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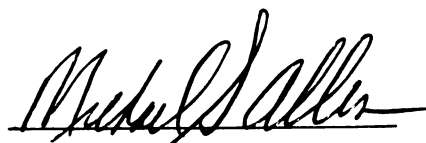
**EFFECTS OF DIETARY LACTOSE COMPARED WITH GROUND
CORN ON THE GROWTH RATE OF RUMINAL PAPILLAE AND RATE
OF VALERATE ABSORPTION FROM THE RUMEN**

presented by

JING XU

has been accepted towards fulfillment
of the requirements for

M.S. degree in ANIMAL SCIENCE


Major professor

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THE GROWTH RATE OF RUMINAL PAPILLAE AND RATE OF VALERATE
ABSORPTION FROM THE RUMEN**

By

Jing Xu

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ABSTRACT

EFFECTS OF DIETARY LACTOSE COMPARED WITH GROUND CORN GRAIN ON THE GROWTH RATE OF RUMINAL PAPILLAE AND RATE OF VALERATE ABSORPTION FROM THE RUMEN.

By

Jing Xu

Eight ruminally cannulated dry, non-pregnant Holstein cows were used in a crossover design experiment to evaluate the effect of diet on growth rate of ruminal papillae and rate of valerate absorption from the rumen. Periods consisted of three, 14-day sub-periods. In the first sub-period (S), a diet of wheat straw was offered at 1% of body weight to shrink the ruminal papillae. This was followed by two sub-periods (G1 and G2) in which treatment diets containing either ground corn or a mixture of ground corn and food grade lactose were offered at 1.5% of body weight. Papillae surface area increased with time for both treatments and a significant treatment by sub-period interaction indicated that growth rate of ruminal papillae was greater with lactose treatment. Rate of absorption of valerate increased with both treatment diets from sub-period S (0.14 /h) to sub-periods G1 and G2 (0.24, and 0.23 /h, respectively) but no effect of treatment diet was detected. Although papillae surface area might have limited rate of valerate absorption from sub-periods S to G1, other factors probably limited rate of valerate absorption later in the period.

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LIST OF ABBREVIATIONS AND SYMBOLS

ATP	adenosine tri-phosphate
BW	body weight
CP	crude protein
DNA	deoxyribonucleic acid
DM	dry matter
DMI	dry matter intake
MCY	microbial cell yield
MM	molecular mass
NAD⁺	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
OM	organic matter
RFA	fermentation acid produced in the rumen
VFA	volatile fatty acids
WH	correction for water added from hydrolysis of OM to hexose
TMR	total mixed ration

INTRODUCTION

High producing dairy cows are challenged by a shortage of energy in early lactation (NRC, 1989). This is caused by depressed feed intake and high energy demand for milk production. In order to meet energy requirements, high producing dairy cows consume highly fermentable diets which result in the production of large quantities of volatile fatty acids. However, these acids must be either neutralized or absorbed, otherwise acidosis will occur (Allen, 1997). Ruminal acidosis results in lower dry matter intake (DMI) and might result in laminitis, rumen ulcers, liver abscesses, and other health problems such as displaced abomasum, ketosis, and hepatic lipidosis. These metabolic diseases will result in lower milk production, loss of body weight, and decreased reproductive performance. Therefore it is very desirable to increase the absorptive capacity of the rumen to prevent excess accumulation of VFA in early lactation.

Preliminary evidence suggests a positive relationship between papillae surface area and VFA absorption rate, and papillae size is affected by the fermentability of diets (Dirksen et al., 1985). Highly fermentable diets such as cereal grains result in larger size while poorly fermented diets such as high forage diets result in smaller size of ruminal papillae. Ruminal papillae utilize fermentation acids for growth and butyrate is more stimulatory than other VFA (Weigand and Young, 1971; Fell and Weekes, 1974; Sakata and Tamate, 1978). Adding lactose and whey products which are rich in lactose resulted in increased molar proportion of butyrate and decreased molar percentage of propionate in rumens of cattle and sheep (Schingoethe, 1975; Schingoethe, 1980; Bragg, 1985). Thus, addition of

lactose to diets of cows prior to calving might accelerate adaptation of ruminal papillae and reduce incidence of ruminal acidosis and associated metabolic diseases.

LITERATURE REVIEW

The rumen and reticulum can account for more than 70% of total gastrointestinal tract mass in a mature ruminant (Church, 1975). Nearly all the rumen wall, except the dorsal rumen, is heavily populated by ruminal papillae which greatly increase the surface area available for absorption of VFA (Hofmann, 1973; Hofmann, 1982). The distribution, number and size of ruminal papillae are closely related to feeding habits, feed availability and digestibility. Under adverse feeding conditions, papillae size can be very small, rounded, thin and filiform. Otherwise they can be very large, thick and tongue shaped (Hofmann, 1993). It has been observed that ruminal papillae in 0 to 3 day old calves were extensively developed on the rumen wall with slender, finger-like shape and maximum length around 0.26 cm. As calves age and dry feeds are fed, papillae length can increase from 0.2 cm to 1.5 cm, depending on nutrition status (Beharka, 1992). Color of ruminal papillae can be pale, brownish, or black, depending on the diets cows are fed. Epithelial thickness of ruminal papillae is determined by rate of cell division in the basal layers and on transit time from proximal to exfoliating layers. Besides this, single cell death (apoptosis) is also important in regulating thickness of cell layers (Fell and Weekes, 1974). Blood and lymph vessels are densely distributed in papillae.

Ruminal epithelium is stratified squamous with four layers: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum (Steven and Marshall, 1970). However, it is difficult to identify the boundary between the stratum basale and stratum spinosum, while the borders of the stratum granulosum and stratum corneum are

relatively well defined (Steven and Marshall, 1970). The cells from strata basale and spinosum have abundant numbers of mitochondria, contribute most to metabolic properties of the tissue, and might be the most important cells in the metabolism of VFA. The stratum corneum plays a role as a barrier to nutrients, e.g., VFA, minerals, absorption across the ruminal wall. The functions of the most external cell layers of stratum corneum are: (1) as a barrier against the physical environment of the lumen; (2) as a site for attachment of ruminal microflora (Steven and Marshall 1970).

The stratified, horny, epithelial lining of the rumen plays a key role in several important physiological functions such as absorption, transport, metabolic activity, and protection, which presupposes their integrity and ready adaptation to the internal and external environment (Fell and Weeks, 1974; Gálfi et al., 1991). The development of the ruminal epithelium is especially important in affecting the absorption of VFA. Stevens (1970) promoted a hypothetical model of a ruminal epithelial VFA transport. He showed that the lumen-facing membrane of ruminal papillae was permeable to both dissociated and un-dissociated forms of VFA, whereas the blood-facing membrane was only permeable to the un-dissociated forms. First, intracellular carbonic acid is formed from CO_2 , which is produced by intracellular metabolism or absorbed from the rumen or blood, then, the intracellular carbonic acid acts as hydrogen ion donors to the dissociated forms of VFA to produce the un-dissociated form VFA. Thus, the increase in un-dissociated VFA form results in the increase of net fatty acid transport into blood.

Gaebel et al. (1987) showed that the membrane of ruminal papillae is permeable to minerals like sodium, chloride, and magnesium, and indicated that ruminal papillae provide the most important interface for ruminants to absorb minerals and water. Rumen movements are needed for eructation, rumination, inoculating the feed with microorganisms, and to help absorption by transferring VFA to the surface of the rumen wall (Ruckebusch, 1988).

The ruminal epithelium contributes approximately 1 – 2% of the total body mass of ruminants (Weeks, 1971; Harmon, 1986). The gut tissues are responsible for 18% of whole body O₂ consumption (Huntington and Tyrell, 1985). Ruminal papillae provide the interface for the absorption of most nutrients for the ruminant and they have an extensively distributed blood vessel system that provides for transportation and absorption of nutrients from the rumen. Metabolism of VFA occurs in ruminal papillae when VFA are absorbed from the rumen into the blood (Mailman, 1982; Dobson, 1984). Among VFA, butyrate is the most extensively metabolized in the rumen. It has been shown that the portal-arterial concentration differences in butyrate were significant and that 33 to 78% of butyrate was converted into ketone bodies (Weigand et al., 1971). Propionate is much less metabolized in the rumen epithelia compared with butyrate. Less than 5% of propionate absorbed is metabolized to lactate in the rumen epithelia, and most of the propionate reaches the liver unchanged (Elliot, 1980). Very little acetate is metabolized by ruminal papillae (Elliot, 1980).

Transition cows are defined as cows approximately 3-wk prepartum until 3-wk postpartum, and have been the subject of relatively little research until recently (Grummer, 1995). Cows experience a dramatic challenge such as parturition and lactogenesis during this period. It is also well known that immediately after calving, due to limited feed intake, mobilization of fat and protein to meet requirements for milk production occurs, which results in body weight and body condition loss during the first few months after calving (NRC, 1989). Excessive mobilization of the cow's body tissue can cause health problems such as poor reproductive performance, ketosis, and low milk yield (Kronfeld, 1970; Butler and Smith, 1989; Staples et al., 1990). Thus, increasing absorptive capacity might result in increased DMI by stabilizing ruminal pH and avoiding acidosis and improvement in energy balance is important for dairy cows.

Absorptive surface area is a function of rumen size, degree of fill, papillae surface area, and degree of hyperkeratosis (Dirksen et al., 1985; Dijkstra et al., 1993). Dirksen et al. (1985) demonstrated a positive relationship between papillae surface area and absorption rate of VFA. Gaebel et al. (1987) also found that ruminal papillae size was increased by high-energy diets and the absorptive capacity of rumen was increased with greater size of ruminal papillae.

