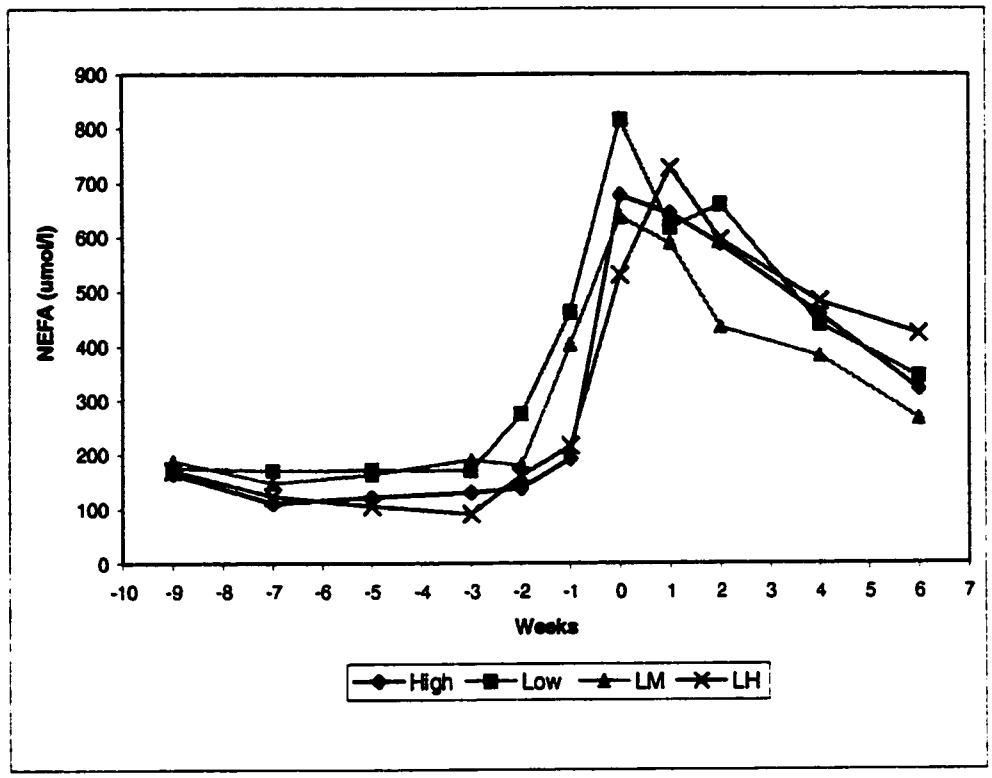

Figure 2.11. Effect of treatments on prepartum and postpartum serum NEFA concentrations.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

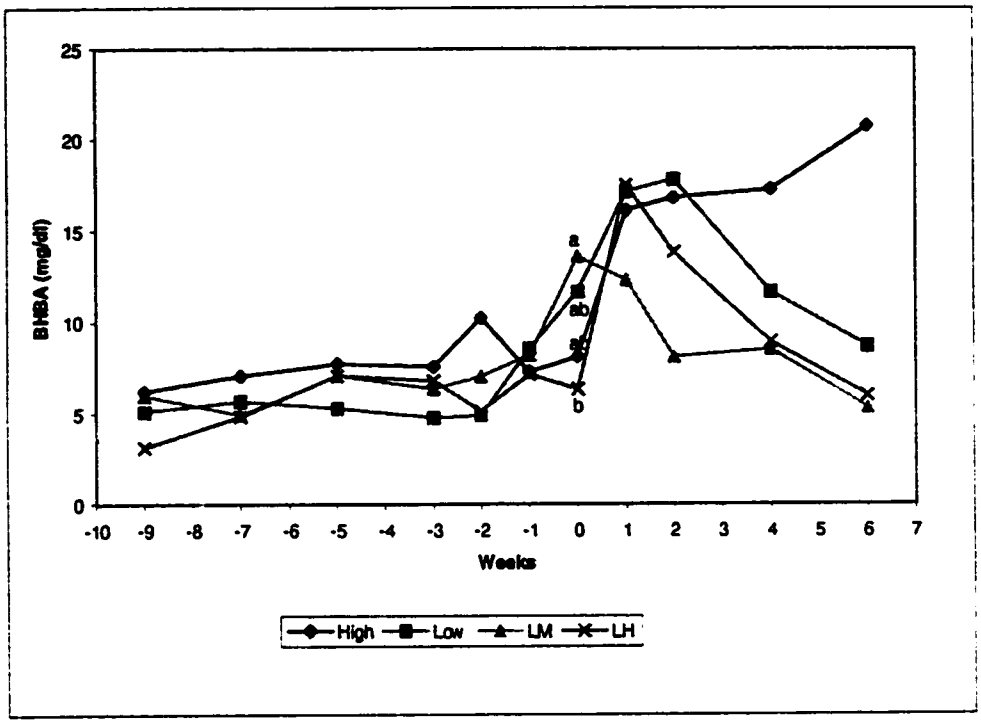

Figure 2.12. Effect of treatments on prepartum and postpartum plasma BHBA concentrations.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

