INFLUENCE OF CANE MOLASSES INCLUSION TO DAIRY COW DIETS DURING THE TRANSITION PERIOD ON RUMEN EPITHELIAL DEVELOPMENT AND A PROPOSED MECHANISM OF RUMEN EPITHELIAL DEVELOPMENT

by

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B.S., New Mexico State University 2000
M.S., Kansas State University 2005

AN ABSTRACT OF A DISSERTATION

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Abstract

Research regarding rumen epithelial adaptation and potential mechanisms during the transition period of the dairy cow is lacking. The rumen epithelium has a tremendous capacity for the absorption of volatile fatty acids (VFA) produced from microbial fermentation in the rumen. Absorption of VFA from the rumen pool delivers energy substrates to the animal and provides stability to the rumen environment. Increased epithelial surface area from the development and adaptation of rumen papillae facilitates VFA absorption. Manipulation of the diet to alter rumen fermentation can have positive effects upon the rumen papillae development supporting VFA absorption. We hypothesized that enhancing rumen epithelial surface area through dietary alterations could lead to greater VFA absorption and improve rumen stability. Experiments were conducted to determine the effects of diets formulated with cane molasses to stimulate the production of ruminal butyrate and thereby increase rumen epithelial surface area and to investigate a potential mechanism for glucagon-like peptide-2 (GLP-2) to impact epithelial development. Feeding cane molasses in the dry period improved dry matter intake during the close-up period and during lactation. Milk production was increased for cows that were fed cane molasses during the dry period. Ruminal absorption of valerate was greater during the close-up period than the far-off period but was not influenced by the addition of cane molasses. Total VFA concentration measured during the dry period was not affected by the addition of cane molasses to the diet. The presence of glucagon-like peptide receptor (GLP-2R) mRNA was confirmed in bovine tissue obtained from rumen epithelium, omasum, abomasum, duodenum, jejunum, ileum, large intestine, and pancreas. The greatest level of expression of mRNA for GLP-2R was in the small intestine and large intestine. Expression of GLP-2R mRNA during the prepartum period tended to be increased with the addition of cane molasses.

Postpartum expression of GLP-2R was not increased by supplementing cane molasses in the dry cow diet. Results from these experiments indicate that dry cow diets formulated to contain cane molasses can positively influence transition cow performance and that the presence of glucagon-like peptide-2 receptor could play a pivotal role in rumen epithelial development.

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Chapter 1. Literature Review

Introduction

Dairy cow nutrition during the prepartum period has become a topic of great interest in research as the importance of sound nutrition during this time is associated with a successful transition from gestation to lactation. Coordinated management of nutrition during the prepartum period brings together postpartum phases in the life cycle of a dairy cow as productive performance, reproductive performance, and animal health directly impact the profitability of the dairy operation. With the increased attention in research regarding the transition period, a predominate theory has evolved suggesting it is critical to minimize the degree of DMI depression during the pre-fresh period. As the dairy cow approaches parturition, DMI can decline as much as 20 to 40% (Grummer, 1993) leading to mobilization of body tissues increasing circulating NEFA levels prior to calving in turn negatively affecting hepatic function. Bertics et al. (1992) compared metabolism and lactation performance of cows allowed to voluntarily reduce DMI prior to calving to those force-fed via rumen fistula; upon parturition both groups were allowed ad libitum intake of a lactation diet. Cows that were force fed had lower hepatic triglyceride accumulation at calving and performed better during lactation. Rabelo et al. (2003) found a positive relationship between prepartum voluntary intake immediately prior to calving and 21-day postpartum intake, which reinforces the value of minimizing the loss of DMI prepartum. Dietary manipulation to attenuate the decrease in DMI during the final days of gestation is likely to improve the transition into lactation and alleviate numerous metabolic disorders associated with this time period.

Numerous publications, reports, and symposia speculate on the importance of rumen papillae development in conjunction with diet change for the absorption of VFA from ruminal fermentation, but very little evidence exists to support this. Dirksen et al. (1985) is the sole foundation for this notation; non-lactating non-pregnant cows were adapted from a poor-quality forage diet to a diet containing a greater amount of concentrate, and VFA absorption from the rumen was measured. Rumen papillae from cows consuming a diet rich in concentrate generally are longer than those from cows offered a high forage diet. Adaption by the rumen epithelium is likely a response to the increased VFA load from ruminal fermentation of concentrates and subsequent metabolic use by the epithelium. Promoting rumen papillae development increases epithelial surface area and likely VFA absorptive ability, thus preventing accumulation of VFA in the rumen, decreased rumen pH, and decreased DMI. It has been suggested that cows with longer papillae are better able to absorb VFA from the rumen and would be less likely to incur ruminal disruptions such as acidosis. Perhaps papillae length is indicative of rumen epithelial health and integrity as well as absorptive ability. Regardless of the effect on rumen epithelium, diets containing greater proportions of concentrates promote rumen microbial adaptation to diets typical of those offered during lactation. Thus, diets with higher NFC levels during the close-up period would promote microbial adaptation and reduce the anomalies associated with diet change during the transition period for the dairy cow.

Research pertaining to mechanisms associated with rumen epithelial development are lacking. A plausible mechanism that can be extrapolated from other areas of research revolves around glucagon-like peptide-2 (GLP-2) and glucagon-like peptide-2 receptor (GLP-2R). Stimulation of intestinal villus growth from GLP-2 has been reported in mice and rats (Drucker et al., 1996; Tsai et al., 1997; Litvak et al., 1998). Increased nutrient absorption from the small intestine of humans has been observed with the administration of GLP-2 (Jeppesen et al., 2001).

Extrapolating these results to the physiological status of the dairy cow during the transition period could prove invaluable. With the lack of research surrounding rumen epithelial development and absorptive ability it would be logical to explore mechanisms associated with rumen epithelial development and maintenance of integrity during the dietary changes associated with the transition period for the dairy cow.

The Transition Cow

The most challenging time of the dairy cow's production cycle is during the transition period, a duration of time that has been defined as three weeks prior to parturition to three weeks postpartum (Grummer, 1995). The dairy cow undergoes hormonal and metabolic changes as well as management changes during the transition to lactation. Nutrient requirements of the dairy cow increase during the last months of gestation by more than 20% to support fetal development (Moe and Tyrrell, 1972). Prepartum DMI diminishes gradually as parturition approaches and may decrease as much as 30% (Bertics, 1992). Increased nutrient requirements coupled with the typical reduction in DMI creates a prepartum period of negative energy balance for the dairy cow. Body tissue is mobilized leading to elevated plasma NEFA and hepatic triglyceride content at parturition (Vazques-Anon et al., 1994). These perturbations in the metabolism of the lactating cow contribute to postpartum metabolic disorders. Prior to parturition, diet formulation during the close-up period is used to slowly adapt the rumen environment by increasing the dietary energy content via increased diet NFC content. This approach promotes rumen epithelial development prior to parturition and enhances the transition to lactation and consumption of a diet containing a greater proportion of concentrates.

During the final weeks of gestation, circulating hormone levels undergo an ebb and flow situation. Plasma insulin levels decrease (Kunz et al., 1985), progesterone levels decline rapidly immediately prior to parturition while plasma estrogen levels surge (Chew et al., 1979). A

variety of theories have been hypothesized regarding the influence of these hormones on the metabolic state of the dairy cow. Researchers at the University of Wisconsin hypothesized that increasing levels of circulating estrogen contribute to increased lipolysis of adipose tissue (Grummer et al., 1990). Mobilization of adipose tissue leads to increased circulating NEFA levels that are taken up by hepatic tissue. Upon extraction from circulation by the hepatic tissue, NEFA can undergo oxidation for energy or esterification and export in the form of a triglyceride. In ruminant animals, the export of triglycerides are inefficient compared to extraction rates for NEFA from circulation. This discrepancy in efficiency leads to accumulation of triglycerides within hepatic tissue contributing to the metabolic disorder of fatty liver. The gluconeogenic capacity of hepatic tissue is negatively impacted with the condition of fatty liver (Overton et al., 1998).

Reducing the degree of depression in DMI during the close-up period associated with the prepartum period should be the goal of the transition nutrition program and appears significant for the prevention of postpartum metabolic perturbations. In a recent investigation, Wallace et al. (1996) examined the relationship between periparturient metabolic disorders, DMI, and milk yield using 48 cows following them through the first 20 days in milk. Dry matter intake was significantly lower for cows affected by an associated disorder versus cows without a disorder (Wallace et al., 1996). Milk yield was 8,952 kg for cows experiencing metabolic anomalies versus 9,424 kg for cows that transitioned normally (Wallace et al., 1996). Numerous postpartum disorders exist that are not mutually exclusive which include milk fever, ketosis, retained placenta, fatty liver, metritis, and displaced abomasum.