Ruminal pH is primarily determined by balance between the production of fermentation acids by microbes in the rumen, and neutralization and absorption of fermentation acids produced (Allen, 1997). Continuous removal of hydrogen ion by absorption of VFA into the blood and passage to the lower gut, and neutralization from salivary buffer secretion

is important to maintaining ruminal pH (Allen, 1997). Ingestion of large amounts of rapidly fermentable carbohydrates within a short time can cause a sharp drop of ruminal pH, which may result in high concentration of lactic acid and acidosis (Dunlop, 1972; Counotte and Prins, 1979; Kezer and Church, 1979; Devisser and De Groot, 1981; Crichlow and Chaplin, 1985). Low ruminal pH might decrease DMI, fiber digestibility, and microbial yield and thus decrease milk yield and increase feed cost (Allen, 1997). However, maximizing energy intake usually results in slightly acidic conditions in the rumen. Considering the advantages and disadvantages of lowering ruminal pH, Allen and Beede (1996) recommended that ruminal pH range from 6.0 to 6.3 is the optimal point which can obtain maximal absorption of fatty acids without causing acidosis to dairy cows.

Causes of Acidosis

Acidosis in ruminants is often separated into several forms, including acute, chronic (sub-clinical), and subliminal types (Owens et al., 1998). Usually during the transition period, cows switch from high forage diet to high energy diet to meet the energy requirements for milk yield. However, if this switch occurs drastically, ingestion of a large amount of fermentable organic matter will produce a large amount of fermentation acids in the rumen, decreasing ruminal pH. Lactic acid can have a major effect on ruminal pH during adaptation to dietary changes (Counotte and Prins, 1981; Allen, 1997). Lactate-utilizing microbes are sensitive to low pH, whereas, lactate-producing microbes are not (Owen et al., 1998). *Streptococcus bovis* produces L-lactate and is the predominant lactate producer at pH 5.2 or greater (Slyter, 1976). When pH decreases below pH 5, lactobacilli

which can produce both L-lactate and D-lactate predominate (Hungate et al., 1952; Slyter, 1976). At normal rumen pH, *Streptococcus bovis* produces acetate and ethanol and little lactic acid (Russell and Allen, 1983). The production of lactate produced under this condition contributes little to the total VFA production in the rumen (Slyter, 1976; Gill et al, 1986). When pH in the rumen decreases to 5.0 or lower, many fiber utilizing rumen bacteria and protozoa die (Hungate, 1952; Krogh, 1961). Thus, abrupt increases in intake of highly fermentable carbohydrates could cause a sharp drop in pH, death of fiber digesting microbes, proliferation of lactate producers, and accumulation of lactate in the rumen (Hungate et al., 1952; Krogh, 1961; Slyter, 1976). Under this situation, the isomer accumulated in the rumen may shift from predominately L-lactate to 50:50 L-lactate: D-lactate (Essig et al., 1988).

Lactate (D or L) can be removed from the rumen by lactate-using microbes (e.g. *Megasphaera elsdenii*), by passage through the ruminoreticular orifice, or absorbed across the ruminal wall (Counotte and Prins, 1981). However, metabolization of lactic acid declines as numbers of lactate utiliziers decline with pH. In a destructive spiral, accumulation of lactic acid drives pH down further (Russell and Allen, 1983). Accumulation of acids increases osmolality in the rumen. When fluid in the rumen is hypertonic to blood plasma, water flow into the rumen could result in systemic dehydration (Allen and Beede, 1996). A greater absorption of acids occurs when acid concentrations are high, pH is low, and osmolality is normal (Tabaru et al., 1990). As L-lactate and D-lactate are absorbed into blood, L-lactate is usually converted into glucose through the pyruvate pathway in the liver (Armstrong, 1964). However, D-lactate can

not be metabolized in the liver, accumulation of lactate occurs in the blood, decreases the normal base-excess in the blood, resulting in decrease in blood pH and systemic acidosis (Owen et al., 1998). The acute acidosis usually results in tissue damage such as ruminal ulcers, liver abscesses and even possibly death (Allen and Beede, 1996).

Most VFA produced is removed from the rumen by absorption across the ruminal wall (Allen, 1997). Therefore the absorptive surface area is one of the important factors in determining the rate of absorption (Dijkstra et al., 1993). A greater absorptive surface area results in a greater absorption rate of VFA, which in turn, helps to decrease the deviation from the optimal pH. Thus, formulating a diet for cows prior to calving to increase rate of growth of ruminal papillae should help to reduce the incidence of acidosis for dairy cows when they are switched to the highly fermentable diets.

Factors Affecting VFA Production

The VFA are short-chain fatty acids, including acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and others. Usually valeric acid and higher chain acids constitute propionate, and butyrate, which account for 95% of the total VFA in the rumen. VFA are end products of anaerobic fermentation by microbial degradation of organic matter. The concentration of VFA in the rumen of the dairy cows is highly variable, usually ranging between 50 to 160 mM (Thurston, et al., 1968; Hoppe, 1984). It takes 2-4 hours for VFA to reach maximal concentration after feeding and it might take up to 48 hours for ruminal fermentation and VFA absorption to decline to less than 5% of its maximal value (Roe et al., 1966; Bergman and Wolff, 1971; Thomas and Martin, 1988). Under normal

conditions, ruminal pH is usually within the range of 5.5 and 6.5. VFA are weak acids ($pK_a \approx 4.8$) and are present in the gastrointestinal tract primarily in anion form (Bergman, 1990). Allen (1996) predicted the VFA production with the following equation based on estimation of organic matter truly digested in the rumen (RDOM) and the assumption of a constant microbial cell yield from the hexose equivalent fermented (MCY):

$$RFA = ((OMI \times RDOM)/(MM \times WH)) \times (1.0 - MCY) \times VFA$$

where RFA: fermentation acid produced in the rumen, OMI = organic matter intake, MM = molecular mass of hexose (0.180 kg/mol), WH = correction for water added from hydrolysis of OM to hexose (0.9). VFA = yield of VFA per mole of available hexose determined by fermentation balance (1800 meq of VFA/mol of hexose for 60% acetic acid, 25% propionic acid, and 15% butyric acid).

During the process of VFA absorption, each of the individual VFA is metabolized to a different extent by the ruminal epithelium, so there is a difference between the concentrations of VFA in the portal blood and the concentrations of VFA in the rumen.

VFA ratios are remarkably stable in spite of wide variation in microbial populations. The molar proportion of acetate: propionate: butyrate is usually around 65:25:10 with roughage diets and 50:40:10 for high concentration diets (Owens and Goetsch, 1993). Wolin and Miller (1983) estimated that in the rumen 58 glucose molecules will ferment to 65 Acetate + 20 Propionate + 15 Butyrate + 60 CO₂ + 35 CH₄ + 25 H₂O. In general,

75% of energy content of carbohydrate goes to VFA, 25% is used by microbes for growth or lost by as hydrogen and methane (Bergman, 1990).

Increasing Production of Butyrate

Dietary carbohydrates degraded in the rumen are comprised of starch, soluble sugars, pectin, hemicellulose, and cellulose. Usually diets rich in lactose such as whey favor butyrate production in the rumen (Grummer et al. 1983; Bragg et al, 1985). Whey is a by-product of the cheese manufacturing and contains mainly soluble sugar, predominately lactose (Schingoethe, 1976; Mackie et al., 1978). It was noticed that by adding small amount of lactose (1.1% of total dry matter intake), or small amount of dried whey (5% of concentrate mixture), there was a slight increase in butyrate concentration, and slight decrease in propionate concentration compared with control diet (Schingoethe and Rook, 1975; Schingoethe et al., 1976). However, substitution of 74% dried whole whey for corn in the concentrate mixtures resulted in a much greater molar percentage of butyrate and more fluid in rumen contents (Bragg et al., 1986). More fluid in rumen contents was probably caused by increased water intake in an effort to regulate the osmolality of the rumen fluid. Schingoethe et al. (1980) demonstrated molar percentages of butyrate increased, propionate decreased, and acetate percentage remained essentially unchanged in rumen contents of steers by feeding lactose or dried whey in different proportions (10, 20%, 30%, and 40% of concentrate mixes). This result is consistent with previous results (Schingoethe et al., 1972), which showed a linear increase in butyrate from either lactose or whey, and usually a decrease in propionate for

feeding whey or lactose. The acetate percentage was not changed, so increases in butyrate appear to be from reduced propionate.

Bragg et al. (1986) reported the factors influencing molar percentages of the rumen VFA when whey or lactose was fed to ruminants. First, the relative contents of these products in the diet and the absolute amounts consumed at each meal and per day have a significant effect on the VFA molar percentage. If the lactose and dried whole whey amount to less than 20% of the dietary DM and the animals are fed below ad libitum, increases in molar percentages of butyrate will be relatively low. Second, both frequency and pattern of feeding have large effects on the percentage of fermentation acids. Increasing meal frequency with feeding high lactose or whey diets seems likely to increase the butyrate percentage and decrease propionate percentage. This might be because variation in ruminal pH is decreased which could avoid the reduction in numbers of ciliate protozoa that produce butyrate. Therefore, high lactose content and increased frequency of feeding are desirable to obtain a high butyrate concentration in the rumen (Schingoethe, 1976; Poncet and Rayssiguier, 1980; Grummer et al., 1983).

Molasses and molasses-type liquid feeds are important byproducts of the sugar production industry; they usually contain high proportions of soluble carbohydrates. They are used extensively in feed to improve palatability, reduce dustiness, and added as readily fermentable carbohydrate source (Church, 1986; Wing et al., 1986). Kellogg and Owen (1969) reported that sucrose is the primary energy source of molasses. Waldo and Schultz (1960) demonstrated that sucrose reduced acetic acid and increased butyric acid

in rumen fluid. Wing et al. (1986) found that ruminal acetic acid proportion increased linearly and propionate decreased curvilinearly by adding 0%, 6%, 12%, and 18% of DM dietary citrus molasses distillers solubles in the diet. There was little change in butyrate concentration with 0, 6, and 12% solubles, but a dramatic increase with 18% solubles. Schingoethe et al. (1972) reported higher total VFA concentration and higher butyrate concentration by adding 11.8% demineralized whey and 5% molasses, compared with rolled shelled corn treatment.

VFA Absorption from the Rumen

The VFA produced in the rumen are removed by absorption across the ruminal wall and by passage through the omasal orifice (Ash and Dobson, 1963). Most acid is removed from the rumen by absorption across the ruminal wall in dairy cattle (Allen, 1997). Rate of absorption is determined by pH, absorptive surface area, the ability of the animal to metabolize absorbed fermentation acids, and ruminal motility (Dijkstra et al, 1995).