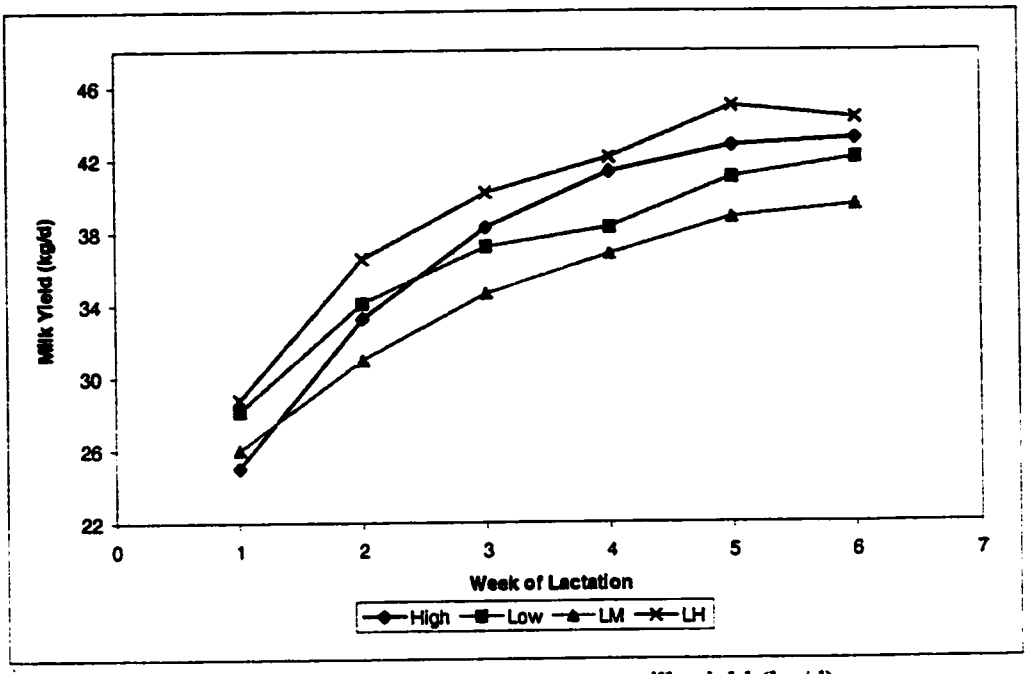

Figure 2.13. Effect of prepartum treatments on milk yield (kg/d).

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

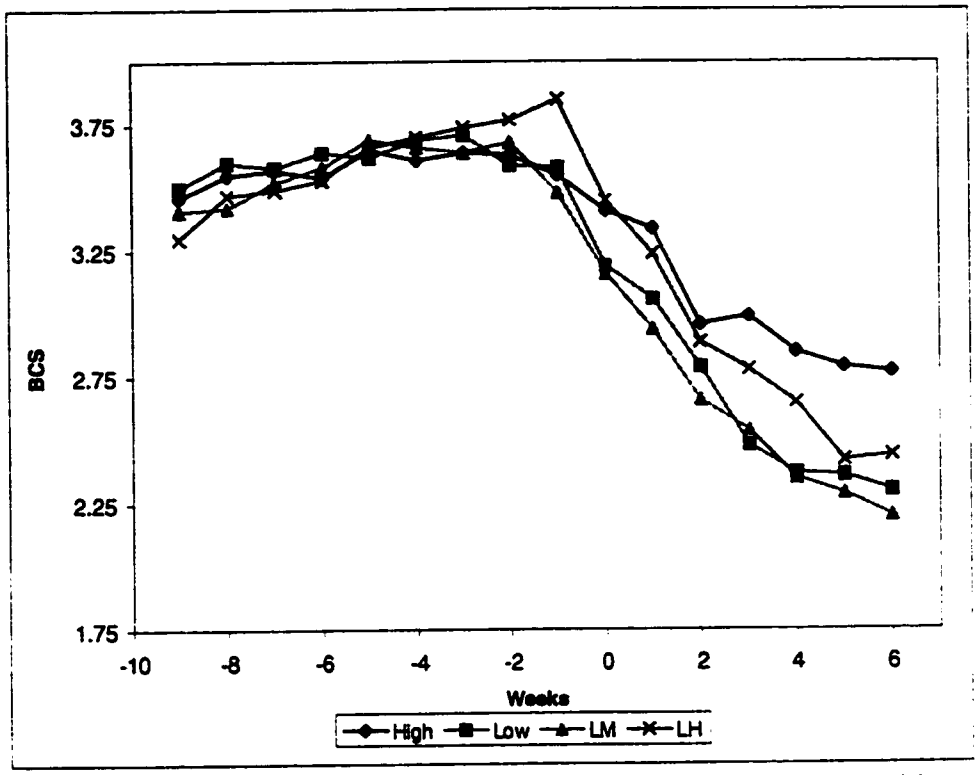

Figure 2.14. Effect of treatments on prepartum and postpartum body condition score (BCS).

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

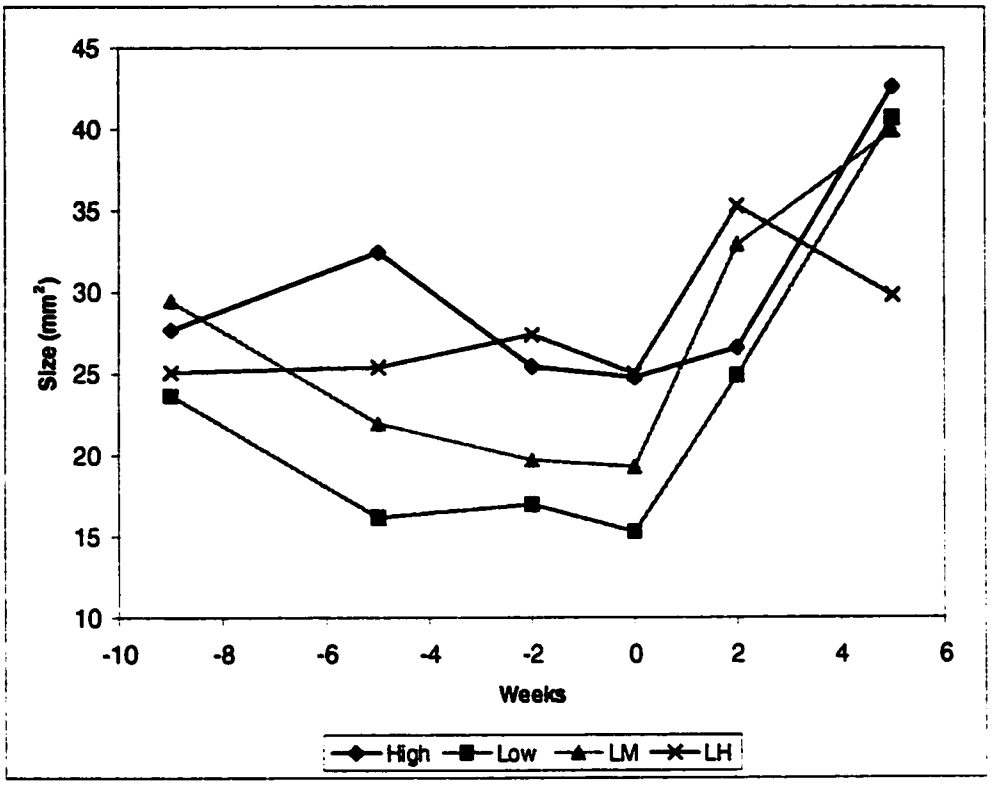

Figure 2.15. Effect of treatments on prepartum and postpartum rumen papillae located in the blind sac.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