Dry Matter Intake

The central most important aspect of dairy nutrition and of a profitable dairy operation is dry matter intake. Diminished prepartum dry matter intake is correlated with the prevalence of

metabolic disorders during the initial days postpartum (Wallace et al., 1996). During the last days of gestation DMI may decrease 20 to 40% (Grummer, 1995). Improved DMI during the prepartum period has been correlated with positive production responses and DMI at 21 days postpartum (Grummer, 1995). Following parturition DMI is insufficient to meet the all energy requirements of the lactating cow (Bell, 1995; Grummer, 1995). This lack of energy intake drives the mobilization of body tissue to support lactation (Bell, 1995). Mobilization of excess body reserves with the concomitant sluggish increase in DMI during the initial days of lactation can exacerbate metabolic disorders and diminish milk production. Prevention of precalving decreases in DMI and subsequently supporting a prompt increase in DMI postpartum should be the goal of all dairy nutritionists. Minimizing the degree of DMI depression during the prepartum has become a common theme to ameliorate postpartum disorders.

Diet Strategies Applied During the Close-up Period

Prevention of prepartum negative energy balance has been explored by increasing the energy density of the diet during the last three weeks of gestation. Generally, increasing the energy density of the close-up diet increases energy intake. Dry matter intake was increased when dietary NFC content was increased during the last weeks of the prepartum period (Minor et al., 1998; Rabelo et al., 2003). Increases in the dietary energy density have also been shown to diminish DMI prepartum (NRC, 2001). In contrast several studies suggest that feeding a diet containing a greater proportion of NFC during the late prepartum period did not negatively influence postpartum performance (Holcomb et al., 2001; Mashek and Beede, 2000). Adding supplemental fat to the close-up diet also has been utilized to increase the energy density. Grum et al. (1996) demonstrated that adding fat to the close-up diet may have positive postpartum effects on hepatic tissue by reducing fatty acid accumulation, but prepartum DMI was diminished.

Manipulating the content of crude protein fed during the close-up period has been investigated. Improving the protein content of the close-up diet to promote fetal tissue deposition and reduce mobilization of maternal protein to support fetal growth is presumed to be beneficial. Doepel et al. (2000) indicated higher levels of crude protein offered during the close-up period are utilized as an energy source by the fetus. Dry cows offered a close-up diet containing 15.3% CP with increased rumen undegradable protein versus a diet containing 14.1% CP had similar DMI during the close-up period and improved milk protein content postpartum (Van Saun et al., 1993). Upon entering lactation, no differences among close-up diets containing 10.6, 12.7, or 14.5% CP were observed with regard to milk production and milk protein content (Putnum and Varga, 1998).

Ruminal Butyrate Production

Microbial fermentation of substrate in the rumen leads to the production of VFA. The pool of VFA in the rumen is predominately made up of acetate, propionate, and butyrate. Acetate, a two-carbon fatty acid, is the major VFA present in ruminal fluid. Propionate, a three-carbon fatty acid, is the second most abundant VFA. Butyrate, a four-carbon fatty acid, the third most abundant, generally makes up 10% to 15% of the molar proportion. Rumen epithelial tissue metabolizes up to 90% of the butyrate produced in the rumen from microbial fermentation (Stevens and Stettler, 1966). Butyrate has a stimulatory effect upon rumen papillae development (Van Soest, 1994). Accordingly, feeding substrates that promote butyrate production in the rumen have the possibility to enhance rumen papillae development. Diet manipulation to alter VFA proportions to increase butyrate concentrations are likely to positively impact rumen epithelial development.

Effects of feeding sucrose on rumen fermentation suggest an increase in ruminal butyrate concentration is possible. Inclusion of sucrose into a diet for lactating cows increased butyrate

molar percentage from 15.9 to 22.4% in rumen fluid with no effect on rumen pH (Kellog and Owen, 1969). Similarly molasses supplementation to provide 6% sucrose to lactating cows increased ruminal butyrate proportions (Owen et al., 1969).

The Rumen

Four distinct anatomical regions, reticulum, rumen, omasum, and abomasum, make up the forestomach area in the bovine with the rumen being the largest component. The rumen of the bovine is a specialized multi-compartmental structure that is a component of the digestive tract. Within the rumen are ruminal pillars corresponding with external grooves separating it into distinct areas referred to as cranial, ventral, dorsal, and blind sacs. The mucosal epithelium of the rumen is composed of four distinct morphological layers called stratum. Beginning at the lumen surface is the stratum corneum, the stratum granulosum, the stratum spinosum, and the stratum basale (Graham and Simmons, 2005). The stratum corneum is non glandular and composed of keratinized stratified squamous epithelium which contains numerous flat leaf-like papillae that protrude into the lumen. Rumen papillae lining the outermost stratum corneum are responsible for the vast absorptive ability of the rumen. Protrusion of the papillae into the rumen increase the surface area of the rumen epithelium and are considered responsible for VFA absorption from the rumen pool (Hofmann, 1988). It is the stratum corneum that is responsible for protection of the underlying layers from physical injury due to rumen contents and bacterial invasion. Less distinctive are the stratum granulosum and stratum spinosum layers. Stratum granulosum cells can be distinguished from the stratum corneum due to tight junction opening outward and the presence of an intercellular space. Within the cells of the stratum granulosum lies granular matter and fibrils. Cells of the granulosum are positioned parallel to the corneum cells and have very small interstitial spaces. Stratum spinosum cells appear oval with serrated edges. Desmosomes within the spinosum layer are associated with cytoplasmic processes

creating the serrated appearance. Underlying the three outermost layers are the columnar cells of the stratum basale. Stratum basale cells are firmly attached to the basement membrane. Within the cells of the basale layer are fully functional mitochondria, golgi vesicles, ribosomes, sodium pump, and other cellular organelles. These are the cellular components responsible for assimilation and metabolism of nutrients absorbed from the rumen pool. Cells of the stratum basale progressively migrate outward becoming spinosum, granulosum, and lastly corneum cells.

Development of Rumen Epithelium

Investigation into the development of the rumen epithelium and rumen papillae mostly has entailed use of young calves and sheep. Stimulation of epithelial development and rumen papillae involves age and diet. As the animal becomes older the rumen becomes larger in overall size, but papillae development may be lacking (Hamada et al., 1962). More importantly diet factors contributing to rumen growth and papillae development include a chemical component and a physical component. Fermentation end-products of feedstuffs produced by rumen microflora stimulate development of the epithelium and subsequently the growth of rumen papillae. Calves offered milk alone demonstrate only small gains in rumen development, whereas counterparts offered milk and alfalfa hay demonstrated greater ruminal tissue weight and increased ruminal papillae length (Warner et al., 1956). Papillae development was greater in calves fed milk and a complete feed containing pellets than in calves offered milk only (Tamate et al., 1962) suggesting the importance of chemical stimulation. Muscular development of the rumen has been influenced by the addition of some interesting materials such as plastic sponges. The epithelium and rumen papillae lacked development with the addition of physical components alone due to the lack of chemical influence from feedstuff fermentation (Tamate et al., 1962). Infusion of propionate and butyrate into the rumen of young calves stimulated rumen epithelial and papillae development, but growth of the underlying musculature was severely

lacking (Tamate et al., 1962). Increasing proportions of butyric and propionic acids from ruminal fermentation stimulates epithelial mitosis and epithelial cell proliferation enhancing papillae development (Hoffman, 1988). The presence of physical feedstuffs to develop the underlying muscle layer for rumen contractions and ultimately mixing of the digesta is important for ruminal fermentation. Microbial fermentation provides the chemical stimulation from VFA for morphological development of the rumen epithelium and papillae. Physical and chemical components are both significant to the normal development of the rumen epithelium and thus animal performance (McGavin and Morrill, 1976).

Rumen papillae development and regression in mature animals is directly related to the diet being consumed. The surface area of the rumen is increased with the development of papillae. Increasing the surface area of the rumen epithelium in turn increases the ability of the epithelium to absorb VFA from the rumen (Dirksen et al., 1985). Moon and Campbell (1973) followed rumen development and papillae growth in ewes during gestation and into lactation and reported increased weight of rumen epithelium at 45-d postpartum. Papillae length and surface area was improved during the postpartum sampling period when animals consumed a diet with a greater proportion of concentrates (Moon and Campbell, 1973). Gaebel et al. (1987) demonstrated a significant increase in rumen papillae surface area from sheep fed a diet containing additional concentrates. Likely the most cited research regarding cattle and rumen adaptation to diet is Dirksen et al. (1985). Surface area of biopsied papillae increased from 24 mm^2 at the end of an energy poor feeding period to over 60 mm^2 at the end of an energy rich feeding period then regressed to 15 mm^2 when returned to the energy poor diet for 5 weeks (Dirksen et al., 1985). Additionally the ability of the rumen epithelium to absorb VFA was increased 337% with energy rich diet, from 4.8 mmol/min to 16.2 mmol/min then diminished with an energy poor diet (7.4 mmol/min) indicating a correlation between surface area and

absorptive ability (Dirksen et al., 1985). The ability of the rumen epithelium to absorb VFA at an increased rate may play a positive role in the stability of the rumen environment. Accumulation of VFA within the rumen would decrease rumen pH and interrupt the normal fermentation process. During the initial feeding of high concentrate diets, the rumen epithelium undergoes an adaptive transition at the cellular level. Fell and Weekes (1974) reported cellular apoptosis occurs rapidly at the papillae during the initial days of high-concentrate feeding and then slows. Apoptotic rates were lower after consumption of the high concentrate diet had taken place for several weeks (Fell and Weekes, 1974). Thickness of the stratum corneum layer can be affected by the feeding regime as well. Rumen epithelium samples from sheep fed a diet containing greater amounts of concentrate feedstuffs demonstrated a cell thickness of 12 to 15 cells whereas the cell thickness for sheep fed a high-forage diet was 2 to 4 cells (Gaebel et al., 1987). Similar to previous research, the increased thickness of cell layers regressed when sheep were offered a high forage diet (Gaebel et al., 1987).