The gradient between VFA concentration in the rumen and that in the blood is an important factor in determining the rates of transfer (Dijkstra et al., 1993). A higher gradient results in greater diffusion of VFA across the ruminal wall. Other factors such as ruminal pH, VFA type, osmolality, blood flow, absorptive surface area affect the absorption rate as well (Dirksen et al., 1985). Absorptive surface area is a function of rumen size, degree of fill (Dijkstra et al., 1995), papillae length (Dirksen et al., 1985), and degree of hyperkeratosis (Hinders and Owens, 1965). All these factors affecting

absorption rate of VFA are related with each other, a change from one will result in a corresponding change of all other factors (Masson and Phillipson, 1951).

Ruminal pH influences rate of VFA absorption by the chain length of the individual acid. Danielli et al. (1945) demonstrated that shorter chain VFA were absorbed relatively faster than longer chain VFA at high pH. He indicated that the rate of absorption would be acetate > propionate > butyrate when $\text{pH} > 7$, while at $\text{pH} < 7$, the order would be reversed. At pH 7, the absorption rate would be equal. As pH on the luminal side decreases, the proportion of acid present in undissociated form increases, and rate of VFA absorption is increased. While pH increases, more VFA are present in the rumen in the dissociated form, thus, decreasing rate of absorption. At pH 7, more than 99% of each VFA present in the rumen is in the dissociated form; at pH 6, this decreases to about 6% of the total. In spite of the large difference in the percentage associated with slight pH variation, efficient removal of these fermentation end-products occurs because the equilibrium is maintained between free acids and dissociated forms (Allen, 1997).

The VFA are the major determinants of ruminal fluid osmotic pressure, both directly and by co- or countertransport of other ions (Dijkstra et al., 1993). Warner and Stacy (1972) concluded from several studies that no net flux of water across the ruminal wall occurred at osmolality in the range of 295 –360 mOsmol/l. When the osmolality of ruminal fluid is outside of this range, the osmotic pressure between the rumen and body fluids is not in balance and water will flush into or out of the rumen until equilibrium between the rumen fluid and blood is reached.

VFA Metabolism in the Rumen

Extensive metabolism occurs when VFA are absorbed across the ruminal epithelium.

The extent and site of metabolism of each individual VFA are different. Unlike butyrate and propionate, acetate passes through ruminal epithelium unchanged but is extensively used by smooth muscle of the gut wall and the adipose tissue in the omentum (Pethick et al., 1981). Propionate is the major precursor of glucose in the fed ruminant animals. From 3 to 15% is metabolized into lactate and pyruvate by ruminal epithelium during absorption (Weigand et al., 1972; Weekes, 1974; Weigand et al., 1975; Nocek et al., 1980). Under normal conditions, lactate will be converted to glucose in the liver but the quantity is very small (Armstrong, 1964). Most propionate is absorbed into blood unchanged and either oxidized or converted into glucose in the liver (Fahey and Berger, 1993). Propionyl-CoA synthetase activity is much less active in the rumen epithelium than in the liver, consistent with propionate being extensively metabolized in the liver, not in ruminal epithelium (Ash and Baird, 1973).

Butyrate is the most extensively metabolized VFA by ruminal epithelium. The extent of butyrate conversion into ketone bodies is negatively correlated to the absorption rate which in turn is influenced by factors such as ruminal pH and ruminal butyrate concentration (Weigand et al., 1971). Results from *in vivo* and *in vitro* studies of VFA uptake by the ruminal epithelium showed that 80% of the absorbed butyrate was converted into ketone bodies before it reached the portal vein (Bergman, 1990).

Research on cattle showed that the conversion rate of butyrate to ketone bodies was 49% (Weigand et al., 1971), whereas in goats 80 – 85% of butyrate was converted into ketone

bodies (Ramsey and Davis, 1965). It was reported that up to 90% of butyrate could be metabolized in the ruminal wall by comparing the net appearance of VFA in the portal blood of sheep with the net production rates of VFA in the rumen (Bergman et al., 1965; Bergman and Wolff, 1971; Beck et al., 1984). The production of ketone bodies includes acetoacetate (20%) and β -hydroxy-butyrate (80%) (Baldwin and Jesse, 1996).

Post-absorptive VFA Metabolism

The first step in the metabolism of VFA is activation. However the activities of the enzymes catalyzing this step are comparatively low. Therefore, the activities of these enzymes which regulate the rate of metabolism of VFA are more likely a limiting factor (Ash and Baird, 1973). Studies on the activity of mitochondrial Acyl Coenzyme A synthetase elucidate why acetate is mainly utilized by extrahepatic tissue, propionate by the liver, and butyrate by the ruminal epithelium (Ash and Baird, 1973; Ricks and Cook, 1978). Enzymes including acetyl-CoA synthetase, propionyl-CoA synthetase, and butyryl-CoA synthetase are found in ruminal epithelium, liver and other tissue such as the kidney. The activity of acyl-CoA synthetase is very low at birth, but it increases significantly in concert with development of ruminal fermentation indicating that acyl-CoA plays a very important role in VFA metabolism (Ricks and Cook, 1978). In ruminal epithelium, the amount of acetyl-CoA synthetase is too low to cause any significant utilization of VFA (Bergman and Wolff, 1971). Results from Ash and Baird's work (1973) indicate that acetyl-CoA synthetase activity is the lowest of the three in both liver and ruminal epithelium.

The ruminal epithelium has a significant potential to metabolize propionate without the interference of butyrate; presence of butyrate extensively reduces the activity of propionyl-CoA synthetase. It was reported that in the absence of acetate and butyrate, ruminal epithelium could metabolize 60-70% of propionate absorbed (Ash and Baird, 1973). By measuring the rate of formation of the corresponding CoA esters in homogenates of ruminal epithelium of cattle, it was found that propionate-activating capacity was reduced by approximately 87% by butyrate (Ash and Baird, 1973) indicating that most propionate would escape catabolism in the ruminal epithelium under normal ruminal condition.

Butyral-CoA synthetase is the most active of the three in ruminal epithelium and high butyral-CoA synthetase activity provides rapid activation of butyrate for oxidation from butyrate to β -hydroxy-butyrate by the epithelium (Cook et al., 1968). Other than this limiting enzyme, β -hydroxy- β -methylglutaryl-CoA synthetases is another limiting factor that may regulate the rate of the conversion of butyrate to ketone bodies by ruminal epithelium. The major pathway of physiological ketone-body formation is via the β -hydroxy- β -methylglutaryl-CoA pathway, however, this enzyme is the least active enzyme among the enzymes involved in this pathway (Baird et al., 1970). Another enzyme, β -hydroxy dehydrogenase likely plays key role in reducing acetoacetate to β -hydroxy-butyrate in the ruminal epithelium. It regulates the conversion of butyrate to β -hydroxy-butyrate by maintaining the $[NAD^+]/[NADH]$ ratio.

In the rumen, the presence of butyrate inhibits activation of acetate and propionate, most probably by decreasing the activity of acetyl-CoA synthetase and propionyl-CoA synthetase in ruminal epithelium. In general, activity of these enzymes is consistent with the high rate of conversion of butyrate into ketone bodies in the ruminal epithelium.

Opposite effects of butyrate on ruminal epithelial cells *in vivo* and *in vitro* identified the importance of hormone function on growth of ruminal epithelia (Sakata and Tamate, 1976; Neogrdy et al., 1989). Sakata and Tamate (1976) showed that butyrate administration on sheep enhanced growth rate of ruminal epithelial cells *in vivo*, whereas Neogrdy et al. (1989) found butyrate inhibited both DNA synthesis and the entire mitotic process *in vitro*. This suggested a possible endocrine mediator role *in vivo*, which is not present *in vitro*. Glfi et al. (1991) postulated that collaboration of butyrate, insulin, and glucagon might be responsible for the progressive adaptation of ruminal mucosa while collaboration of cortisol and butyrate influence both progressive and regressive adaptation. More work is needed to test the postulation.

Effects of Blood Flow on VFA Absorption and Metabolism

It is well recognized that digestion and absorption increase blood flow to the gastrointestinal tract of all animal species (Sellers, 1964; Thorlacius, 1972). In ruminants, large quantities of VFA and CO₂ are produced in the rumen from microbial fermentation which are the two most important factors affecting blood flow in the reticulorumen (Sellers, 1964; Thorlacius, 1972; Bergman, 1990). Annison (1964) demonstrated that the increasing CO₂ and VFA concentration could increase blood flow

in the ruminal artery, which is the major supply for the posterior and ventral parts of the rumen. Butyrate is the most effective VFA in stimulating right ruminal arterial flow, and there is a linear relationship between butyrate and blood flow rate with concentrations from 0 to 20 mM (Thorlacius, 1972). At 20 mM or higher, increasing concentration of butyrate will still result in increased blood flow but the relationship is not proportionate. This is probably because the increase of blood flow was caused by dilation of the blood vessels, reaching the maximal of dilation at 20 mM or higher. At 40 mM, butyrate tends to inhibit motility of the rumen (Sellers, 1964).

Thorlacius (1972) found that applied carbon dioxide and VFA in the rumen could increase the content of hemoglobin and the effect was localized to the mucosal area of application. Buffered solutions of butyrate, acetate, saline, or saline solution gassed with carbon dioxide were individually sprayed into the caudodorsal blind sac and a small area of the ventral sac. Ruminal papillae samples were taken from the area where these buffer solutions had been applied and hemoglobin content of the ruminal papillae was measured. It was observed that butyrate (200mM) greatly increased hemoglobin content in the area to which it was applied, acetate solution (200mM) and saline solution gassed with carbon dioxide also significantly increased hemoglobin content in the area where they were applied, but to a lesser extent. The areas where solutions of acids were applied appeared redder. Results showed that VFA and carbon dioxide caused 40 to 100% increase in blood content of ruminal papillae which was localized and that butyrate had the greatest effect (Thorlacius, 1972).