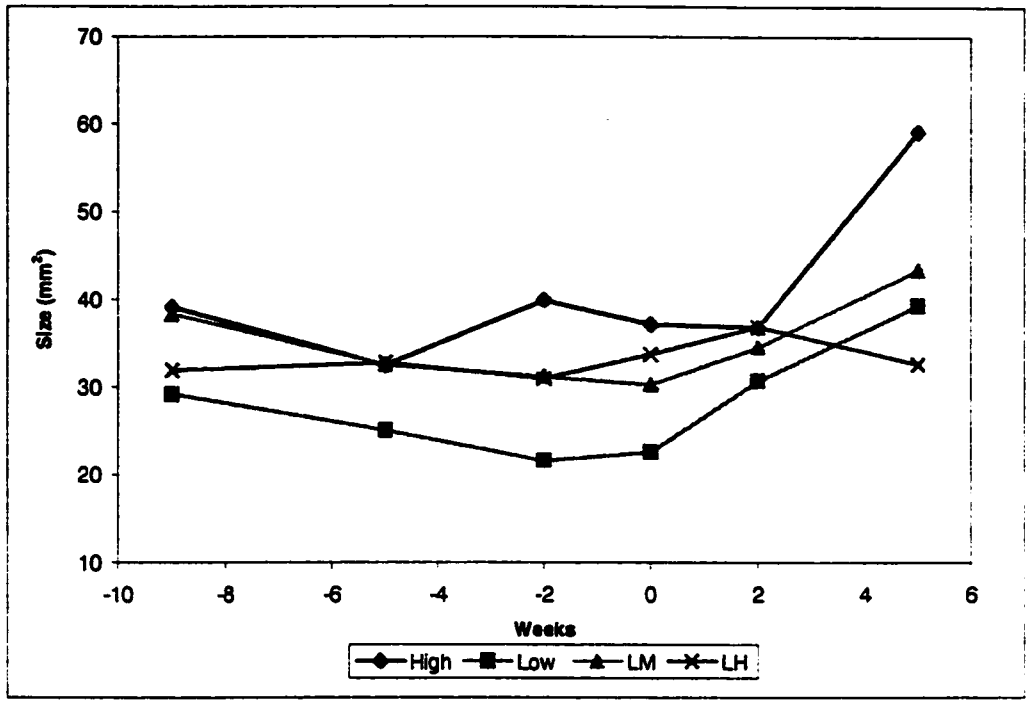

Figure 2.16. Effect of treatments on prepartum and postpartum rumen papillae located in the ventral sac.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

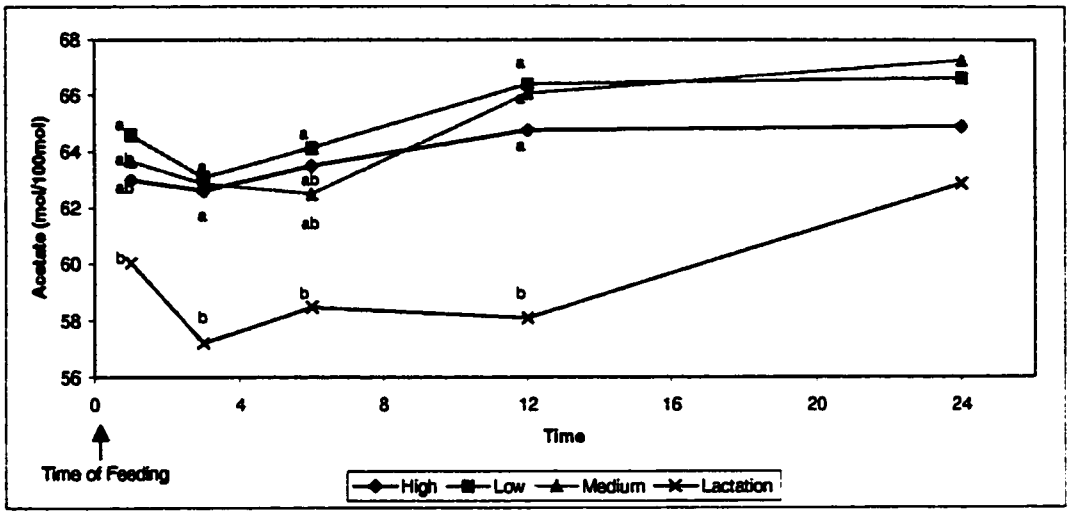

Figure 2.17. Diurnal patterns of acetate in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

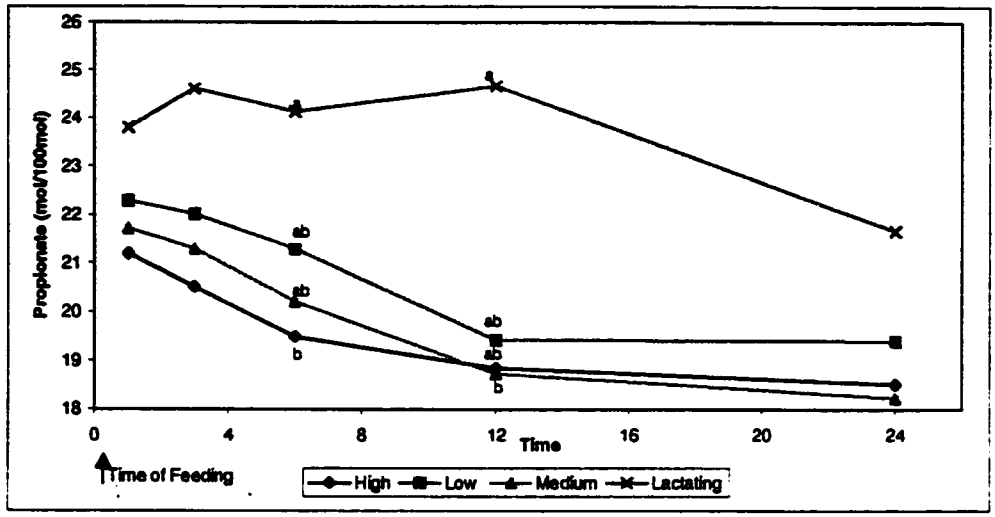

Figure 2.18. Diurnal patterns of propionate in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