Absorption of VFA by the Rumen Epithelium

The goal of feeding prepartum diets is to adapt the rumen epithelium to absorb VFA at a rate similar to production so as to stabilize rumen pH immediately postpartum. This stabilization is conducive to a smooth dietary transition that has positive postpartum effects on the animal. Absorption of VFA from the rumen pool predominately occurs via diffusion and is influenced by rumen pH and VFA concentration. More than 90% of the VFA produced within the rumen are absorbed through the rumen wall with less than 10% flowing out of the rumen to the lower alimentary tract (Harfoot, 1978). Rumen pH has a direct effect upon the disassociation of VFA and ultimately the absorption. The pH of the rumen affects the disassociation state of the VFA as does the pKa of each individual fatty acid. Rumen VFA are weak acids with an approximate pKa value of 4.8, whereas lactate is a stronger acid (pKa=3.86). Rumen pH is consistently above

the pKa values of the individual fatty acids and thus each VFA mostly exists in the disassociated state. Fatty acids can be absorbed across the rumen wall in both the undisassociated form and disassociated form. Undisassociated forms of the individual fatty acids are the predominate form that diffuse across the rumen wall (Gaebel et al., 2002). Disassociated VFA are absorbed by electroneutral anion exchangers (Gaebel and Sehested, 1997). In the anionic form VFA are exchanged for bicarbonate anion across the epithelium (Gaebel and Sehested, 1997). The rate of VFA absorption from the rumen pool increases as the chain length increases, thus acetate < propionate < butyrate (Dijkstra et al., 1993). As each VFA passes through the rumen epithelium and into portal circulation a differing degree of metabolism takes place in the epithelium. Butyrate is predominately utilized by the rumen epithelium and metabolized to BHBA (Bergman, 1990). Propionate is approximately 10 to 50% utilized by the epithelium, whereas acetate is utilized minimally by the epithelium (Bergman, 1990). Each appears in portal circulation at various rates due to the extent of metabolism by the epithelial tissue. It is this coordinated absorption and metabolism by the epithelium that plays an important role in maintaining the pH of the rumen environment.

Rumen Epithelial Proliferation

Glucagon-Like Peptide-2

In 1996, the 33-amino acid hormone, glucagon-like peptide-2 (GLP-2), gained attention as it was first reported to stimulate intestinal mucosal epithelial growth and cell proliferation in the mouse (Drucker et al., 1996). Subsequent research has demonstrated that GLP-2 participates in numerous functional activities of the small intestine. GLP-2 peptide is a component of proglucagon cosecreted with GLP-1 from enteroendocrine L cells in the small intestine. Intestinal proglucagon yields numerous peptides that include glicentin, oxyntomodulin, GLP-1, GLP-2, and intervening peptide-1 and -2 that promote physiological activities. The proglucagon

gene is highly conserved as is the sequence responsible for GLP-2 (Irwin et al., 1999) suggesting the importance in physiological processes with the homology of GLP-2 reported to be 87 to 97% among human, rat, mice, pig, and cow (Baksheev and Fuller, 2000).

Synthesis and Secretion of Glucagon-Like Peptide-2

Glucagon-like peptide 2 is synthesized as proglucagon, a prohormone, and then undergoes post-translational processing. Post-translational processing of proglucagon is tissue specific and distinction among tissues is important. Intestinal enteroendocrine L cells express prohormone convertase1/3 (PC1/3) ultimately responsible for conversion of proglucagon to GLP-1, GLP-2, glicentin, oxyntomodulin, and intervening peptide-1 and -2 (Furuta et al., 1997). Prohormone convertase 2 (PC2) from pancreatic α cells forms two products, glucagon and a proglucagon fragment (Furuta et al., 1997). Thus, the presence of tissue specific enzyme complexes leads to the differing end products. Hypoglycemia is the major stimulatory factor for the release of proglucagon from pancreatic α cells. Increases in intestinal GLP-2 mRNA transcript are present when nutrients are ingested and travel to the small intestine (L'Heureux and Brubaker, 2001). Luminal presence of nutrients is largely responsible for stimulating GLP-2 secretion from the enteroendocrine L cells of the small intestine (Thulesen et al., 1999; Xiao et al., 1999). Luminal presence of nutrients includes neural and chemical stimuli presented to the enteroendocrine L cell (L'Heureux and Brubaker, 2001). Notably the presence of carbohydrates and VFA provide stimulatory effects at the enterendocrine L cell (Xiao et al., 1999). Indirect stimulatory effects from feeding have been observed for the secretion of GLP-2 from the intestinal L cell also. Upon ingestion of a meal for piglets and humans, GLP-2 release from the small intestine occurred in as little as 15 minutes and then a secondary phase of GLP-2 release was observed at 60 minutes (Burrin et al., 2000; Jeppesen et al., 1999). Extrapolating on the

effect of VFA upon GLP-2 secretion, researchers have shown the infusion of butyrate to provide a stimulatory effect at the enteroendocrine L cell (Bartholome et al., 2004).

Glucagon-Like Peptide-2 Receptor

Glucagon-like peptide-2 receptor (GLP-2R) is a 7-domain transmembrane G-protein coupled receptor (Munroe et al., 1999) comprised of 550 amino acids (Drucker, 2002). Actions of GLP-2 are mediated through its receptor and receptor specificity. Expression of GLP-2R mRNA transcripts has been detected in tissues of the stomach, small intestine, large intestine, central nervous system, and lungs (Yusta et al., 2000). Further investigations reveal GLP-2R mRNA expression may be localized to specific subsets of enterocytes (Yusta et al., 2000). Trophic responses observed in intestinal epithelium have led to the belief that the epithelium must express a receptor responsible for GLP-2 actions. While much debate still remains, studies have tried to shed light on the debate of receptor presence in specific cell types. Injection of radiolabeled GLP-2 in rats demonstrated binding along the villus epithelium (Thulesen et al., 2000). In a study using in situ hybridization ??with?? digoxigenin labeled GLP-2R DNA probes, receptor expression was found in neurons underlying the intestinal epithelium but not the epithelium (Bjerknes and Cheng, 2001). Results from these studies reiterate the possibilities that the physiological actions and trophic effects of GLP-2 are mediated indirectly or through secondary messengers (Burrin et al., 2001).

Agonist binding to GLP-2R is highly specific and the major activator of trophic effects in the mucosa of the gastrointestinal tract. Degradation of GLP-2 by dipeptidyl peptidase IV leads to GLP-2^{3-33} that is also able to interact with GLP-2R with less affinity, but still demonstrates agonist properties (Thulesen et al., 2002). Antagonist binding by Exendin which blocked the trophic effects of GLP-2, has been discovered in previous research (Tang-Christensen et al.,

2000). Competitive binding may play a role in the number of receptors available to be bound by GLP-2 and thus impact the effects of the hormone.

Biological Importance of Glucagon-Like Peptide-2 in Epithelial Development

GLP-2 effects of greatest interest are the trophic effects exhibited by the epithelium of the small intestine when GLP-2 is administered. Murine studies have demonstrated the dramatic intestinal mucosa response to GLP-2 due to crypt cell proliferation and inhibition of epithelial cell apoptosis (Burrin et al., 2000b; Yusta et al., 2000). Crypt cell proliferation was increased 24% in conjunction with a 110% decrease in epithelial apoptosis when GLP-2 was administered (Tsai et al., 1997a). Increased tissue RNA content and total protein content was observed for mice injected with GLP-2 versus control as well as increased small intestine weight (Brubaker et al., 1997). Dramatic results have been observed in human patients fed via total parenteral nutrition (TPN) when GLP-2 is concomitantly infused. Gastrointestinal tissue normally undergoes severe hypoplasia during TPN administration. With the added venous infusion of GLP-2 with TPN, small intestinal epithelium is greater in mass, contains greater amounts of DNA and protein, greater villus height, and greater mucosal thickness than in rats infused with TPN alone (Chance et al., 1997). Burrin et al. (2000b) demonstrated that GLP-2 administration in conjunction with TPN could maintain similar intestinal development and integrity to neonatal piglets fed a normal diet. Piglets on the GLP-2 treatment had similar small intestine weights and villus height versus normally fed piglets.