Anatomic studies showed that ruminal epithelium is highly vascularized, having lavish blood supply, a remarkably dense capillary network and a high blood content (Hofmann, 1973). Physiological studies showed that in conscious sheep, ingestion of feed increased blood flow in the epithelium of rumen and reticulum (Dobson et al., 1981; Edelstone and Holzman, 1981). The increase in blood flow to the rumen and reticulum could be matched by an increase in cardiac output and /or a decrease in flow to other vascular beds (Chou, 1983). Stephenson (1997) reported that in resting animals, only 20% of the systemic blood is found in arteries, arterioles, and capillaries, with most of the blood residing in veins which are known as blood reservoirs. VFA and carbon dioxide can directly act as vasodilators on the blood vessels in the epithelium and in the ruminal wall (Fleming and Arce, 1986). Another theory is that animal can mobilize the 'resting' blood out of veins into arteries, and this could account for 30 to 40% increase or decrease of total blood flow (Folkow et al., 1963). Although other factors could influence blood supply in the rumen as well, the vasodilation of blood vessels and mobilization of more blood into the rumen are the two most important factors for regulating blood flow in the rumen (Folkow et al., 1963; Annison, 1964; Thorlacius, 1972; Chou, 1983; Fleming and Arce, 1986).

Mechanism of Papillae Growth

Cells constantly adapt themselves to changes of environment. Under normal conditions, the adaptation is called physiologic adaptation, which is usually induced by hormones or endogenous chemical substances; under abnormal conditions, the adaptation is called pathologic adaptation (Cotran et al., 1994). Adaptations include changes in cell number,

size, and differentiation. An increase in cell number is called hyperplasia which is usually a response to hormones and tissue healing. Hyperplasia involves an initial mitogenic stimulus, followed by the synthesis of a number of proteins that regulate a cascade of events governing progression through each cell cycle (Preisig and Franch, 1995). Increases in cell size but no recruiting of new cells is called hypertrophy.

Hypertrophy is a response to hormonal stimulation and environmental change. Both synthesis of more structural components (cell cycle-dependent hypertrophy) and decreased protein degradation (cell cycle-independent hypertrophy) account for the increase of cell size (Preisig and Franch, 1995). Atrophy is the shrinkage in the size of the cell by loss of cell substance. Causes of atrophy include decreased workload, loss of innervation, diminished blood supply, poor nutrition, loss of endocrine stimulation, and aging (Cotran et al., 1994).

Protein to DNA ratio has been used as an indicator of cell size and RNA to DNA ratio has been used as indicator of protein synthetic capacity (Clowes et al, 1998). Both of these two parameters are broadly used in the study of muscle fiber. Hyperplasia does not result in the change of protein to DNA ratio and hypertrophy results in increased protein to DNA ratio.

Bevington et al. (1994) reported that metabolic acidosis can lead to tubular hypertrophy *in vivo*, and low pH exerts more direct effect on cell growth than higher pH *in vitro*.

It is well recognized that high energy diets are more stimulatory to papillae growth; low pH and acidosis occur to the ruminant as a result of switching from high forage to high

concentrate diets. The factors that regulate papillae growth and mechanisms involving hyperplasia and hypertrophy need further study.

Papillae size can increase very quickly. Early studies on ruminal papillae development with calves showed that the oxygen consumption and ketone-body production by ruminal epithelia increased with age in the presence of butyrate. VFA, especially butyrate stimulate blood flow in ruminal papillae, and blood flow can be used as an index of energy expenditures by the body organs or tissues (Hird and Weidemann, 1964). Elevation of ruminal butyrate resulted in an increase in mitotic indices and in blood flow to ruminal papillae (Sakata and Tamate, 1978; Sakata and Tamate, 1979). In sheep rumen, papillae and muscle portion have about the same weight but about 90% of blood goes to papillae. Greater blood flow can supply more oxygen to ruminal papillae and remove VFA at a greater rate. Energy expenditures are closely correlated with papillae size. Measurement of O₂ consumption with adult sheep showed that conversion of the butyrate into ketone-bodies accounts for 70% of the oxygen consumption, and this metabolic activity can supply substantial proportion of energy requirement for the papillae development (Hird and Weidemann, 1964). Walker and Simmonds (1962) suggested that metabolism of butyrate as ruminal epithelium ketone-body-forming system, and hypothesized that this system was responsible for the development of ruminal papillae. This system is present when ruminant animals are still on milk diets, even though its level is very low compared with normal activity.

ATP is the universal currency of energy and FADH_2 and NADH can produce 2 and 3 high-energy bonds, respectively. These high-energy bonds supply energy for biosynthesis of ruminal papillae. During the metabolism of butyrate to acetoacetate, ruminal epithelial tissue derives a net yield of three 3 high-energy phosphate bonds. A favorable NADH to NAD^+ ratio which is maintained by β -hydroxy-butyrate dehydrogenase is important in regulating the energy supply in the ruminal epithelium (Weigand et al., 1971).

Epithelial cells especially in the basal and intermediary sections of the epithelium are rich in mitochondria that could provide adequate energy supply for papillae growth (Hydén and Sperber, 1965; Henrikson, 1970; Steven and Marshall, 1970). Other factors such as the level of hormones, the amount of enzymes, as well as their activities are also important in regulating biosynthesis of papillae. As previously mentioned, among important enzymes in ruminal papillae, butyral-CoA synthetase is the most active in the ruminal epithelium. This high butyral-CoA synthetase activity provides rapid activation of butyrate for the following metabolism to β -hydroxy-butyrate by the epithelium, securing the production of ATP for the ruminal epithelium (Cook et al., 1968).

The studies mentioned above indicate that conversion of butyrate to ketone bodies is the main source of energy supply, and this is controlled by butyral-CoA synthetase which is very active in ruminal epithelium. Faster blood flow removes VFA quickly, increasing the concentration gradient and rate of absorption of VFA from the rumen and replenishes the supply of oxygen to ruminal papillae. Ruminal epithelia have abundant mitochondria,

which are able to extensively metabolize VFA, especially butyrate to obtain energy for growth. These factors help explain why papillae can increase size in such a short time and why butyrate is so stimulative to papillae growth.

OBJECTIVE

Increasing absorptive surface area in the rumen of dairy cows prior to calving might result in increased rate of volatile fatty acid absorption, higher ruminal pH, and decreased incidence of acidosis in early lactation. Positive relationships were reported among amount of grain feeding, ruminal papillae surface area, and absorption rate of volatile fatty acids (Dirksen et al. 1985) and minerals (Gaebel et al. 1987). Lactose feeding has been reported to increase ruminal butyrate concentration (Schingoethe et al., 1972; Schingoethe et al., 1980; Windschitl and Schingoethe, 1984; Bragg et al., 1986) which is the predominant energy source for ruminal papillae. The objective of this experiment was to compare the effects of partial substitution of lactose for ground corn in the diet on growth rate of ruminal papillae and rate of absorption of valerate from the rumen.

MATERIALS AND METHODS

Experimental Design

Eight ruminally cannulated dry, non-pregnant Holstein cows were used in a crossover design experiment. Rumens were evacuated 7 days before initiation of the experiment and cows were blocked by rumen volume and assigned randomly to a replicated ($n = 4$) crossover design (Figure 1). Periods were 42 days in length and consisted of three, 14-day sub-periods. In the first sub-period (S), a diet of wheat straw was offered at 1% of body weight to shrink the ruminal papillae. This was followed by two sub-periods (G1 and G2) in which treatment diets were offered (Figure 2). Treatments were ground corn or food grade lactose and treatment diets are described below.

Cow Management

An animal use form was submitted and approved by the Michigan State University All-University Committee on Animal Use and Care and the Department of Animal Science, Michigan State University. Surgery for ruminal cannulations was performed at the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, MSU. Cows were housed individually in comfort stalls in the south barn at the MSU Dairy Cattle Teaching and Research Center. These stalls were bedded twice daily at 0500 h and 0800 h with sawdust. Fresh water was accessible 24 h per day. Diets were offered at 1% BW during sub-period S and 1.5% BW during sub-periods G1 and sub-period G2 to avoid excessive fattening. Cows were fed once daily at 0800 h except during sample and data collection on d 12, 13, and 14 of sub-periods G1 and G2 when cows were fed twice

daily at 0800 h and 2000 h. On d 12, 13, and 14 of sub-periods G1 and G2 treatment diets for cows within a block were mixed and fed to both cows.

Diet Formulation

During sub-period S, a diet of wheat straw was offered at 1% of BW to shrink the ruminal papillae. Trace mineral salt blocks were accessible for each animal. Treatment diets consisted (on DM basis) of chopped grass hay (36.1%), ground corn grain (21.2%), soybean meal (16.5%), mineral and vitamin premix (3.3%) and either additional corn (23%) for the corn treatment or food grade lactose (18.6%) and additional soybean meal (4.4%, to equalize the crude protein content of treatments) for the lactose treatment (Table 1). Diets were mixed and diet dry matter was offered at 1.5% of body weight. Diets were formulated to contain 1.63 Mcal NE_L / kg, 29% NDF, and 16% CP. The forage used was an orchard grass-timothy hay mixture which was chopped and mixed with water in a ratio of two parts hay to three parts water before mixing with treatment concentrates in a total mixed ration.

Sample and Data Collection

Samples of ruminal liquid for analysis of VFA concentrations were taken every 6 h for 24 h beginning at 1300 h on d 12 of each sub-period. Ruminal fluid pH of each sample was measured immediately after sampling. Ruminal contents were evacuated at 1400 h on d 13 of each sub-period and ruminal papillae were biopsied from 3 sites in the rumen. The centerpoint of site 1 was on the left wall of the ventral sac approximately 14 cm below the lower lip of ruminal cannula. The centerpoint of site 2 was on the right side of the

rumen directly opposite site 1. The centerpoint of site 3 was on the cranial pillar facing the ventral sac. At least 12 ruminal papillae were biopsied from each site each time. Three ruminal papillae were placed in a plastic bag and frozen immediately with dry ice for DNA content and weight analysis (DM basis). Three ruminal papillae were fixed in 10% formalin solution for mitotic index and blood vessel analysis, and three ruminal papillae were stored at 4 °C in an isotonic saline solution for image analysis.

Following biopsy of ruminal papillae, half of the ruminal digesta of each cow was exchanged with the half of the ruminal digesta from the other cow within each block. The ruminal digesta from both cows within a block were mixed and placed back into the rumens of both cows. The purpose of this procedure was to minimize differences in characteristics of digesta such as ruminal pH on the disappearance of Co-EDTA and valerate from the rumen.