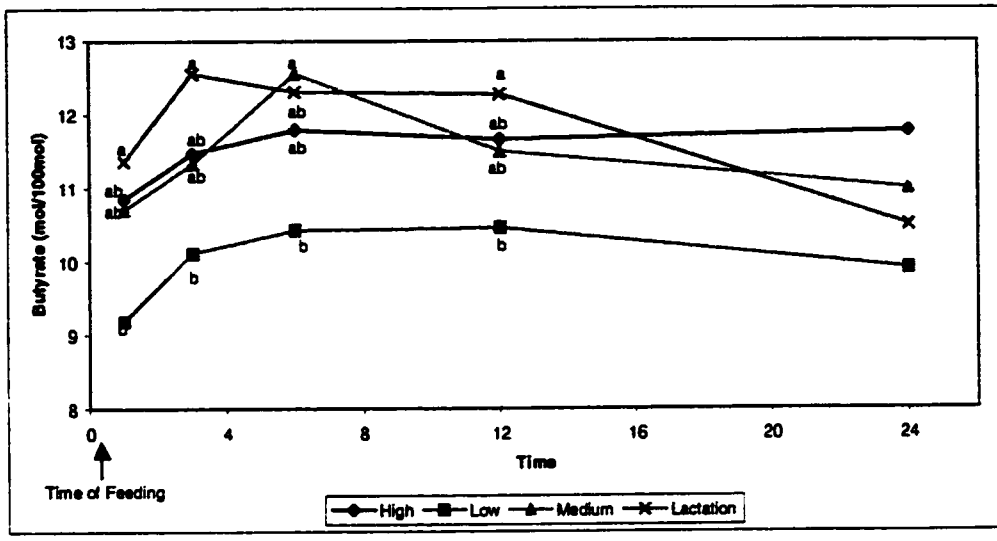

Figure 2.19. Diurnal patterns of butyrate in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

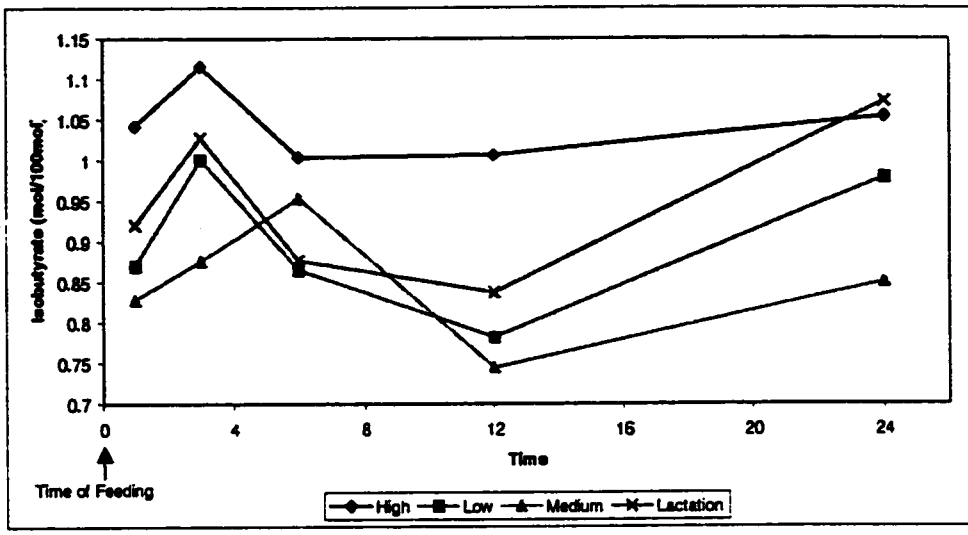

Figure 2.20. Diurnal patterns of isobutyrate in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

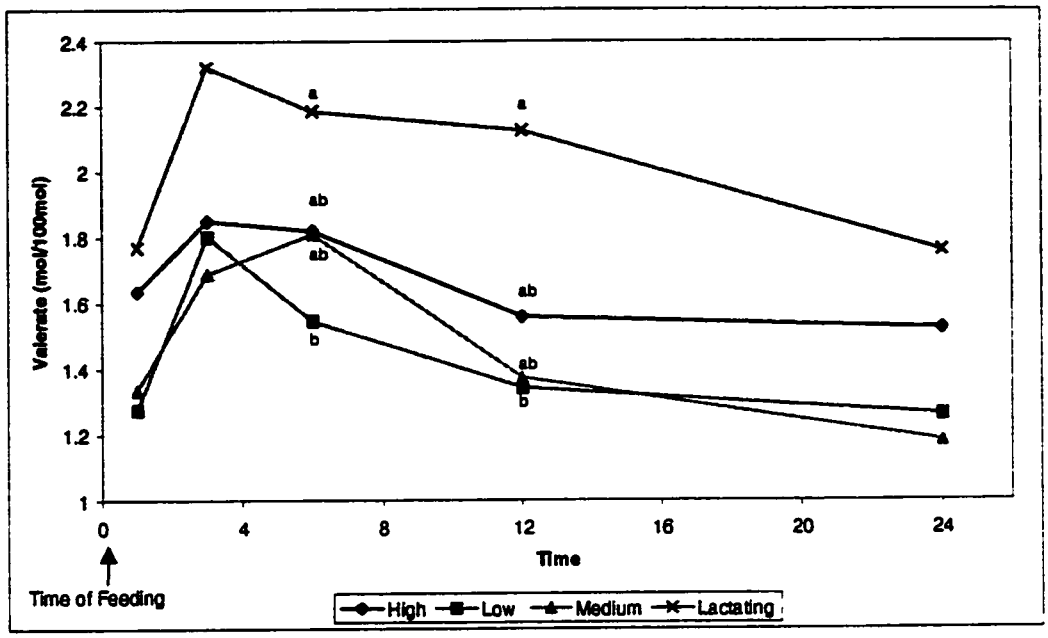

Figure 2.21. Diurnal patterns of valerate in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet **(High)** (♦), low concentrate diet **(Low)** (■), medium concentrate diet **(Medium)** (Δ), and the lactating diet **(Lactation)** (×).