GLP-2 biological activities reach beyond the trophic effects in the small intestine. Enzymatic activity of sucrase and lactase within the murine small intestine has been enhanced when GLP-2 is infused (Brubaker et al., 1997). Exogenous GLP-2 attenuated weight loss through enhanced nutrient absorption from the lumen of the small intestine (Brubaker et al., 1997; Cheeseman et al., 1996). Reduced gut motility, increased diet digestibility, and increased

nutrient absorption (Wojdemann et al., 1998) have been observed with the venous infusion of GLP-2 in piglets. In support of improved nutrient absorption, numerous studies with GLP-2 have found increased absorption of amino acids and sugars in conjunction with GLP-2 (Au et al., 2002; Prasad et al., 2000; Kato et al., 1999). Interestingly the permeability of the intestine has been shown to be decreased leading to reduced bacterial translocation within the gut tissue and suppression of cytokine release that causes inflammation (Benjamin et al., 2000; Boushey et al., 1999). Conflicting results have been reported surrounding mechanisms associated with nutrient transport and exogenous GLP-2 administration. Increased action of SGLT-1 was observed by Cheeseman et al. (1997), whereas no differences were observed in protein content of SGLT-1 or GLUT2 by Brubaker et al. (1997). Translocation of GLUT-2 to the intestinal brush border from the basolateral membrane was rapid with GLP-2 infusion (Au et al., 2002), suggesting a mechanism for nutrient absorption without the increase in transporter protein levels.

Summary

Perhaps the most stressful period of time in the production cycle of the dairy cow is the transition period. During late gestation the nutrient requirements of the cow increase to support fetal development at the same time that there is a decrease in DMI. To overcome the energy deficit, the cow mobilizes body tissue to support fetal and mammary tissue development. Strategies to reduce the degree of DMI depression during late gestation are a logical approach to support DMI and improve transition performance.

Speculation revolving around the development of rumen papillae with regard to diet manipulation is found throughout research in dairy nutrition. However, little research has been undertaken to support the central belief that increased rumen papillae development leads to increased VFA absorption from the rumen. Rumen papillae development as the dairy cow enters the transition period is completely unexplored. Rumen epithelial development has not gone

completely uninvestigated as the importance of butyrate to stimulate epithelial growth and papillae development is evident. This suggests that diet manipulation to promote butyrate formation in the rumen would also stimulate papillae growth. Mechanisms associated with papillae development have been contemplated in numerous research articles and symposia, again with little merit. Proposing a mechanism associated with glucagon-like peptide 2 and its receptor is certainly original. Research outside of animal science demonstrates the potency of GLP-2 in promoting villus growth in the small intestine.

Sucrose from molasses increases ruminal butyrate proportions, which are likely to promote rumen epithelial development. Increases in papillae surface area associated with rumen epithelial development would benefit the transition dairy cow during periods of diet change and rapid ruminal fermentation changes.

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Chapter 2. Effect of dietary inclusion of cane molasses in dry cow diets on performance and rumen absorptive ability

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Abstract

Two experiments were conducted to evaluate the effect of cane molasses addition to dry cow diets on periparturient performance and rumen absorptive ability. Experiments used a randomized complete block design with cows blocked by projected calving date and randomly assigned to treatment. Treatments were control diets without molasses (NM) or diets containing molasses (M) fed for 60 d prior to projected calving date in the far-off and the close-up periods. During lactation all cows were individually fed a common lactation diet. In Exp. 1, primiparous and multiparous Holstein dairy cows were used to evaluate animal performance. Cows fed M during the dry period consumed more DM during the close-up period and during the postpartum period. Milk yield and energy corrected milk yield was greater for cows fed M. Composition of milk did not differ between diets. Yield of butterfat and protein from milk was greater for cows offered the M diet. In Exp. 2, 6 multiparous Holstein cows with rumen cannulas were used to evaluate performance and absorptive ability of rumen epithelium in response to the addition of cane molasses to diets during a 60-d dry period. Dry matter intake was greater for cows offered the M diet during the close-up period and tended to be greater during the lactation period. Valerate was bolus dosed into the rumen via a solution containing 2 mol of valeric acid and 4.0 g Co-EDTA adjusted to a pH of 6.0 using sodium hydroxide. Valerate absorption was greater during the close-up than the far-off period but did not differ for cows fed NM (31.2%/h) or M (32.8%/h) diets. During lactation, valerate absorption did not differ for cows previously fed NM

(35.3%/h) or M (43.2%/h). Ruminal liquid volume, dilution rate, and outflow were similar between diets during the dry period. Total VFA concentration during the dry period did not differ between cows fed control and molasses, nor did molar percentages of acetate, propionate, butyrate, or isovalerate. Total VFA concentration and molar percentage of propionate were greater, whereas acetate molar percentage was less, during the close-up period versus the far-off period. Feeding cane molasses during the dry period demonstrated positive effects on DMI during the periparturient period and subsequently milk production during lactation. Inclusion of cane molasses in diets for non-lactating cows did not significantly improve ruminal absorptive ability.

Introduction

The rumen epithelium of dairy cattle undergoes a transformation in response to diet change. Rumen papillae development and adaptation from a typical dry cow diet to a lactating diet can require up to 7 wk postpartum (Overton and Waldron, 2004) limiting the amount of VFA absorbed from the rumen during early lactation. This delay in adaptation can negatively affect rumen environment and the cow if VFA accumulate in the rumen. Adaptation of rumen papillae to an energy-rich lactating diet from an energy-poor dry cow diet was investigated by Dirksen et al. (1985). Surface area of biopsied papillae increased from 24 mm^2 at the end of an energy-poor feeding period to over 60 mm^2 at the end of an energy-rich feeding period. Rumen papillae then regressed to 15 mm^2 when the cow was fed an energy poor diet for 5 wk (Dirksen et al., 1985). The ability of the rumen epithelium to absorb VFA was increased from 4.8 mmol/min to 16.2 mmol/min when cows were fed an energy-rich diet (Dirkesen et al., 1985). Epithelial absorptive ability then regressed when cows were returned to an energy-poor diet, to 7.4 mmol/min, indicating a correlation between surface area and absorptive ability (Dirksen et al., 1985).

Increasing NFC content of the prepartum diet over traditional dry cow diets has been investigated to promote a smooth transition to a lactating diet (Doepel et al., 2002, Grum et al., 1996, Rabelo et al., 2003). These studies demonstrated positive effects on prepartum DMI and energy intake, postpartum DMI, and subsequently milk yield. Results from these studies provide evidence that manipulation of prepartum diets influence lactation performance. Confounding the increase in NFC content during the prepartum period is the increased dietary energy. None of the studies investigating increased NFC or energy consumption measured the response of the rumen epithelium.

Promotion of rumen epithelial development prior to parturition with dietary components known to influence rumen fermentation could be advantageous. Butyrate is the fatty acid most extensively metabolized by the rumen epithelium (Stevens and Stettler, 1966), whereas propionate is secondary. Butyrate is largely responsible for the development of epithelial tissue (Sakata and Tamate, 1976, 1978). Sucrose supplementation to steers fed low quality hay increased butyrate production without diminishing propionate production (Heldt et al., 1999). Moreover, ruminal infusion of butyrate in sheep promoted the proliferation of rumen epithelial cells (Sakata and Tamate, 1978) as did the infusion of propionate to the rumen of sheep (Sakata and Tamate, 1979).

Our hypothesis was that supplementation with cane molasses containing 34% sucrose during the prepartum period would stimulate ruminal butyrate production, ultimately promote ruminal papillae development and absorptive ability, increase DMI postpartum due to less ruminal VFA accumulation, and thereby improve early lactation performance.

Materials and Methods

All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Experimental Design and Treatments

Cows were housed at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) during the trials. Using a randomized complete block design cows were blocked by projected calving date and assigned to dietary treatment. Experimental diets were a control diet (NM) or a diet with cane molasses (M) (Table 1 and 2) and were offered during the far-off period (60 to 30 d prior to projected calving) and the close-up period (30 to 0 d prior to projected calving). Cane molasses was added to experimental diets after the TMR was mixed to add a covering of molasses to all dietary components. Upon freshening, all cows were offered a common lactation diet for the duration of the experiment. Diets were offered twice daily at 0600 and 1600 h as a TMR for ad libitum intake. Amount of TMR offered and refused was recorded daily. Dry matter determination of corn silage and wet corn gluten feed was performed weekly at 105°C for 24 h, and diets were adjusted to maintain proportions of each on a DM basis. Samples of TMR and orts were collected weekly and dried at 105°C for 24 h for DM determination. Corn silage, alfalfa hay, whole cottonseed, wet corn gluten feed, and individual grain mixes were sampled weekly and composited by period for analysis of chemical composition by Northeast DHI Forage Testing Laboratory (Ithaca, NY). Cows were weighed and body condition scored using a 5 point scale (Wildman et al., 1982) on consecutive days prior to the beginning of each period and then immediately following the a.m. milking. Cows were milked 4 times daily for the initial 21 DIM then twice daily with milk yield recorded at each milking. Milk samples were obtained weekly (a.m./p.m. composite) and analyzed for fat, protein, lactose, milk urea-N (MUN), and somatic cell count (SCC). Coccygeal blood was collected into 10-mL EDTA Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ) at 0900 h (approximately 3 h after feeding) on d 1, 3, 5, 7, and 15 of lactation. Plasma was harvested and stored at -20°C until analyzed for albumin, NEFA, and PUN concentrations.