Co-EDTA solution was prepared by the method described by Udén et al. (1980). Co-EDTA solution contained 5 grams of Co-EDTA dissolved in 250 ml dH₂O. Two hundred milliliters of valeric acid solution (>99%) was adjusted to pH 6 with NaOH (12.5%), and brought up to 384 ml in volume by addition of distilled water. Solutions containing valeric acid (384 ml) and Co-EDTA (250 ml) were pulse-dosed into five sites of the rumen at 1700h on d 13 of each sub-period. Ruminal fluid was sampled at 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 13, 16, 19, 25, 31, 38 h after dosing.

Approximately 90 ml of ruminal fluid was collected at each time after dosing. Rumen digesta was collected from 5 sites in the rumen and strained through nylon mesh to obtain

the rumen fluid samples. Sampling sites were anterior dorsal sac (ADS), anterior ventral sac (AVS), caudo-dorsal blind sac (CDS), and the caudo-ventral blind sac (CVS).

Ruminal fluid samples were frozen at -20°C until analysis.

Sample Analysis

VFA analysis Ruminal liquid samples were thawed at room temperature and centrifuged at $13,000 \times g$ for 30 min at 4°C for VFA and valeric acid determination by HPLC as described by Dado and Allen (1993). Samples were then re-frozen.

Cobalt concentration analysis In preparation for cobalt analysis, rumen fluid samples were thawed at room temperature, centrifuged at $13,000 \times g$ for 30 min at 4°C . Fifteen milliliters supernatant were removed and placed into 100 ml volumetric flasks with flat bottoms and dried overnight in an oven at 110°C . The residue was digested with sulfuric acid and H_2O_2 (Hach et al., 1987), and diluted to 100 ml with distilled H_2O . The concentration of cobalt in each sample was measured by atomic absorption spectrometry (SpecAA 200 Varian, Palo Alto, CA). Standard curves for cobalt were created in a ruminal fluid matrix to correct for mineral interactions. The ruminal fluid for the curve was a composite of 15 ml sub-samples of the 0 h samples of ruminal fluid from all cows and all periods.

Image analysis For determination of size, ruminal papillae were washed in an isotonic saline solution (0.9%) to remove particulate matter, and blotted with absorbent tissue to remove the liquid on the surface before analysis. The papillae were stained with black

tissue dye (The Davidson Marking System®, Bloomington, MN) before they were scanned (Hewlett Packard Scanjet II cx, Palo Alto, CA). Image analysis software (NIH Image 1.61, National Institutes of Health, Bethesda, MD) was used to determine surface area, length and width of each papillae.

DNA content analysis To prepare ruminal papillae for analysis of DNA content, frozen ruminal papillae were thawed at room temperature, washed with an isotonic saline solution to remove particulate matter, then placed in a 5 ml pre-numbered snap-top vials and freeze dried for 24 h in a lyophilizer (Speed VAC SC110, Savant Company, Farmingdale, NY). The dry weight of papillae sampled from each site was recorded. The freeze-dried ruminal papillae were stored for subsequent DNA content analysis.

The DNA content of ruminal papillae was determined with a Hoechst H 33258 kit (Sigma-Aldrich, St. Louis, MO), using the method described by Labarca and Paigen (1980). The freeze dried ruminal papillae samples were soaked in 650 ml phosphate-saline buffer solution (0.05 M NaPO₄, 2.0 M NaCl, pH 7.4) for one hour, homogenized for 1 min with a tissue homogenizer (Tekmar®, Cincinnati, Ohio) followed by sonification for 30 sec (Sonicator Cell Disruptor, Plainview, NY). The samples were then centrifuged at 8,000 x g for 30 min at 4 °C. Twenty microliters of supernatant were collected and diluted by adding 3180 µl of the phosphate-saline buffer solution. The diluted sample was then pipetted (160 µl) into a well of a microtiter immunoassay plate (Dynex, Chantilly, VA). Forty microliters of Hoechst 33258 dye solution (10 µg/ml) was added to each well. Samples were read in a multi-well plate reader (CyToFLUOR®

series 4000, Sunnyvale, CA) with excitation at 360nm and emission at 460 nm. Calf thymus DNA (Sigma Chemical Co, St. Louis, MO) was used to for preparation of the standard curve.

Mitotic index and blood vessel number analysis of site 1 Tissue slides were prepared with the ruminal papillae fixed in 10% formalin solution. Samples were dehydrated with alcohol for 24 hours and embedded in paraffin. Sections (1 µm thick) were cut and stained with hematoxylin and eosin stains. Mitotic index was calculated as the number of basal cell nuclei showing mitotic figures as a percent of the total number of basal cell nuclei observed. Vessel number and surface area were analyzed for each section of rumen papillae by JAVA® Video Analysis Software (Jandel Scientific, Corte Madera, CA). Vessels were identified as veins and lymph or arteries.

Determination of Rate Constants

Rate of concentration decline of valerate from the rumen over time was estimated by nonlinear regression using JMP® (Version 3.2, SAS Institute Inc., Cary, NC) with the equation:

$$A = A_0 \times e^{-kt}$$

Where

A = valerate concentration at time = t,

A₀ = initial valerate concentration in the rumen at time = 0,

k = rate of concentration decline of valerate over time, h⁻¹

t = time postdosing (h).

Rate of concentration decline of cobalt over time was estimated by nonlinear regression using JMP® (Version 3.2, SAS Institute Inc., Cary, NC) with the equation:

$$A = A_0 \times e^{-kt} + BG$$

Where

A = cobalt concentration at time = t,

A₀ = initial cobalt concentration in the rumen at time = 0,

k = rate of concentration decline of cobalt overtime, h⁻¹

t = time postdosing (h), and

BG = background concentration of cobalt in the rumen.

Rate of absorption of valerate was estimated by subtracting rate of concentration decline of cobalt over time from rate of concentration decline of valerate over time.

Statistical Analysis

All data for papillae measurements except calculated changes over time were analyzed using the fit model procedure of JMP® (Version 3.2, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijklmn} = \mu + P_i + D_{j(i)} + T_k + B_l + C_{m(i)} + S_n + T_k * D_{j(i)} + T_k * S_n + T_k * B_l + S_n * D_j + B_l * S_n + B_l * D_{j(i)} + T_k * D_j * S_n + T_k * B_l * S_n + T_k * B_l * D_{j(i)} + B_l * D_{j(i)} * S_n + B_l * D_{j(i)} * S_n * T_k + e_{ijklmn}$$

μ = overall mean;

P_i = random effect of period (i = 1 to 2);

$D_{j(i)}$ = fixed effect of sub-period (ordinal, $j = 1$ to 3);
 T_k = fixed effect of treatment ($k = 1$ to 2);
 B_l = fixed effect of block ($l = 1$ to 4);
 $C_{m(l)}$ = random effect of cow within block ($m = 1$ to 2);
 S_n = fixed effect of site ($n = 1$ to 3);
 $T_k * D_{j(i)}$ = interaction of treatment by sub-period;
 $T_k * S_n$ = interaction of treatment by site;
 $T_k * B_l$ = interaction of treatment by block;
 $S_n * D_{j(i)}$ = interaction of site by sub-period;
 $B_l * S_n$ = interaction of block by site;
 $B_l * D_{j(i)}$ = interaction of block by sub-period;
 $T_k * D_{j(i)} * S_n$ = interaction of treatment, sub-period, and site;
 $T_k * B_l * S_n$ = interaction of treatment, block, and site;
 $T_k * B_l * D_{j(i)}$ = interaction of treatment, block, and sub-period;
 $B_l * D_{j(i)} * S_n$ = interaction of block, sub-period, and site;
 $B_l * D_{j(i)} * S_n * T_k$ = interaction of block, site, and treatment; and,
 e_{ijklmn} = residual, assumed to be normally distributed.

Data for VFA concentration, rates of Co and valerate concentration decline, and rate of absorption of valerate, mitotic index, blood vessels were analyzed using the following model:

$$Y_{ijklmn} = \mu + P_i + D_{j(i)} + T_k + B_l + C_{m(l)} + T_k * D_{j(i)} + B_l * T_k + B_l * D_{j(i)} + B_l * T_k * D_{j(i)} + e_{ijklmn}$$

- μ = overall mean;
- P_i = random effect of period ($i = 1$ to 2);
- $D_{j(i)}$ = fixed effect of sub-period (ordinal, $j = 1$ to 3);
- T_k = fixed effect of treatment ($k = 1$ to 2);
- B_l = fixed effect of block ($l = 1$ to 4);
- $C_{m(l)}$ = random effect of cow within block ($m = 1$ to 2);
- $T_k * D_{j(i)}$ = interaction of treatment by sub-period;
- $B_l * T_k$ = interaction of block by treatment;
- $B_l * D_{j(i)}$ = interaction of block by sub-period;
- $B_l * T_k * D_{j(i)}$ = interaction of block, treatment, and sub-period; and,
- e_{ijklmn} = residual, assumed to be normally distributed.

Calculated changes in data over time for papillae measurements were analyzed using the following model:

$$Y_{ijklmn} = \mu + P_i + T_j + B_k + C_{l(k)} + S_m + T_j * S_m + P_i * B_k + P_i * S_m + B_k * T_j + B_k * S_m + P_i * B_k * S_m + B_k * T_j * S_m + e_{ijklm}$$

- μ = overall mean;
- P_i = random effect of period ($i = 1$ to 2);
- T_j = fixed effect of treatment ($j = 1$ to 2);
- B_k = fixed effect of block ($k = 1$ to 4);
- $C_{l(k)}$ = random effect of cow within block ($l = 1$ to 2);

S_m = fixed effect of site ($m = 1$ to 3);
 $T_j * S_m$ = interaction of treatment by site;
 $P_i * B_k$ = interaction of period by block;
 $P_i * S_m$ = interaction of period by site;
 $B_k * T_j$ = interaction of block by treatment;
 $B_k * S_m$ = interaction of block by site;
 $P_i * B_k * S_m$ = interaction of period, block, and site;
 $B_k * T_j * S_m$ = interaction of block, treatment, and site; and,
 e_{ijklm} = residual, assumed to be normally distributed.