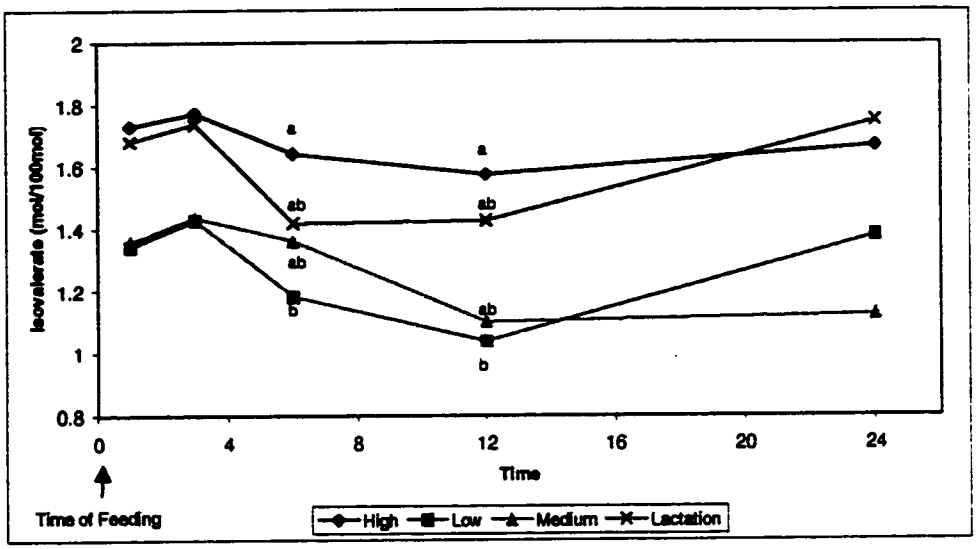

Figure 2.22. Diurnal patterns of isovalerate in the rumen fluid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

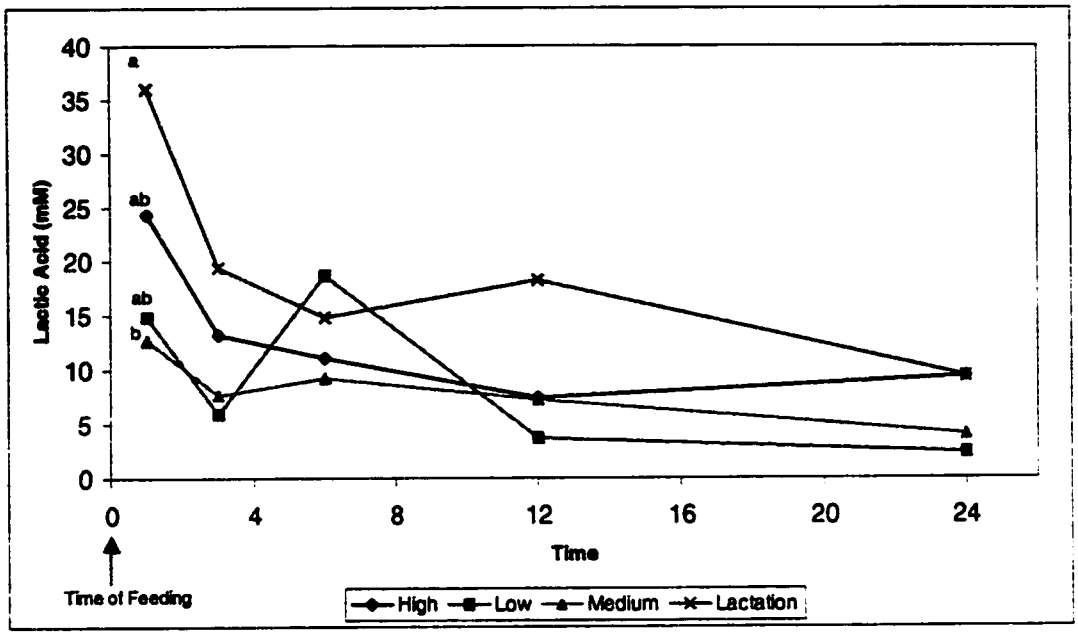

Figure 2.23. Diurnal patterns of ruminal lactic acid as influenced by diets.

Legend: Cows fed the high concentrate diet (**High**) (♦), low concentrate diet (**Low**) (■), medium concentrate diet (**Medium**) (Δ), and the lactating diet (**Lactation**) (×).

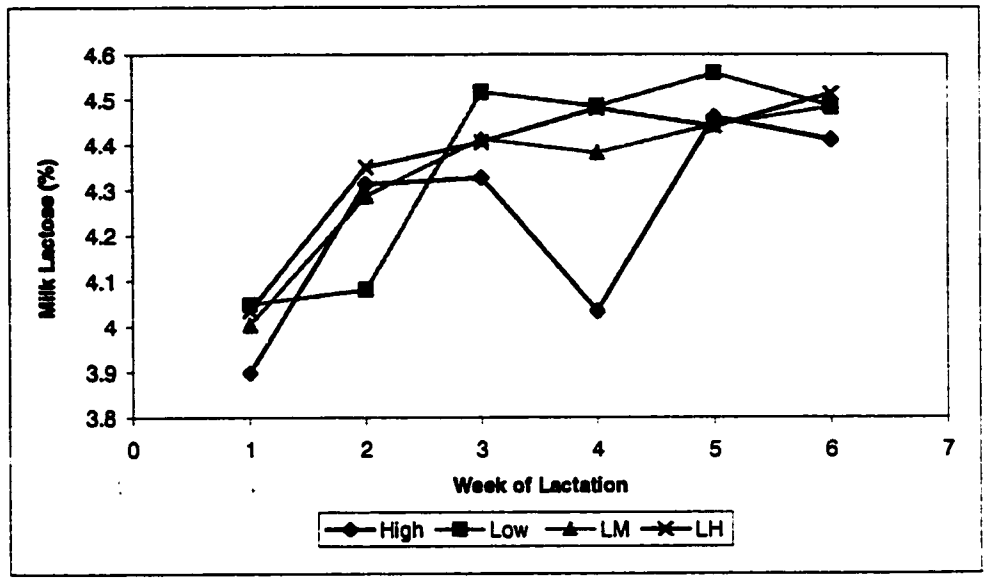

Figure 2.24. Effect of prepartum treatments on milk lactose %.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