Experiment 1. Twenty six primiparous and 28 multiparous Holstein cows were used to evaluate cane molasses addition to dry cow diets on animal performance. During the far-off period, cows were housed in pens and then moved into tie stalls during the close-up period. Cane molasses was added to the TMR at 3.2% of DM during the far-off period and 3.3% of DM during the close-up period (Table 1). During experiment 1, cows were housed in group pens during the initial 30 d of the 60 d dry period removing opportunity for replication for statistical analysis. Pen averages for DMI are reported in Table 3 for this period without statistical evaluation.

Experiment 2. Six multiparous cows fitted with rumen cannulas were used to measure performance, diet digestibility, and rumen parameters. Cows were housed in a tie-stall barn beginning 74 d prior to projected calving for adaptation and were scheduled to undergo a dry period of 60 d. Cane molasses was added to the far-off TMR at 3.3% of DM and to the close-up TMR at 3.7% of DM (Table 2). Experiment 2 was conducted with second lactation cows that entered a tie-stall barn for adaptation approximately 14 d prior to the end of lactation. Prepartum and postpartum experimental diet composition and nutrient profiles for experiments 1 and 2 are provided in Tables 1 and 2. Diets were designed to be isocaloric and isonitrogenous. Initial cow BW and BCS are reported in Tables 4 and 8.

Apparent Total Tract Digestion. Diet digestibility was determined during the far-off and close-up periods using ADIA as an internal marker. Diet and fecal grab samples were obtained over 3 d (d 1: 0200, 0800, 1400, 2000 h; d 2: 0400, 1000, 1600, 2200 h; d 3: 0600, 1200, 1800, 2400 h) for a 24-h collection advanced 2 h at each fecal grab sample and pooled by cow for determination of ADIA content (n = 3).

Rumen Parameters. Rumen pH was recorded as an average of 3 samples obtained from different sites in the rumen using a suction strainer device and portable pH meter. For

determination of VFA concentration, 8 mL rumen fluid and 2 mL of 25% (wt/wt) metaphosphoric acid were mixed and frozen. Ruminal fluid samples were later thawed and centrifuged at 30,000 x *g* for 20 min, and a portion of the supernatant fluid was analyzed for VFA concentrations using gas chromatography (Hewlett-Packard 5890A, Palo Alto, CA; 183 x 0.635-cm column with GP 10% Sp-1200/1% H_3PO_4 on 80/100 Chromosorb W AW; Supelco, Bellefonte, PAP, with N_2 as the carrier gas, and flow rate at 80 mL/min with column temperature of 130°C.

Rumen Papillae Development. Rumen papillae morphology was determined at -60, -30, and -2 d prior to projected calving date and 16, 30, 44, 58, and 72 d postpartum. Rumen contents were evacuated through the rumen cannula. Tissue from the ventral sac was used to ensure use of proliferative epithelial tissue. A portion of the ventral sac was exteriorized through the rumen fistula and 5 rumen papillae within a 2.5 x 2.5 cm area were selected, excised, and measured for length and width. The sampling site in the ventral sac area was selected to ensure that the immediate area had not been previously biopsied and that papillae were healthy in appearance. Individual papillae were selected at random from the 2.5 x 2.5 cm area. Rumen contents were returned to the rumen upon completion of the biopsy.

Rumen Absorptive Ability

The absorptive ability of the rumen was measured using a technique of valerate dosing (Resende et al., 2006, Allen et al., 2000) at -60, -30, and -2 d prior to projected calving and 2, 16, 30, 44, 58, and 72 d postpartum. Valerate was bolus dosed into the rumen via a solution containing 2 mol (204 g) of valeric acid and 4.0 g Co (as CoEDTA) adjusted to a pH of 6.0 using sodium hydroxide. Ruminal samples were obtained at 0 h for baseline valerate determination and at 0.5, 1.0, 1.5, 2, 3, 4, 6, and 8 h following the dosing of the valeric acid/Co-EDTA solution. Only samples obtained from 1 to 8 h were used in statistical analysis because the 0.5-h samples

did not appear to demonstrate complete mixing in the rumen . Valerate concentrations were determined as described for VFA above. The rate of valerate disappearance was determined as the negative of the slope of the line when the natural logarithm of valerate concentration was regressed against time. Ruminal fluid Co concentrations were determined by atomic absorption spectrophotometry and were used to calculate liquid passage rates as the negative of the slope of the line when the natural logarithm of Co concentration was regressed against time. Valerate absorption rate was then calculated as liquid passage rate subtracted from the rate of valerate disappearance (valerate absorption rate = valerate disappearance rate – liquid passage rate). Rumen liquid volume was calculated as the intercept from the regression of Co concentration.

Laboratory analyses

Diet and fecal samples were dried at 55°C for 24 h and ground in a Wiley mill to pass a 1-mm screen. Ground samples were then ashed at 450°C in a muffle oven for determination of ash and OM concentration. Neutral detergent fiber and ADF contents of diet and feces were determined using the ANKOM filter bag technique (ANKOM Technology Corp., Fairport, NY). Concentration of CP was completed using Leco FP-528 combustion analysis (AOAC 990.03) with protein degradability determined by *Streptomyces griseus* enzymatic digestion (Coblentz et al., 1999; Roe and Sniffen, 1990). Ether extract was determined gravimetrically following extraction with anhydrous diethyl ether (AOAC 2003.05) and the nonfiber carbohydrate (NFC) fraction was calculated by difference (NFC = 100 – [% NDF + % CP + % ether extract + % ash]) (NRC, 2001). Net energy of lactation was calculated using dairy NRC (2001) computer program from chemical analysis of composites. Samples of milk were analyzed for fat, protein, lactose, somatic cell count (SCC), and urea nitrogen (MUN) content by Heart of America DHI Laboratory (Manhattan, KS). Fat, protein, and lactose content in milk were determined using near infrared spectroscopy (Bentley 2000 Infrared Milk Analyzer, Bentley Instruments Inc.). A

flow cytometer laser (Somacount 500, Bentley Instruments Inc.) was used to determine SCC, and chemical methodology from a modified Berthelot reaction (ChemSpec 150 Analyzer, Bentley Instruments Inc.) was used to measure MUN. Plasma samples were analyzed for albumin concentration using Sigma Diagnostics procedure no. 631 Albumin Reagent, St. Louis, MO. Plasma NEFA content was determined with a colorimetric assy, NEFA-C Kit (Wako Chemicals, Richmond, VA) as modified by Eismann et al. (1988). Plasma urea nitrogen was determined by a diacetyl-monozime assay using a Technicon Auto Analyzer III (Technicon Industrial Method no. 339-01; Tarrytown, NY). Plasma glucose concentration was determined using a Technicon Auto Analyzer III (Technicon Industrial Method no. SE-4-0036FJ4; Tarrytown, NY) utilizing a peroxidase indicator reaction. Concentrations of VFA in ruminal samples was measured using gas a chromatograph (Hewlett-Packard 5890, Avondale, PA) with N_2 gas as the carrier, a flow rate of 80 mL min, 1.9 m x 6.35 mm i.d. Supelco column #1-1965 with flame ionization detection, and a column temperature of 130°C.

Statistical Analysis

Data were analyzed as repeated measures with an autoregressive covariance structure (Littell et al., 1996) using PROC MIXED of SAS version 8.01 (SAS Institute Inc., Cary, NC). Analyses were completed separately for prepartum data and postpartum data with the model including diet, time, and diet x time interactions as fixed effects. Random effects included cow, block, and cow x block. Statistical significance was declared at $P < 0.05$ and trends noted at $P > 0.05$ to $P < 0.15$. Least square mean separation for treatment and parity effects were conducted using the PDIFF statement in SAS.

Results

Experiment 1

Dry Matter Intake, Milk Yield, and Milk Composition

Dry matter intake, milk yield, and milk composition for experiment 1 are shown in Table 3. As expected BW was greater for multiparous cows and BCS was greater for primiparous cows throughout the experiment (Table 4). Significant diet x parity interaction was observed for milk yield, ECM, and ECM/DMI (Table 3). Increases in milk yield ($P < 0.0001$) and ECM were observed for multiparous cows and for cows previously fed the molasses diet during the dry period. Percentage fat in milk was greater for primiparous cows than for multiparous cows. Yield of fat and protein from milk was greater for multiparous cows than for primiparous cows. Intake of dry matter was greater ($P < 0.0001$) for multiparous cows than for primiparous cows.

Addition of cane molasses to the dry cow diet increased DMI ($P < 0.0001$) during the close-up period and translated into an increase ($P < 0.0001$) in DMI during the postpartum period. Milk fat and protein percentages were not affected by prepartum diet. Feeding molasses during the dry period increased the yield of fat ($P = 0.003$) and protein ($P = 0.0006$) in milk. Concentrations of MUN showed a tendency to be greater ($P = 0.054$) for cows previously fed molasses.