For all models interactions were not included in the model when the effect was not significant ($P > 0.10$). Treatment effects were declared significant at $P < 0.05$ and interactions were declared significant at $P < 0.10$, unless otherwise note.

RESULTS

Dry Matter Intake

Refusals of wheat straw of up to 30% were common for up to 3 days following introduction of wheat straw diets. However, refusals were rare after this adaptation period. When cows were switched to treatment diets, refusals were common (up to 80%) for cows consuming the lactose diets for up to 3 days after introduction, but no refusals were observed for cows consuming the diets with corn treatments. After adaptation, cows consumed both diets within 1 h of feeding. No feed refusals were noticed during sub-period G2 for either treatment.

Volatile Fatty Acid Concentration

There was a significant effect of treatment on concentration of total VFA with higher concentration for corn treatment compared with lactose treatment (60.8 mM vs. 53.7 mM, $P < 0.05$, table 2). There also was a significant treatment by sub-period interaction ($P = 0.01$). During sub-periods S and G1, no significant effect of treatment on concentration of total VFA was detected. However, during sub-period G2, corn treatment had a greater concentration of total VFA (81.9 mM vs 60.3 mM, $P < 0.0001$, table 2). Corn treatment increased acetate, and butyrate but not propionate concentrations compared with lactose treatment. Treatment by sub-period effects were significant for acetate, propionate, and butyrate concentration for probably different reasons. There was no effect of treatment on acetate or butyrate concentrations for sub-periods S and G1, but both acetate and butyrate concentrations were higher for corn treatment in sub-period G2. Differently,

there was no effect of treatment on propionate concentration in sub-periods S and G2, but propionate was higher for lactose treatment in sub-period G1.

Papillae Growth

There was a significant effect of sub-period on papillae length ($P < 0.001$), width ($P < 0.001$), and surface area ($P < 0.001$) indicating papillae growth when more fermentable diets were fed. Lactose treatment increased papillae length (10.2 vs. 8.7 mm), width (3.14 vs. 2.87 mm), and surface area (26.1 vs. 20.0 mm²), compared to corn treatment (Table 3). No difference in length, width, or surface area was detected after the wheat straw diet in sub-period S ($P > 0.10$), however, all size measurements were different ($P < 0.05$) for sub-periods G1 and G2. A significant interaction of treatment and sub-period for all size measurements ($P < 0.02$) indicated that growth rate of papillae differed by treatment over time. In addition, treatment contrasts for differences between sub-periods G2 and S and sub-periods G1 and S indicate that growth rate was greater for lactose treatment for all measurements than for corn treatment. Growth rate of lactose treatment for length, width, and surface area was greater across sub-periods G1 and G2 ($P < 0.02$) and within sub-period G1 ($P < 0.01$) but not for sub-period G2 ($P > 0.10$).

DNA Concentration

There was a significant effect of sub-period on DNA content ($P < 0.01$) which increased from sub-period S to G2 (Table 4). No significant effect of treatment or treatment by sub-period interaction was observed for DNA (0.0113 vs 0.0107, $P > 0.10$, Table 4).

However a trend ($P < 0.08$) for higher DNA concentration for corn treatment was observed for sub-period G1 (Table 4).

Mitotic Index

There was a significant effect of sub-period on mitotic index ($P < 0.01$) which decreased when the more fermentable diets were fed (Table 5). There was no significant effect of treatment on mitotic index of ruminal papillae ($P > 0.10$, table 5) and no interaction of treatment and sub-period on mitotic index ($P > .10$, table 5).

Blood Vessel Number and Area of Crosscut Section of Ruminal Papillae

No significant effect of sub-period on V/Area ($P = 0.18$), $(V+A)/\text{Area}$ ($P = 0.20$), and A/V ($P = 0.77$) were noticed, a tendency of significant effect on A/Area ($P = 0.09$) was observed. No significant effect of treatment or treatment by sub-period interaction was observed on number of vein and lymph vessels per mm^2 , number of arteries per mm^2 , total vessels per mm^2 , or ratio of vein and lymph vessels to arteries.

Relative Rate of Absorption of Valerate from the Rumen

A significant effect of sub-period on rate of valerate concentration decline in rumen fluid ($P < 0.0001$), was detected, but there was no sub-period effect on rate of cobalt concentration decline in rumen fluid ($P = 0.78$). A significant effect of sub-period on estimated rate of valerate absorption was detected ($P < 0.0001$).

There was no significant effect of treatment and no interaction of treatment by sub-period on rate of valerate concentration decline over time ($P > 0.10$, table 7). There was no significant effect of treatment and no interaction of treatment by sub-period on rate of cobalt concentration decline over time ($P > 0.10$, table 7).

No significant effect of treatment or interaction of treatment and sub-period on rate of absorption was observed ($P > 0.10$, table 7). However, the corn treatment tended to have higher rate of valerate absorption for sub-period S. Although difference in absorption rate between sub-periods G1 and S ($P > 0.10$) were not different by treatment, lactose tended ($P = 0.08$) to have a greater increase in rate of valerate absorption between sub-periods G2 and S.

DISCUSSION

DMI, Meal Patterns and VFA Concentration

Voluntary feed intake has been reported to be regulated by energy density or physical fill (Conrad, et al., 1964). Other factors such as taste, smell, texture, and visual appearance also act to regulate voluntary feed intake (Forbes, 1977). Rumens were all fully packed with wheat straw during rumen evacuation for sub-period S. However, DMI was not limited by physical fill because refusals were rare except for the first few days of sub-period S. The purpose of the wheat straw diet was to provide a diet with low fermentability diet and low production of butyrate or propionate in order to shrink the ruminal papillae. During sub-periods G1 and G2, cows were offered diets with either corn or lactose treatments at 1.5% BW to provide substrate for papillae growth. Intake was restricted to 1.5% BW to prevent excessive gain in body condition of the dry, non-pregnant cows consuming these high energy diets for a relatively long time period (8 weeks total) and to remove the potentially confounding effect of variable DMI. Although large feed refusals were observed for diets containing lactose for the first few days of sub-period G1, there were no refusals for either diet after adaptation. Initial refusals for diets containing lactose treatment were probably because cows had to adjust to the new taste of lactose.

Restricting intake might have been a problem in this experiment because the cows consumed the treatment diets very quickly (within 1 h) and rumens were not more than one-half full. Lactose is a very fermentable sugar which is rapidly fermented to VFA

(Bragg et al., 1985). Restricted DMI, rapid consumption of diet, and rapid fermentation all act to provide a very pulsatile flow of energy substrates to ruminal papillae within a day. These conditions might not accurately reflect those of dry cows close to calving that are consuming diets ad libitum with multiple meals throughout the day. In addition, concentration of VFA measured might not accurately reflect average daily concentration because of the rapid consumption of meals. Although diets were fed twice per day for the last 3 days of each period when samples were collected, samples for VFA analysis were taken 5 and 11 h after each feeding. The relatively low VFA concentrations observed in ruminal fluid might be because meals were rapidly consumed and largely fermented before the samples were taken and high VFA concentration for the corn treatment diets might reflect a slower rate of fermentation of corn relative to lactose.

The effect of treatment on butyrate concentration in ruminal fluid was unexpected.

Lactose feeding increased butyrate concentration in ruminal fluid in several experiments (Schingoethe and Rook, 1975; Schingoethe et al., 1976; Bragg et al. 1986; Schingoethe et al., 1980) and was expected to increase butyrate concentration in this experiment.

Although butyrate was not higher in sub-period G1 and was lower in sub-period G2 for lactose compared with corn treatment, it is possible that these relative differences are not representative of what occurred over time because an inadequate number of samples were taken. If lactose was fermented more quickly after meals and did result in greater butyrate production than corn, it might have been largely absorbed before the first sample was taken 5 h later.

Growth Rate of Ruminal Papillae

The effect of treatment on size and surface area of ruminal papillae is not consistent with the lower butyrate concentration for the lactose treatment. Butyrate is metabolized by ruminal papillae and supplies the majority of energy required for papillae growth (Weigand, 1971; Sakata and Tamata, 1979; Hoffman, 1993). Although lactose treatment resulted in increased papillae size and surface area, growth rate was higher for lactose treatment for sub-period G1 only. This indicates that maximum benefit of feeding lactose on papillae size was obtained within 2 weeks when lactose was fed at the level used in this experiment. Lower rates of growth for lactose treatment in sub-period G2 compared to sub-period G1 might have been because concentration of energy substrates for papillae growth became more limiting as the amount of papillae tissue increased. Although the production of energy substrates for papillae growth was similar between sub-periods G1 and G2, the amount of papillae tissue was greater for sub-period G2, reducing the supply of energy substrate per gram of papillae tissue.

DNA Content and Mitotic Index

The trend for lower DNA concentration ($P < 0.08$) for lactose treatment in sub-period G1 indicates that the increase in papillae size might be partly from hypertrophy. No treatment difference in DNA concentration ($P > 0.10$) in sub-period G2 is consistent with no treatment differences in growth rate for sub-period G2.

No significant difference was observed in mitotic index between the two treatments.

Sakata and Tamate (1978) reported that mitotic index increased 1 day after dosing

sodium butyrate into rumen, and tended to decline on following days. Sakata and Tamate (1979) reported that mitotic index of ruminal epithelium also responded to propionate and acetate, but the mitogenic effect was weaker than butyrate. No treatment differences for mitotic index in this experiment might be because the papillae were sampled too late after diet changes. Therefore, the effect of treatment on hyperplasia of papillae cells is not certain. Cotran et al. (1994) reported that although hypertrophy and hyperplasia are two distinct processes, both occur together frequently; they may well be triggered by the same mechanism. Therefore, it is probably true in the development of ruminal papillae, both hyperplasia and hypertrophy occurred concomitantly. However, one might be more important than the other during a certain time after diet change. Similarly, cell death (apoptosis) and atrophy together probably account for decreased papillae size when cows were switched to the wheat straw diet.