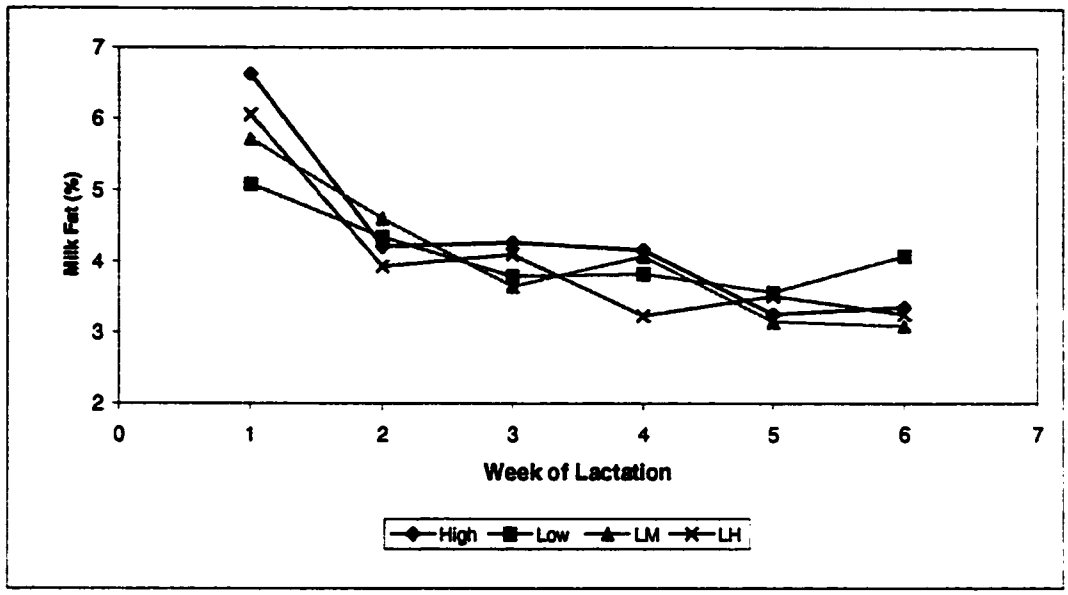

Figure 2.25. Effect of prepartum treatments on milk fat %.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

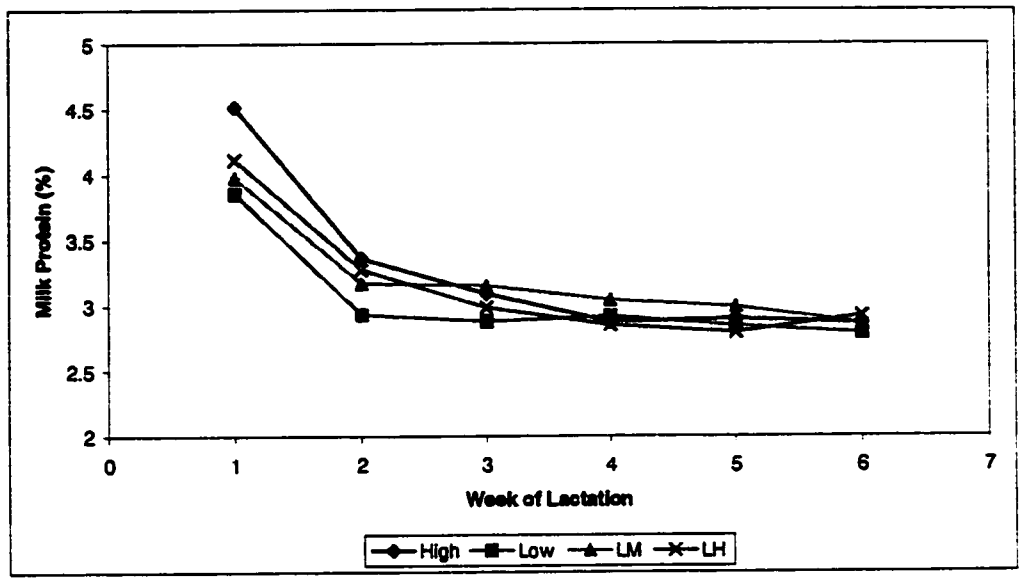

Figure 2.26. Effect of prepartum treatments on milk protein %.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

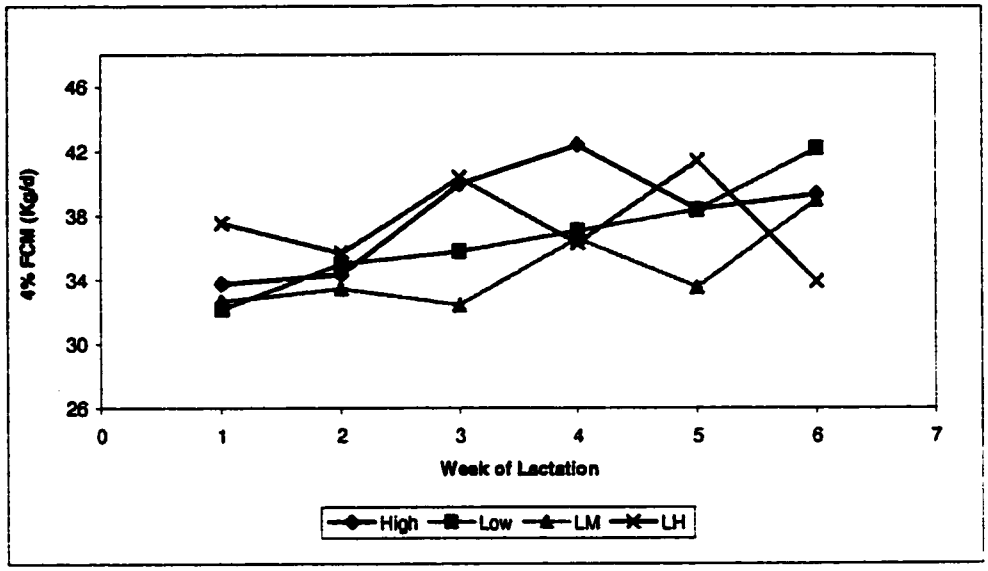

Figure 2.27. Effect of prepartum treatments on 4% fat corrected milk.

Legend: Cows fed the high concentrate diet (**High**) for the entire dry period (61 d) (♦), low concentrate diet (**Low**) for the entire dry period (61 d) (■), low concentrate diet changed to a medium concentrate diet 32 d prepartum (**LM**) (Δ), and low concentrate diet changed to a high concentrate diet 32 d prepartum (**LH**) (×).

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Chapter 3: General Discussion and Conclusions

3.1. Introduction

The transition period between late pregnancy and early lactation is certainly one of the most interesting stages of the lactation cycle in dairy cows. Nutrition and management of cows during this transition period has received tremendous attention and has been well-documented in recent reviews (Bell, 1995; Grant and Albright, 1995; Grummer, 1995; Goff and Hurst, 1997; Drackley, 1999; Ingvartsen and Anderson, 2000).