Plasma Metabolites

Effects of experimental diets on postpartum plasma constituents are shown in Table 6. Plasma urea nitrogen concentration was greater ($P < 0.0001$) for multiparous cows than for primiparous cows. Plasma glucose concentration tended ($P = 0.09$) to be greater and NEFA were greater ($P = 0.002$) for primiparous cows than for multiparous cows. During the initial 3 d postpartum primiparous cows previously consuming molasses diets showed reduced NEFA levels in plasma (Figure 2-1) relative to primiparous cows previously consuming control. For multiparous cows previously consuming molasses diets, plasma NEFA concentrations were lower (Figure 2-1) initially (d 1 postpartum) relative to cows fed control diets.

Experiment 2

Dry Matter Intake, Milk Yield, and Milk Composition

Dry matter intake, milk yield, and milk composition are reported in Table 7. Molasses did not improve DMI when added to the far-off diet. Dry matter intake during the close-up period was greater (P = 0.002) for cows fed molasses diet than for cows fed control diet. A tendency (P = 0.08) was observed for cows fed molasses during the prepartum period to have greater DMI during lactation relative to cows fed the control diet. The numerical improvement in DMI for cows that were fed the molasses diet did not translate into a significant improvement in milk yield for cows in this experiment. Yield of ECM tended to be greater (P = 0.07) for cows fed molasses than for cows fed control during the prepartum period. Percentage of fat in milk did not differ, whereas percentage of protein in milk tended to be greater (P = 0.06) for cows fed molasses. Yield of fat was greater (P = 0.002) and protein tended to be greater (P = 0.07) from cows fed molasses compared to cows fed control. Milk urea nitrogen concentration was greater (P = 0.001) for cows consuming the molasses diet during the prepartum period. Body weight and BCS were not different nor did the change in BW or BCS differ between cows fed control or molasses diets (Tables 8 and 9). Plasma NEFA levels were lower for the initial 3 d of lactation for cows that were fed molasses relative to control during the dry period (Figure 2-2).

Diet Digestibility

Digestibility of OM tended (P = 0.052) to be greater for diets offered during the close-up period relative to those offered during the far-off period (Table 10). The addition of molasses to dry cow diets did not affect digestibility of OM for this experiment. A tendency (P = 0.07) for a diet x period interaction was observed for OMD (Table 10). Digestibility of NDF and ADF did not differ between the far-off period and the close-up period nor with respect to molasses addition.

Rumen Fluid VFA Proportions and Kinetics

Rumen fluid measurements and kinetics are presented in Table 11. Rumen pH was not affected by diet during the prepartum period. During the close-up period, rumen pH was lower ($P = 0.04$) than during the far-off period. Total VFA concentration was greater ($P = 0.02$) in rumen fluid from cows during the close-up period compared to rumen fluid from cows during the far-off period. Ruminal molar acetate percentage was greater ($P = 0.04$) during the far-off period compared to the close-up period. Molar propionate percentage in ruminal fluid was greater ($P = 0.003$) during the close-up period relative to the far-off period. Percentage of butyrate and isovalerate did not differ between prepartum periods or between diets.

Liquid dilution rate was 7.9 and 12.0%/h for control and molasses diets during the prepartum period, but differences were not detected ($P = 0.32$). Liquid dilution tended ($P = 0.15$) to be greater during the far-off period than during the close-up period. Absorption of valerate from the rumen was similar for cows fed control (31.2%/h) and cows fed molasses (32.8%/h) during the prepartum period. Valerate absorption was greater ($P = 0.02$) during the close-up period compared to the far-off period. Liquid volume of the rumen, liquid outflow, and liquid turnover did not differ between diets or periods.

Postpartum rumen measurements are shown in Table 12. Rumen pH during the postpartum period did not differ between cows previously fed control or molasses. Total VFA did not differ in ruminal fluid from cows previously fed control or molasses diets. Molar percentages of acetate, propionate, butyrate, and isovalerate in ruminal fluid did not differ during the postpartum period for cows fed control or molasses during the prepartum period. Effects of diets on rumen variables during the postpartum were not significant during the experiment. A significant ($P = 0.02$) day effect was observed for molar percentage of propionate in ruminal fluid during the postpartum period. Day was also significant for liquid dilution rate ($P = 0.03$) and liquid outflow ($P = 0.002$).

Prepartum rumen papillae measurements are shown in Figures 2-3 to 2-5. Rumen papillae length was longer ($P = 0.001$) for cows fed control diet compared to cows fed molasses during the prepartum period. Width of rumen papillae was unaffected by prepartum diet. Papillae width was greater ($P = 0.02$) for the close-up period relative to the far-off period. Feeding molasses during the dry period did not enhance rumen papillae surface area as surface area of the rumen papillae was greater ($P = 0.02$) for cows fed the control diet compared to cows fed the molasses diet.

Postpartum rumen papillae measurements are shown in Figures 2-7 to 2-9. Papillae length, width, and surface area were greater ($P < 0.0001$) during the postpartum period for cows that were previously fed control compared to molasses. Effect of day was significant for rumen papillae length, width, and surface area. Length of rumen papillae was greatest on d 30 and d 44 than on d 16, d 58, or d 72. Postpartum papillae width was greatest on d 30 and d 44 than on d 16, d 58, or d 72. Papillae surface area was greater on d 30 and d 44 than on d 16, d 58, or d 72. Diet x day interaction was significant for papillae length, width, and surface area.

Discussion

The 2 experiments were conducted to determine if the prepartum dietary addition of cane molasses, a source of sucrose, can influence rumen epithelial adaptation and subsequently lactation performance. Absorption of VFA from the rumen pool is commonly attributed to the development of papillae lining the inside of the rumen. Greater surface area leads to greater absorption (Dirksen, et al. 1985) and attenuates VFA accumulation which can cause ruminal acidosis. Dietary strategies that promote the development of the rumen papillae would be advantageous to the animal by improving the rumen's ability to adapt to diet changes, specifically regarding the diet switch from late gestation to lactation. Logically, it would be appropriate to employ a dietary strategy to promote rumen epithelial development, prior to

parturition and for a sufficient duration to allow for rumen papillae transition to take place. We hypothesized that sucrose addition via cane molasses to dry cow diets would positively influence prepartum intake and rumen papillae development subsequently enhancing VFA absorption and easing the transition to the lactation diet containing a greater proportion of rapidly fermented carbohydrates.

The increases in prepartum DMI in response to molasses are consistent with improvements in rumen adaptation during the prepartum period for the upcoming lactation diet that contains greater proportions of concentrates. Increases in prepartum DMI have been observed when increasing dietary concentrate levels in numerous studies (Rabelo et al., 2003; 2001, Minor et al., 1998). Sucrose addition to the lactation diet has stimulated DMI during the early lactation period (Nombekela et al., 1994). Although the addition of lactose at 15.7% of the diet did not improve prepartum DMI, the nadir in DMI associated with the approaching parturition was diminished (Defrain et al., 2006). Decreasing the DMI depression immediately prepartum is positively correlated with reduced circulating NEFA concentrations and postpartum DMI (Bertics et al., 1992). Inconsistent with our results is a study where the addition of 2.7% sucrose to a prepartum diet 21 d prior to projected calving did not improve DMI (Ordway et al., 2002), perhaps a consequence of the short feeding duration or form of supplementation. Our study resulted in greater prepartum DMI which translated to greater postpartum DMI for cows offered the molasses diet during late gestation. The improved DMI during early lactation translated into increased yield of milk and ECM in Exp. 1. The lack of ability to detect differences in experiment 2 may be due to the low cow numbers. Yields of milk fat and milk protein were the directly related to milk production as differences in milk composition were not observed. Reduction in initial NEFA levels for both primiparous and multiparous cows in Exp. 1 suggests an improved energy status immediately postpartum resulting from greater intake during

initial lactation for cows that were offered molasses diets during late gestation. Significant diet by parity interactions were present for milk yield, yield of ECM, ECM/DMI, and yield of fat from milk. Primiparous and multiparous cows offered molasses diets during the dry period experienced greater milk yield. The improvement for primiparous cows was 0.9 kg/d versus 4.4 kg/d for multiparous cows resulting in an interaction for milk and ECM yield. Yield of fat from milk experienced a diet by parity interaction resulting from the larger increase in milk production for multiparous cows. Efficiency of ECM production was numerically less for primiparous cows and numerically greater for multiparous cows when previously fed molasses diets, creating the interaction. Initial improvement in efficiency for primiparous cows fed control is linked to the mobilization of body tissue as evidenced by the initial increase in circulating NEFA levels creating an artificially high efficiency.

A tendency for greater OM digestibility during the close-up period compared to the far-off period is likely a function of diet composition as the diet was formulated to be more digestible during the close-up period. The tendency for improved OM digestibility in response to molasses during the far-off period was observed during a time when DMI was not affected. In contrast, the tendency for dietary molasses addition to reduce OM digestion during the close-up period was associated with an increase in DMI, which may have contributed to the decrease in digestion.