Blood Vessel Number

Increased rate of VFA absorption observed by Dirksen (1985) after papillae adaptation might be attributable to increased papillae surface area or to increased blood flow within papillae. It is well recognized that VFA stimulate ruminal arterial blood flow and that butyrate is the most effective of the VFA (Annison, 1964; Sellers, 1964). However, the effect of VFA on blood vessel size and blood vessel number in the ruminal papillae has not been reported. We found no significant effect of treatment on number of blood vessel and lymph vessels per mm² of crosscut section of papillae. Although we had intended to evaluate the effect of treatment on size of blood and lymph vessels, it was not measured

because we could not develop a suitable method for the large number of samples collected.

Relative Rate of Absorption of Valerate from the Rumen

Although Dirksen (1985) reported a relationship between papillae surface area and rate of VFA absorption from the rumen, other factors such as pH and the gradient between VFA concentration in the rumen and that in the blood also affect rate of VFA absorption.

Weigand et al. (1971) demonstrated that high hydrogen ion concentration and VFA concentration in rumen resulted in greater absorption rate. In this experiment, we minimized differences of VFA concentration and ruminal pH by mixing rumen digesta and diets prior to dosing the valerate and Co-EDTA. This was so that differences in absorption rate observed between treatments were primarily because of differences in papillae surface area and (or) blood flow.

Although there was no effect of treatment on absorption rate of valerate from the rumen detected for the experiment, the difference between absorption rate measured at the end of sub-period G2 minus that measured at the end of sub-period S tended to be different by treatment. This is because the corn treatment tended to have a greater absorption rate of valerate at sub-period S ($P = 0.08$) and had a numerically lower absorption rate at sub-period G2. This indicates that absorption rate continued to increase at a greater rate for lactose treatment than that for corn treatment during the 4-week treatment period. This is consistent with the greater rate of growth of papillae size for the lactose treatment and the observation by Dirksen (1985) that rate of absorption of VFA increased with papillae

size. However, only a marginal increase in absorption rate was detected in spite of a 43% greater surface area by the end of sub-period G2. This might be because the cows used in this experiment were dry cows with relatively low energy requirements compared with lactating cows. Surface area might not have been the limiting factor for absorption of valerate for these cows. Other factors such as blood flow or factors affecting the concentration gradient between the rumen and the blood might have been the limiting factor. However, this does not necessarily mean that surface area is not a limiting factor for VFA absorption under different conditions.

Thorlacius and Lodge (1973) found high concentrate diets cause the rumen epithelium to become very darkened, presumably hyperkeratinized. The color of ruminal papillae from lactose treatment was much darker than ruminal papillae from the corn treatment (personal observation). Fell and Weeks (1974) suggested that keratinized ruminal epithelium may influence absorption rate. Hinders and Owens (1965) also found that calves with induced hyperkeratosis lost about 50% VFA absorptive capacity. The darker color of rumen papillae on lactose treatment in this experiment was presumably caused by keratinization which might have negated any effect of increased papillae size on absorption rate of valerate.

SUMMARY

Lactose treatment did not result in increased butyrate concentration in the rumen at 5 and 11 h after feeding. However, diets were eaten within 1 h after feeding and lactose is quickly fermented, therefore, the times chosen for sampling were probably too late to detect peak fermentation from the lactose diet.

Lactose treatment increased papillae size and growth rate during sub-period G1, but did not increase growth rate during sub-period G2. Lower growth rate for lactose treatment in sub-period G2 compared with sub-period G1 might have been because concentration of energy substrates for growth became more limiting as the amount of papillae tissue increased.

Concentration of DNA (DM basis) tended to be lower for lactose treatment during sub-period G1, but no treatment difference was detected for sub-period G2. Lower DNA concentration indicates hypertrophy of ruminal papillae for the lactose diet. No treatment effect was observed on mitotic index or density of blood and lymph vessels.

No treatment difference was detected for rate of absorption rate of valerate from the rumen at the end of sub-periods G1 and G2 even though lactose treatment increased papillae size. However, absorption rate tended to be lower for lactose treatment at the end of sub-period S and the increase in absorption rate from sub-period S to G2 tended to

be greater for the lactose treatment. The biological significance of the greater increase in absorption rate over time for lactose treatment is difficult to assess.

CONCLUSION

Although lactose treatment resulted in greater papillae growth than corn treatment, absorption rate of valerate was not greater, and more work is required in this area before recommendations can be made regarding lactose addition to diets of cows prepartum.

The lack of relationship between papillae growth and valerate absorption should be investigated. Was absorptive surface area the rate limiting step for valerate absorption from the rumen of these animals? Does papillae surface area relate to absorptive surface area? Does lactose increase keratinization of ruminal epithelial tissue? To what extent will increased ketone production from metabolism of butyrate affect incidence of ketosis? These questions should be answered in subsequent experiments before practical application of this research.

Figure 1 **Timeline of the experiment.**

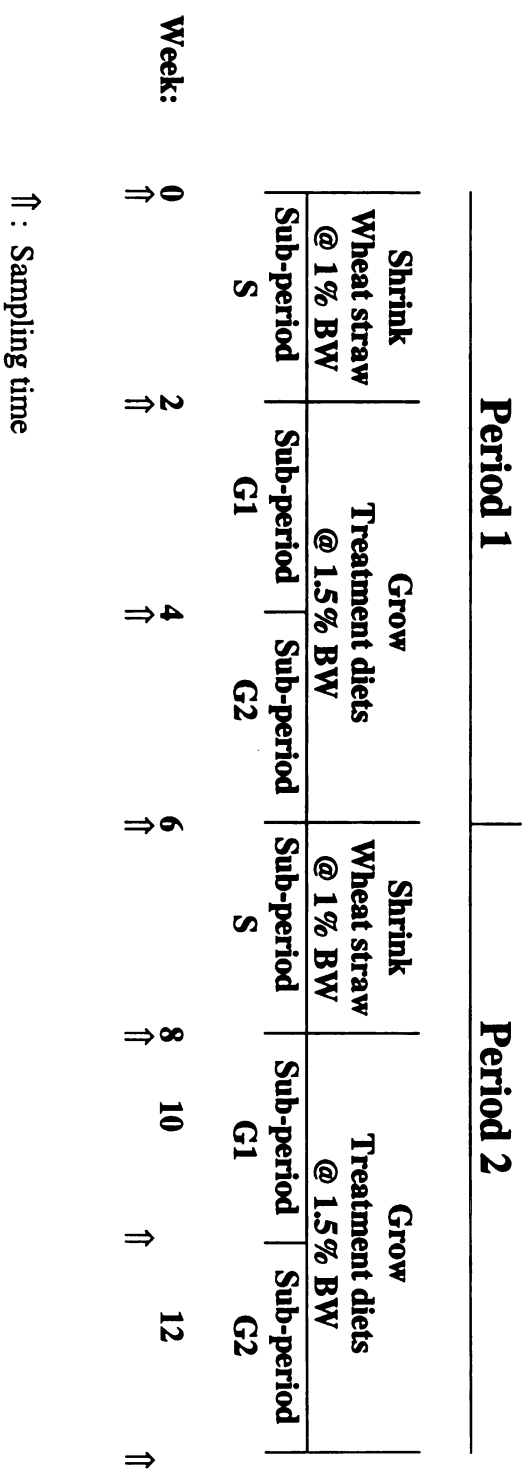


Figure 2 Sequence of treatments for individual cows.

	Sub-period	Cow 1, 3, 5, 7	Cow 2, 4, 6, 8
Period I	S	Wheat Straw	Wheat Straw
	G1	Lactose	Corn
	G2	Lactose	Corn
Period II	S	WS	WS
	G1	Corn	Lactose
	G2	Corn	Lactose

LIST OF TABLES

Table 1 Ingredient and chemical composition of diets.

	Treatment		
	Corn	Lactose	Wheat straw
Ingredients, % DM of diet			
Ground Corn	44.2		
Lactose Mix		44.2	
Grass Hay	36.1	36.1	
Wheat Straw			98.5
Soybean Meal	16.5	16.5	
Mineral & Vitamin	3.3	3.3	1.5
Chemical composition			
Dry Matter, % DM	58.0	59.0	88.2
NE _L (Mcal/kg)	1.63	1.63	0.97
CP, % of DM	16.0	16.0	3.0
NDF, % of DM	29.6	28.2	86.4
Ca, % of DM	0.60	0.60	0.18
P, % of DM	0.41	0.36	0.05

Vitamin & mineral mix composition: 31.2% dry ground corn, 22.7% limestone, 18.0% dicalcium phosphate, 9.3% sodium bicarbonate, 8.7% salt, 6.3 magnesium sulfate, 3.0% trace mineral premix, 0.40% vitamin A, 0.35% vitamin D, and 0.09% vitamin E.
Lactose mix composition: 47.6% dry ground corn, 41.9% food grade lactose, and 10.5% soybean meal.

Table 2 **Effect of treatment on concentration of volatile fatty acids in the rumen.**

	<u>Treatment</u>			<u>P value</u>		
	<u>Sub-period¹</u>	<u>Com</u>	<u>Lactose</u>	<u>SE</u>	<u>Treatment</u>	<u>Sub-period</u> <u>Trt x Sub-period²</u>
pH		6.43	6.57	± 0.03	0.005	<0.0001 0.159
Total VFA, mM	Overall	60.8	53.7	± 2.3	0.03	<0.0001 0.011
	S ³	36.8	38.4	± 3.9	0.77	
	G1 ⁴	64.1	62.7	± 3.9	0.79	
	G2 ⁵	81.9	60.3	± 3.9	<.0001	
Acetate, mM	Overall	41.4	34.4	± 1.5	<0.01	<.0001 0.006
	S	27.8	29.1	± 2.7	0.72	
	G1	41.9	36.5	± 2.7	0.15	
	G2	54.5	37.5	± 2.7	<.0001	
Propionate, mM	Overall	10.9	11.9	± 0.5	0.22	<.0001 0.07
	S	6.5	6.8	± 0.9	0.87	
	G1	12.5	15.8	± 0.9	0.01	
	G2	13.8	13.0	± 0.9	0.54	
Butyrate, mM	Overall	7.3	5.7	± 0.4	<.005	<.0001 0.003
	S	2.0	2.1	± 0.6	0.96	
	G1	8.2	7.6	± 0.6	0.47	
	G2	11.7	7.4	± 0.6	<.0001	

TABLE 2 (Continued)

- ¹Sub-period: include Sub-period S, Sub-period G1, and Sub-period G2;
²Ttt x Sub-period: Interaction of treatment by sub-period;
³S: The 2-week period when cows were on wheat straw diet;
⁴G1: First 2-week period of concentrate treatment;
⁵G2: Second 2-week period of concentrate treatment;

Table 3 Treatment effect on the growth of ruminal papillae.