The transition period generally refers to the final three weeks before calving and the first three weeks of lactation. The close up dry cow period is used to prepare the cow and rumen for higher amounts of concentrate in the diet, which are commonly fed during early lactation. The profitability of the dairy cow is largely influenced by the ability of the animal to make a successful transition from the dry period to early lactation. Therefore, nutritional and management limitations during this time impede the ability of dairy cow to reach maximum potential. Nutritionists and researchers recommend feeding higher levels of concentrate (up to 40%) to the close up dry cow in order to adapt the rumen mucosa and microbial population for the up coming lactation diet.

Dirksen et al. (1985) reported that the length of the rumen papillae declined during the early part of the dry period in cows fed a low energy diet. The reduction in papillae length had a marked effect on the ability of the rumen to absorb volatile fatty acids (VFA). In fact, the absorptive capacity of the rumen

was decreased up to 50% by the end of the dry period in cows fed a low energy diet. Fell and Weekes (1974) reported that animals fed high levels of whole-barley grain for a few weeks developed a mucosal rugae in the rumen. On the rugae the papillae appeared short in length with abnormally clubbed profiles. Fell and Weekes (1974) suggested that much of the damage occurred in the first few weeks of feeding the high grain diet, after which there appeared to be an adaptation. The nature of the adaptive process remains unclear. However, Gaebel et al. (1987) reported significant papillae development in sheep fed a 90% concentrate diet compared to sheep fed hay only. The increase in papillae size was attributed to the higher concentrations of VFA (primarily butyric acid) produced in the sheep consuming the higher concentrate diets. Meon and Campbell (1973) reported that papillae size during the postpartum period (approximately 50 d) were larger than those found in the prepartum period. Dirksen et al. (1985) reported that rumen papillae required a minimum of seven weeks to fully develop, with the greatest increase in papillae length and ruminal absorption capability occurring in the final two weeks of adaptation in dairy cows fed a high-energy diet.

Rumen microbes also need to be acclimatized to changes in the diet. If a cow is abruptly switched to a high-energy diet then she is at risk of developing severe metabolic disorders such as rumen acidosis. *Primary* lactate producing microbes respond rapidly to high starch diets and they can produce large amounts of lactate in the rumen. However, *secondary* lactate-utilizing bacteria respond more slowly to changes in the diet, requiring approximately three to four weeks to

reach levels that will effectively prevent lactate from building up in the rumen (Huntington et al., 1981; Goff and Horst, 1997). More importantly, the poorly developed rumen epithelium of the unadapted cow will not be able to absorb VFA quickly enough to prevent a build up of organic acids in the rumen. These effects lead to one of the important questions that we investigated: how long does it require papillae to achieve maximum surface area and can this be achieved during the dry period?

In view of these critical facts about adapting the rumen, should higher energy diets be introduced earlier than twenty-one days prior to calving? Will this allow enough time for the rumen to develop? And finally, do prepartum diets affect papillae development? The hypothesis of the study was that introducing higher levels of concentrate throughout the dry period would increase the overall surface area of rumen papillae. An increased surface area of rumen papillae should increase the capability of the rumen to absorb the end products of fermentation and in turn allow the dairy cow to better cope with the transition period. Therefore, the objective of this study was to examine the effects of duration and level of concentrate fed to Holstein dairy cows and determine the effects of experimental diets on papillae surface area, dry matter intake, body weight and condition score, ruminal fermentation patterns, blood metabolites, and lactational performance.

3.2. Overall Experimental Conclusions and Implications

Conducting research during the transition period presents several challenges. Measurements during this time are laden with a high degree of variation reflected by the differences among individual cows responding to parturition and the onset of lactation. The incidence of health disorders during this time also contributes to the variation in DMI, energy balance, tissue mobilization, milk yield and responses to imposed treatments. Therefore data during this transition period may be difficult to interpret. Also, a large number of cows are usually required to ensure that true treatment differences can be determined. These are a few important points to take note of when reviewing results from the transition period between late pregnancy and early lactation.

Increasing the level of concentrate fed prepartum did not significantly alter many of the variables measured such as, DMI, energy intake and balance, BW and BCS during the dry period. All of these variables possessed a significant treatment by week interaction indicating that cows did not respond similarly across the weeks measured. Results indicated that the LH treatment cows increased DMI, energy intake and balance during the final weeks of gestation compared to the rest of the cows. The increased energy intake of cows fed the higher levels of concentrate resulted in less mobilization of adipose tissue as parturition approached, shown by the lower serum NEFA concentrations.

This has been the first time anyone has digitally analyzed rumen papillae surface area throughout the dry and early lactation period at the University of Alberta. The image analysis technique is an effective but not widely used method

to determine the surface area of rumen papillae. Generally, this procedure worked well. In the present study, papillae development was not significantly influence by prepartum nutrition. Rumen papillae decreased in surface area as cows approached parturition regardless of prepartum treatment. These results cast doubt on the generally accepted dogma that rumen papillae develop in response to fermentable carbohydrate in the diet during the prepartum period. The linear regression in papillae surface area may be related to the animal's physiological status. The authors speculate that the physiological status of the dairy cow is more important with regard to papillae development than the nutritional regime during the prepartum period. However, papillae surface area tended to increase by the fifth week of lactation. The lactating diet showed significantly higher total VFA concentrations compared to when the cows were fed the low concentration diet. This may have contributed to the increased surface area of papillae found in the rumen. However, the energy demands of the dairy cow dramatically increase during early lactation due to milk production. Therefore, the increased surface area in rumen papillae during early lactation may be attributed to the increased energy requirements needed for milk production. The development of rumen papillae may also be influence by the increased level of fermentable carbohydrates in the rumen based on the increased DMI of dairy cows during the lactation period. The prepartum treatments did not significantly alter milk yield or milk composition during the first six weeks of lactation.

The increase in papillae surface area during the postpartum period was an interesting observation however, our prepartum results did not support Dirksen et

al. (1985) experimental findings. Comparing the Dirksen et al. (1985) study and the present one raises an interesting question: would cows have shown the increase in papillae development if they were continued on a high forage diet during early lactation? I believe these cows would have shown an increase in rumen papillae surface area. The regulation of growth in rumen papillae can not be strictly related to the diet consumed and other factors such as hormonal changes, blood supply to the rumen, and the animal's physiological status should be considered. Although dietary composition has been implicated as a major factor influencing papillae development, prepartum nutrition did not significantly influence papillae development in the present study. Therefore, nutrition may play less of a role in the contribution of rumen papillae development during the prepartum period than previously thought.