Rumen papillae measurements indicate that cane molasses did not positively influence measured papillae parameters, and in fact seemed to lead to smaller papillae. Diets utilized in experiment 2 were designed to be isocaloric and DMI was greater, at least during the close-up period, for the molasses-containing diet; thus, a response to energy intake per se was not expected. However, papillae response was consistent with rumen VFA concentrations in that total VFA concentration was numerically less for the molasses-containing diet prepartum,

demonstrating a positive relationship between VFA concentrations and rumen papillae length and surface area. Moreover the greater total VFA concentration and percent propionate during the close-up period when compared to the far-off period is consistent with greater papillae width and surface area. This response is supported by Lane et al. (2000) where increased rumen papillae size was observed from lambs with greater ruminal VFA concentrations.

Rumen papillae response to diet did not translate into improved absorptive ability; valerate absorption was similar between diets at 31.2%/h for control and 32.8 %/h for molasses diets. Greater valerate absorption during the close-up period than during the far-off period adheres to the paradigm as a numerically greater rumen papillae surface area was observed and is supported by Dirksen et al. (1985). Rumen papillae adaptation during the prepartum period appears to influence valerate absorption, but the response does not appear to be proportional. Postpartum, rumen papillae from cows previously offered the control diet had greater length, width, and surface area. Similar to prepartum diet response, the greater papillae surface area for control cows postpartum did not translate into improved postpartum absorption of valerate from the rumen pool. Although rumen papillae from cows previously fed molasses diets were smaller than those fed control, valerate absorption from the rumen was not affected.

Conclusion

Feeding cane molasses during a 60-d dry period improved prepartum and postpartum DMI and milk yield. Increased postpartum DMI for cows previously offered molasses diets was accompanied by a numeric increase in valerate disappearance from the rumen. This increase indicates enhanced adaptation by the rumen epithelium. The paradigm of greater rumen papillae surface area being fundamental to VFA absorption from the rumen was not supported by our data. The lack of a positive relationship between rumen papillae surface area and epithelial absorptive ability warrants further investigation. These studies indicate that maintenance of DMI

during the prepartum period is beneficial to an efficient transition to lactation and that the addition of cane molasses may stimulate intake during this time.

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Table 1. Ingredient and nutrient composition of control and cane molasses diets offered during a 60-d dry period for Exp. 1

	Far-off		Close-up		Lactation
Ingredient	Control	Molasses	Control	Molasses	
	----------% DM ----------				
Prairie hay	56.3	56.3	36.8	36.8	-
Corn silage	13.0	13.0	13.1	13.1	22.0
Wet corn gluten feed	9.7	9.7	12.0	12.0	20.4
Soybean meal, solvent	7.0	7.0	5.8	5.8	-
Soybean meal, expeller	-	-	-	-	7.6
Alfalfa hay	6.7	6.7	9.0	9.0	15.3
Whole cottonseed	3.2	3.2	5.0	5.0	8.5
Corn, ground	3.2	-	16.4	13.1	20.4
Molasses, sugarcane	-	3.2	-	3.3	0.95
Sodium bicarbonate	-	-	0.48	0.47	0.8
Magnesium oxide	0.12	0.12	0.40	0.40	0.2
Fishmeal, menhaden	-	-	1.2	1.2	2.12
Limestone	0.54	0.53	0.14	0.14	1.4
Trace mineralized salt[1]	0.40	0.40	0.20	0.20	0.34
Vitamin premix[2]	0.14	0.14	0.14	0.14	0.12
Sodium selenite[3]	0.03	0.03	0.02	0.02	0.008
Nutrient					
DM, % as fed	72.6	71.7	70.3	68.9	61.6
CP	12.3	12.1	14.1	14.5	19.1
NDF	53.7	52.5	43.9	43.2	30.6
ADF	33.3	32.5	26.8	25.8	17.1
NE_L, Mcal/kg	1.47	1.47	1.64	1.63	1.59
Calcium	0.61	0.62	0.54	0.53	1.0
Phosphorus	0.36	0.42	0.44	0.51	0.51
Magnesium	0.34	0.32	0.45	0.42	0.41
Potassium	1.56	1.53	1.41	1.39	1.21
Sodium	0.27	0.26	0.25	0.22	0.43

[1]Composition not less than: 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, 0.004% Co.
[2]Provided 4,400 IU Vitamin A; 2,200 IU Vitamin D; and 16 IU Vitamin E per kg of dietary DM.
[3]Provided 0.06 mg Se per kg of diet DM.

Table 2. Ingredient and nutrient composition of control and cane molasses diets offered during a 60-d dry period for Exp. 2

	Far-off		Close-up		
Ingredient	Control	Molasses	Control	Molasses	Lactation
	----------% DM ----------				
Prairie hay	57.5	56.6	37.3	37.3	-
Corn silage	12.6	12.4	12.2	12.2	20.5
Wet corn gluten feed	9.8	9.6	11.9	11.9	20.6
Corn, ground	1.3	-	16.4	12.9	21.7
Alfalfa hay	6.8	6.7	9.3	9.3	15.5
Whole cottonseed	3.4	3.0	5.1	5.1	8.4
Soybean meal, solvent	7.1	7.0	5.1	5.1	-
Soybean meal, expeller	-	-	-	-	7.5
Molasses, sugarcane	-	3.3	-	3.7	0.90
Sodium bicarbonate	-	-	0.48	0.46	0.80
Magnesium oxide	0.14	0.13	0.40	0.40	0.20
Fishmeal, menhaden	-	-	1.25	1.23	2.08
Limestone	0.54	0.53	0.14	0.14	1.36
Trace mineralized salt[1]	0.53	0.52	0.20	0.19	0.33
Vitamin ADE premix[2]	0.14	0.14	0.17	0.16	0.12
Sodium selenite[3]	0.03	0.03	0.02	0.02	0.008
Nutrient					
DM, % as fed	73.2	73.3	70.9	71.6	62.5
CP	12.4	12.2	14.0	13.9	18.8
NDF	53.7	52.4	46.9	45.8	35.8
ADF	33.4	32.6	26.5	25.9	19.1
NE_L, Mcal/kg	1.25	1.29	1.41	1.42	1.63
Calcium	0.51	0.52	0.43	0.46	0.94
Phosphorus	0.34	0.35	0.39	0.39	0.51
Magnesium	0.39	0.29	0.44	0.45	0.42
Potassium	1.67	1.73	1.42	1.55	1.19
Sodium	0.25	0.30	0.27	0.27	0.45

[1]Composition not less than: 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, 0.004% Co.
[2]Contributed 4,400 IU Vitamin A; 2,200 IU Vitamin D; and 16 IU Vitamin E per kg of dietary DM.
[3]Provided 0.06 mg Se per kg of diet DM.
[4]Provided

Table 3. Dry matter intake and milk yield and composition for primiparous and multiparous cows fed control or molasses diets during a 60-d dry period (Exp. 1)

	Control		Molasses			*P* value		
	Primiparous	Multiparous	Primiparous	Multiparous	SEM	Diet	Parity	Diet x Parity
n	13	14	13	14				
DMI, kg/d								
Far-off	11.8	14.9	11.9	15.6	-	-	-	-
Close-up	11.6	16.7	12.9	18.5	0.32	<0.0001	<0.0001	0.45
Lactation	16.9	24.3	18.3	25.7	0.25	<0.0001	<0.0001	0.89
Milk Yield, kg/d	35.8	46.4	36.7	50.8	0.57	<0.0001	<0.0001	0.003
ECM, kg/day	36.3	45.1	37.2	49.5	0.58	<0.0001	<0.0001	0.003
ECM/DMI	2.28	1.94	2.14	2.01	0.029	0.27	<0.0001	0.001
Milk fat								
%	3.84	3.43	3.78	3.51	0.135	0.93	0.01	0.60
kg/d	1.33	1.56	1.36	1.75	0.033	0.003	<0.0001	0.02
Milk protein								
%	2.89	2.96	2.90	2.86	0.078	0.54	0.88	0.45
kg/d	1.02	1.35	1.06	1.43	0.015	0.0006	<0.0001	0.13
Milk lactose, %	4.81	4.78	4.84	4.75	0.039	0.99	0.14	0.45
SCC x 1000/mL	266	125	272	264	49.1	0.10	0.09	0.14
MUN, mg/dL	14.3	16.3	14.7	16.8	0.26	0.05	<0.0001	0.78

Table 4. Prepartum and postpartum body weight and body condition of primiparous and multiparous cows fed control or molasses diets during a 60 d dry period (Exp. 1)

	Control		Molasses			*P* value		
Item/d to parturition	Primiparous	Multiparous	Primiparous	Multiparous	SEM	Diet	Parity	D x P
Body weight, kg								
-60	655	704	662	717	12.5	0.55	0.004	0.86
-30	676	732	687	735	10.4	0.62	<0.001	0.79
0	631	690	645	717	10.8	0.19	<0.001	0.65
30	549	615	576	637	10.0	0.08	<0.001	0.89
75	555	630	584	653	11.5	0.10	<0.001	0.85
Body condition[1]								
-60	3.67	2.79	3.82	2.92	0.057	0.08	<0.001	0.89
-30	3.71	2.96	3.78	3.03	0.054	0.33	<0.001	0.99
0	3.71	3.08	3.75	3.05	0.050	0.93	<0.001	0.64
30	2.83	2.42	2.91	2.52	0.068	0.35	<0.001	0.96
75	2.73	2.22	2.80	2.38	0.069	0.20	<0.001	0.68