	Sub period	Treatment			<i>P</i> value		
		Corn	Lactose	SE	Trt ¹	Sub ²	Trt x Sub ³
Length, mm	Overall	8.70	10.20	± 0.22	<.0001	<.0001	< .01
	S ⁴	7.80	7.96	± 0.38	0.76		
	G1 ⁵	8.60	10.76	± 0.38	<.00001		
	G2 ⁶	9.56	11.91	± 0.38	<.00001		
Growth rate, mm/d	0 – 28	0.063	0.141	± 0.018	<0.02		
	0 – 14	0.057	0.200	± 0.035	<0.01		
	14 – 28	0.068	0.082	± 0.039	0.80		
	Contrast 0 – 14 ⁷				<.0001		
	Contrast 0 – 28 ⁸				<.0001		
Width, mm	Overall	2.87	3.14	± 0.06	< .01	<.0001	0.02
	S	2.57	2.51	± 0.10	0.67		
	G1	2.87	3.22	± 0.10	<.05		
	G2	3.22	3.70	± 0.10	<.001		
Growth rate, mm/d	0 – 28	0.023	0.042	± 0.005	0.018		
	0 – 14	0.021	0.051	± 0.007	< .01		
	14 – 28	0.025	0.034	± 0.010	0.52		
	Contrast 0 – 14				<.05		
	Contrast 0 – 28				<.001		
Surface area , mm ²	Overall	19.96	26.13	± 0.76	<.0001	<.0001	<.001
	S	15.86	16.05	± 1.32	0.92		
	G1	19.56	27.37	± 1.32	< .0001		
	G2	24.46	34.98	± 1.32	< .0001		
Growth rate, mm ² /d	0 – 28	0.307	0.676	± 0.074	< .001		
	0 – 14	0.264	0.809	± 0.103	< .001		
	14 – 28	0.350	0.544	± 0.124	0.30		
	Contrast 0 – 14				<.005		
	Contrast 0 – 28				<.001		

Table 3 (Continued)

¹Trt: Treatment;

²Sub: Sub-period;

³Trt x Sub: Interaction of treatment by sub-period;

⁴S: The 2-week sub-period when cows were on wheat straw diet;

⁵G1: First 2-week sub-period of concentrate treatment;

⁶G2: Second 2-week sub-period of concentrate treatment;

⁷Contrast (0-14): The contrast of the change from sub-period G1 to sub-period S between the two experiments;

⁸Contrast (0-28): The contrast of the change from sub-period G2 to sub-period S between the two experiments;

Table 4 Treatment effect on DNA/DM ratio¹ of ruminal papillae.

	Sub-period ²	Treatment		± SE	Trt ³	<u>P value</u>	
		Corn	Lactose			Sub ⁴	Trt x Sub ⁵
DNA/DM	Overall	0.0113	0.0107	± 0.0005	0.39	< 0.01	0.28
	S ⁶	0.0094	0.0099	± 0.0008	0.63		
	G1 ⁷	0.0128	0.0109	± 0.0008	0.08		
	G2 ⁸	0.0118	0.0115	± 0.0008	0.81		
Contrast	0 – 14 ⁹				0.11		
Contrast	0 – 28 ¹⁰				0.61		

¹DNA/DM: Ratio of DNA content and freeze dried weight of ruminal papillae,(µg/g);

²Sub-period: include Sub-period S, Sub-period G1, and Sub-period G2;

³Trt: Treatment;

⁴Sub: Sub-period;

⁵Trtx Sub: Interaction of treatment by sub-period;

⁶S: The 2-week period when cows were on wheat straw diet;

⁷G1: First 2-week period of concentrate treatment;

⁸G2: Second 2-week period of concentrate treatment;

⁹Contrast (0-14): The contrast of the change from sub-period G1 to sub-period S between the two experiments;

¹⁰Contrast (0-28): The contrast of the change from sub-period G2 to sub-period S between the two experiments;

Table 5 Treatment effect on mitotic index¹ of ruminal papillae.

	Sub-period ²	Treatment			Trt ³	<u>P value</u>	
		Corn	Lactose	SE		Sub ⁴	Trt x Sub ⁵
Mitotic Index	Overall	0.81	0.72	± 0.05	0.21	< .01	0.46
	S ⁶	1.03	0.72	± 0.09	0.13		
	G1 ⁷	0.68	0.84	± 0.09	0.40		
	G2 ⁸	0.71	0.58	± 0.09	0.84		
Contrast	0 – 14 ⁹				0.61		
Contrast	0 – 28 ¹⁰				0.22		

Number of basal cell nuclei showing mitotic figure

$$^1\text{Mitotic Index} = \frac{\text{Number of basal cell nuclei showing mitotic figure}}{\text{Number of basal cell nuclei counted}} \times 100$$

²Sub-period: include Sub-period S, Sub-period G1, and Sub-period G2;

³Trt: treatment ;

⁴Sub: Sub-period;

⁵Trtx Sub: Interaction of treatment by sub-period;

⁶S: The 2-week period when cows were on wheat straw diet;

⁷G1: First 2-week period of concentrate treatment;

⁸G2: Second 2-week period of concentrate treatment;

⁹Contrast (0-14): The contrast of the change from sub-period G1 to sub-period S between the two experiments;

¹⁰Contrast (0-28): The contrast of the change from sub-period G2 to sub-period S between the two experiments;

Table 6 Treatment effect on blood vessel analysis of ruminal papillae.

		Treatment			<u>P value</u>		
	Sub-period ¹	Corn	Lactose	SE	Trt ²	Sub ³	Trt x Sub ⁴
V ⁵ /Area ⁶	Overall	127.6	116.6	± 8.1	0.35	0.18	0.95
	S ⁷	128.9	114.8	± 14.0	0.48		
	G1 ⁸	115.5	102.4	± 14.0	0.51		
	G2 ⁹	138.3	132.7	± 14.0	0.78		
	Contrast	0 – 14 ¹⁰			0.98		
Contrast	0 – 28 ¹¹			0.77			
A ¹² /Area	Overall	8.4	8.0	± 0.79	0.71	0.09	0.56
	S	10.1	9.8	± 1.36	0.88		
	G1	7.9	6.0	± 1.36	0.31		
	G2	7.3	8.3	± 1.36	0.61		
	Contrast	0 – 14			0.54		
Contrast	0 – 28			0.64			
(V+A)/Area	Overall	136.0	124.6	± 8.6	0.36	0.20	0.93
	S	139.0	124.6	± 14.9	0.50		
	G1	123.5	108.4	± 14.9	0.48		
	G2	145.6	141.0	± 14.9	0.82		
	Contrast	0 – 14			0.98		
Contrast	0 – 28			0.75			
V/A	Overall	17.8	16.0	± 1.8	0.50	0.77	0.33
	S	19.0	12.5	± 3.1	0.15		
	G1	15.5	18.6	± 3.1	0.49		
	G2	18.9	17.0	± 3.1	0.66		
	Contrast	0 – 14			0.14		
Contrast	0 – 28			0.47			

Table 6 (Continued)

¹Sub-period: Include Sub-period S, Sub-period G1, and Sub-period G2;

²Trt: treatment;

³Sub: Sub-period;

⁴Trtx Sub: Interaction of treatment by sub-period;

⁵V: Number of vein showed on the crosscut of ruminal papillae;

⁶Area: Area of cross cut of ruminal papillae

⁷S: The 2-week period when cows were on wheat straw diet;

⁸G1: First 2-week period of concentrate treatment;

⁹G2: Second 2-week period of concentrate treatment;

¹⁰Contrast (0-14): The contrast of the change from sub-period G1 to sub-period S between the two experiments;

¹¹Contrast (0-28): The contrast of the change from sub-period G2 to sub-period S between the two experiments;

¹²A: Number of artery showed on the crosscut of ruminal papillae;

Table 7 Treatment effect on rate of valerate concentration decline overtime, rate of cobalt concentration decline overtime, and rate of absorption.

	Sub-period	Treatment		SE	<u>P value</u>		Trt x Sub ³
		<u>Corn</u>	<u>Lactose</u>		Trt ¹	Sub ²	
Kv ⁴	Overall	0.3019	0.2910	± 0.0084	0.36	<.0001	0.30
	S ⁵	0.2457	0.2294	± 0.0145	0.43		
	G1 ⁶	0.3462	0.3154	± 0.0145	0.14		
	G2 ⁷	0.3139	0.3282	± 0.0145	0.49		
Kco ⁸	Overall	0.0877	0.0914	± 0.0054	0.63	0.78	0.29
	S	0.0830	0.1037	± 0.0093	0.12		
	G1	0.0917	0.0837	± 0.0093	0.55		
	G2	0.0883	0.0869	± 0.0093	0.91		
Ka ⁹	Overall	0.2142	0.1996	± 0.0084	0.23	<.0001	0.19
	S	0.1627	0.1258	± 0.0146	0.08		
	G1	0.2545	0.2317	± 0.0146	0.27		
	G2	0.2256	0.2413	± 0.0146	0.45		
Contrast	0 – 14 ¹⁰				0.63		
Contrast	0 – 28 ¹¹				0.08		

¹Trt: Treatment;

²Sub: Sub-period;

³Trt x Sub: Interaction of treatment by sub-period;

⁴Kv: rate of valerate concentration decline over time;

⁵S: The 2-week period when cows were on wheat straw diet;

⁶G1: First 2-week period of concentrate treatment;

⁷G2: Second 2-week period of concentrate treatment;

⁸Kco: rate of cobalt concentration decline over time;

⁹Ka: rate of valerate absorption calculated by subtracting rate of cobalt concentration decline over time from rate of valerate concentration decline over time.

¹⁰Contrast (0-14): The contrast of the change from sub-period G1 to sub-period S between the two experiments;

¹¹Contrast (0-28): The contrast of the change from sub-period G2 to sub-period S between the two experiments;

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