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Appendix 2.2.1. Volatile Fatty Acid Analysis Procedure.

1. Thaw frozen rumen fluid samples.
2. Centrifuge at 4°C for 15 min at 4 000 rpm.
3. After centrifuging, transfer 1 ml of supernatant to gas chromatography vials and 200 µl of internal standard (4-meyl valeric acid).
4. Added 0.2 ml of orthophosphoric acid.
5. Cap vials with a rubber septum, mix and ready for gas chromatography analysis.
6. External VFA standards prepared for the determination of the response factor of each VFA.
   External Standard (in 100 ml of distilled water):
   (0.3017 g acetic acid, 0.1292 g propionic acid, 0.0096 g isobutyric acid, 0.08 g butyric acid, 0.0142 g valeric acid, 0.0187 g isovaleric acid, 0.0099 g caprioc acid).
7. Control rumen fluid was prepared by the addition of 0.80 ml of rumen fluid to 0.2 ml of internal standard. (This sample was to ensure quality and consistency in gas chromatography performance across all runs).

Conditions for Gas Chromatography:

1. Instrument used to analyze the lactic acid derivative was a Varian model 3400 with a megabore capilary column STABILWAX-DA (30 m, 0.5 mm I.D.).
2. Temperature program:
   - injector temperature of 170°C.
   - detector temperature of 200°C
   - initial temperature of 120°C for 1 min.
   - column rate of 10°C/minute, final temperature of 180°C for 2 min.
3. Flow rate of helium was 30 ml/min and a chart speed of 0.5 cm/min.
4. Response factor for each VFA was calculated using the Shimadzu Ezchrom 3.2 version data system (Shimadzu Scientific Instruments, Inc., Columbia, MD.). Reprocessing of the samples gave the concentration of the VFA in mg/ml. For quality control, the control rumen fluid sample was used in every run to ensure consistency in gas chromatography performance.

Appendix 2.2.2. Lactic Acid Analysis Procedure.

1. Thaw frozen rumen fluid sample.
2. Shake well after thawing.
3. Centrifuge at 6°C for 15 min at 4 000 rpm.
4. After centrifuging, add 1 ml of supernatant to screw cap test tube (13 X 100 mm or 16 X 100 mm), 0.2 ml of internal standard (malonic acid) and 0.2 ml of 3N NaOH.
   Internal Standard: malonic acid (disodium salt) (molecular weight = 148 g/mol)
   (Composition: 0.3997 g of malonic acid in 200 ml of distilled water).
   External Standard: lactic acid (lithium salt) (molecular weight = 96.01 g/mol)
   (Composition: 0.2162 g lactic acid per 100ml of distilled water).
5. Mix samples and cover with perforated parafilm and evaporated dryness in a freeze-dryer (-60°C, shelf heat 15°C)
6. Add 1 ml of methanoic-HCL to dry sample (make sure the sample is completely dry) and cap test tubes, vortexed and heated for 25 min at 110°C in a forced air oven.
7. After heating, revortex samples, allow to cool and allow precipitate to settle overnight.
8. After settling, transfer supernatant to gas chromatography vials and analyze using gas chromatography.

Condition for Gas Chromatography:

1. Instrument used to analyze the lactic acid derivative: Varian model 3400 with a megabore capilary column STABILWAX-DA (30 m, 0.5 mm I.D.).
2. Temperature program used for analysis:
   - initial temperature of 80°C for 1 min
   - final temperature of 180°C for 8 min
   - column rate of 20°C/min
   - initial auxiliary temperature (autosampler injector port) of 180°C
   - detector temperature of 190°C
3. Autosampler was bottle filled with methanol.
4. Volume of sample injected was 0.5µl and the solvent plug size was <0.3 µl.
5. Splitter flow rate approximately 40 ml/minute.
6. Column pressure approximately 7psig.

With this temperature setting and a megabore capillary column, the lactic acid derivative eluted at approximately 3.65 min and the internal standard at approximately 5.3 min. The length of one run was 14 min. Calculations were done for impurities in the internal and external standards for malonic acid (disodium salt) and lactic acid (lithium salt), respectively. Automatic calculation for response factors (using the absolute amount of internal standard in the tube in mg) calculated using Shimadzu Ezchrom 3.2 version data system (Shimadzu Scientific Instruments, Inc., Columbia, MD.). Reprocessing of the samples gave the lactic acid derivative concentration in mg/ml. For quality control, control rumen fluid was carried with every sample preparation and each run of samples to ensure consistency in gas chromatography performance.

Appendix 2.2.3. Ammonia Nitrogen Analysis Procedure.

Ammonia Nitrogen Analysis for Rumen Fluid:

1. Thaw rumen fluid.
2. Shake and centrifuge at 5°C for 15 min at 4 000 rpm.
3. After centrifuging, pipette 20µl of supernatant (in duplicate) into 16 X 100 mm borosilicate disposable (glass) test tubes and cover with parafilm.
4. Add reagents to standards and samples and put into a dark cupboard at room temperature for 1 hr for color development.

   Reagents:

   1. Sodium phenate (12.5 g phenol + 6.2 g NaOH in 500 ml volumetric flask made to volume with deionized water.
   2. Sodium Nitropursude – stock solution (1%) – 1 g/100ml water. (working solution (0.01%) – 5 ml stock diluted up to 500 ml (volumetric flask with deionized water)).
   3. Sodium Hyperchlorite – 0.2N, 15 ml NaOCl (4-6%) diluted to 500 ml with deionized water, pH = 12 with 50% NaOH.

Conditions for Spectrophotometer:

1. Absorbance read on a dipping probe at 600 nm (Spectronic 300 Array, The Milton Ray Company, V 1.14 Analytical Products Division) using deionized water to zero spectrophotometer. (Pump speed: 80, pump time: 6, purge time: 3).

2. Software: Rapid Scan Rev. 2.1 (1991, The Milton Ray Company) and standard curves and absorbance/transmittance were the application utilized. A standard curve was used to determine the absorbance of the sample being analyzed and the amount of ammonia nitrogen in the individual samples. The concentration of ammonia nitrogen in rumen fluid was extrapolated directly from the spectrophotometer readings.