[1]Scored on 1 to 5 scale (Wildman et al., 1982)

Table 5. Prepartum and postpartum BW change and BCS change for primiparous and multiparous cows fed control or molasses diets during a 60-d dry period (Exp. 1)

	Control		Molasses			*P* value		
Item/d to parturition	Primiparous	Multiparous	Primiparous	Multiparous	SEM	Diet	Parity	D x P
Body weight change, kg								
-60 to -30d	21.2	27.4	24.8	17.3	5.11	0.65	0.93	0.33
-30 to 0d	35.2	38.6	37.5	62.0	6.79	0.18	0.14	0.26
0 to 30d	-82.3	-75.9	-69.0	-79.8	8.16	0.68	0.85	0.45
0 to 75d	-76.0	-77.3	-60.2	-62.7	12.10	0.34	0.91	0.97
Body condition change[1]								
-60 to -30d	0.04	0.17	-0.04	0.17	0.455	0.30	0.03	0.88
-30 to 0d	0.00	0.12	-0.04	0.02	0.035	0.17	0.09	0.52
0 to 30d	-0.88	-0.65	-0.84	-0.53	0.059	0.31	0.001	0.64
0 to 75d	-1.00	-0.86	-0.95	-0.66	0.077	0.21	0.04	0.47

[1]Scored on 1 to 5 scale (Wildman et al., 1982)

Table 6. Plasma urea nitrogen, glucose, and NEFA content for the initial 15 DIM for primiparous and multiparous cows fed control or molasses diets during a 60-d dry period (Exp. 1)

	Control		Molasses			*P* value		
Item	Primiparous	Multiparous	Primiparous	Multiparous	SEM	Diet	Parity	Diet x Parity
PUN, mg/dL	11.7	13.8	11.7	14.3	0.42	0.52	<0.0001	0.54
Glucose, mg/dL	62.0	59.3	64.0	59.7	2.10	0.55	0.09	0.69
NEFA, umol/L	732	532	620	523	47.7	0.20	0.002	0.28

Table 7. Dry matter intake and milk yield and composition for cows fed control or molasses diets during a 60-d dry period (Exp. 2)

	Diet			
Item	Control	Molasses	SEM	*P* value
DMI, kg/d				
Far-off	13.0	13.1	0.97	0.96
Close-up	12.1	13.6	1.19	0.002
Lactation	23.3	26.5	2.6	0.08
Milk yield, kg/dy	40.4	44.3	4.1	0.41
ECM, kg/day	38.7	45.9	4.9	0.07
Efficiency, ECM/DMI	1.69	1.78	0.047	0.16
Milk fat				
%	3.57	3.62	0.18	0.83
kg/d	1.33	1.68	0.22	0.002
Milk protein				
%	2.89	3.02	0.087	0.06
kg/d	1.17	1.32	0.092	0.07
Milk lactose, %	4.84	4.88	0.056	0.35
SCC, x 1000/mL	25	32	5.4	0.22
Milk urea nitrogen, mg/dL	14.3	17.4	1.7	0.001

Table 8. Prepartum and postpartum body weight and body condition of cows fed control or molasses diets during a 60-d dry period (Exp. 2)

	Diet			
Item/d to parturition	Control	Molasses	SEM	*P* value
Body weight, kg				
-60	675	651	15	0.32
-30	699	680	10	0.27
-2	737	716	23	0.56
0	697	671	24	0.50
15	687	653	24	0.38
30	681	646	15	0.17
60	680	656	20	0.49
Body condition[1]				
-60	2.67	2.58	0.08	0.52
-30	2.75	2.67	0.16	0.73
-2	2.92	3.00	0.12	0.64
0	3.08	3.17	0.08	0.52
15	2.73	2.75	0.01	0.38
30	2.57	2.58	0.13	0.95
60	2.67	2.50	0.13	0.37

[1]Scored on 1 to 5 scale (Wildman et al., 1982)

Table 9. Body weight change and body condition change for cows fed control or molasses diets during a 60-d dry period (Exp. 2)

	Diet			
	Control	Molasses	SEM	*P* value
Body weight change, kg				
-60 to -30d	24.6	29.6	9.0	0.71
-30 to -2d	37.9	35.6	15.3	0.92
0 to 15d	-9.8	-18.2	7.0	0.45
15 to 30d	-6.1	-7.6	18.4	0.96
30 to 60d	-1.5	12.1	11.7	0.46
Body condition change[1]				
-60 to -30d	0.08	0.08	0.08	1.00
-30 to -2d	0.17	0.33	0.08	0.23
0 to 15d	-0.35	-.43	0.08	0.59
15 to 30d	-0.16	0.17	0.13	0.99
30 to 60d	0.09	-0.10	0.08	0.15

[1]Scored on 1 to 5 scale (Wildman et al., 1982)

Table 10. Diet dry matter (DM), organic matter (OM), NDF, and ADF digestibility for control and molasses diets offered during a 60-d dry period (Exp. 2)

	Far-off		Close-up			*P* value		
Item	Control	Molasses	Control	Molasses	SEM	Diet	Period	Diet x Period
OM digestibility, % intake	51.6	55.6	61.5	56.1	2.6	0.75	0.05	0.07
NDF digestibility, % intake	42.7	47.5	49.4	46.3	2.3	0.79	0.40	0.24
ADF digestibility, % intake	34.5	37.9	44.3	39.4	2.9	0.85	0.19	0.32

Table 11. Rumen fluid VFA proportions, pH, and kinetics during the prepartum period for cows fed control or molasses diets (Exp 2).

	Diet			Period			*P* value		
Item	Control	Molasses	SEM	Far-off	Close-up	SEM	Diet	Period	Diet x Period
Total VFA, m*M*	97	88	7.89	87	98	7.72	0.31	0.02	0.53
Acetate, molar %	68.9	67.1	1.36	69.2	66.9	1.1	0.39	0.04	0.36
Propionate, molar %	16.9	18.5	1.15	17.0	18.5	0.9	0.41	0.003	0.95
Butyrate, molar %	11.7	12.3	0.44	11.7	12.2	0.4	0.37	0.42	0.33
Isovalerate, molar %	1.36	1.21	0.11	1.22	1.35	0.1	0.27	0.33	0.68
Rumen parameter									
pH	7.11	7.16	0.19	7.54	7.09	0.164	0.62	0.04	0.73
Liquid dilution, %/h	7.9	12.0	2.32	7.3	12.6	2.48	0.32	0.15	0.61
Valerate absorption, %/h	31.2	32.8	4.43	25.0	39.0	3.55	0.79	0.02	0.69
Rumen liquid volume, L	57.6	51.5	5.63	58.3	50.9	6.171	0.47	0.39	0.85
Outflow, L/h	4.7	5.8	1.19	4.2	6.3	1.175	0.56	0.21	0.77
Turnover, h	12.9	10.8	2.61	14.6	9.0	2.873	0.53	0.14	0.50

Table 12. Rumen fluid VFA proportions, pH, and kinetics during the postpartum period for cows fed control or molasses diets (Exp. 2)

	Prepartum diet			Day postpartum							*P* value		
Item	Control	Molasses	SEM	2	16	30	44	58	72	SEM	Diet	Day	Diet x Day
Total VFA, mM	101.8	101.9	4.33	108.6	97.0	100.9	100.0	100.6	103.9	5.003	0.98	0.20	0.03
Acetate, molar %	58.1	58.1	1.64	58.8	55.5	58.7	58.5	58.6	58.3	1.85	0.99	0.40	0.28
Propionate, molar %	23.4	25.8	0.93	22.8	27.4	24.4	24.8	24.8	23.4	1.16	0.11	0.02	0.16
Butyrate, molar %	15.5	13.9	0.86	16.5	15.0	14.6	14.4	13.7	14.0	0.83	0.21	0.12	0.23
Isovalerate, molar %	1.78	1.34	0.31	1.14	1.32	1.45	1.45	1.65	2.33	0.45	0.38	0.43	0.62
Rumen parameter													
pH	6.87	6.76	0.24	6.99	6.73	6.61	6.75	6.72	6.81	0.19	0.63	0.58	0.74
Liquid dilution, %/h	14.7	17.2	0.92	12.8	17.0	17.4	17.7	14.9	16.3	1.38	0.11	0.03	0.37
Valerate abs., %/h	35.3	43.2	4.53	33.2	50.5	39.2	30.2	43.3	39.0	7.6	0.21	0.48	0.84
Liquid volume, L	68.5	58.7	4.0	62.5	60.6	66.1	68.7	62.9	60.9	4.9	0.20	0.72	0.48
Outflow, L/h	9.9	10.1	1.0	7.9	10.1	11.4	13.1	9.2	9.6	1.0	0.93	0.002	0.19
Turnover, h	9.4	7.4	0.80	9.9	8.1	7.4	7.8	8.8	8.2	0.88	0.17	0.17	0.22

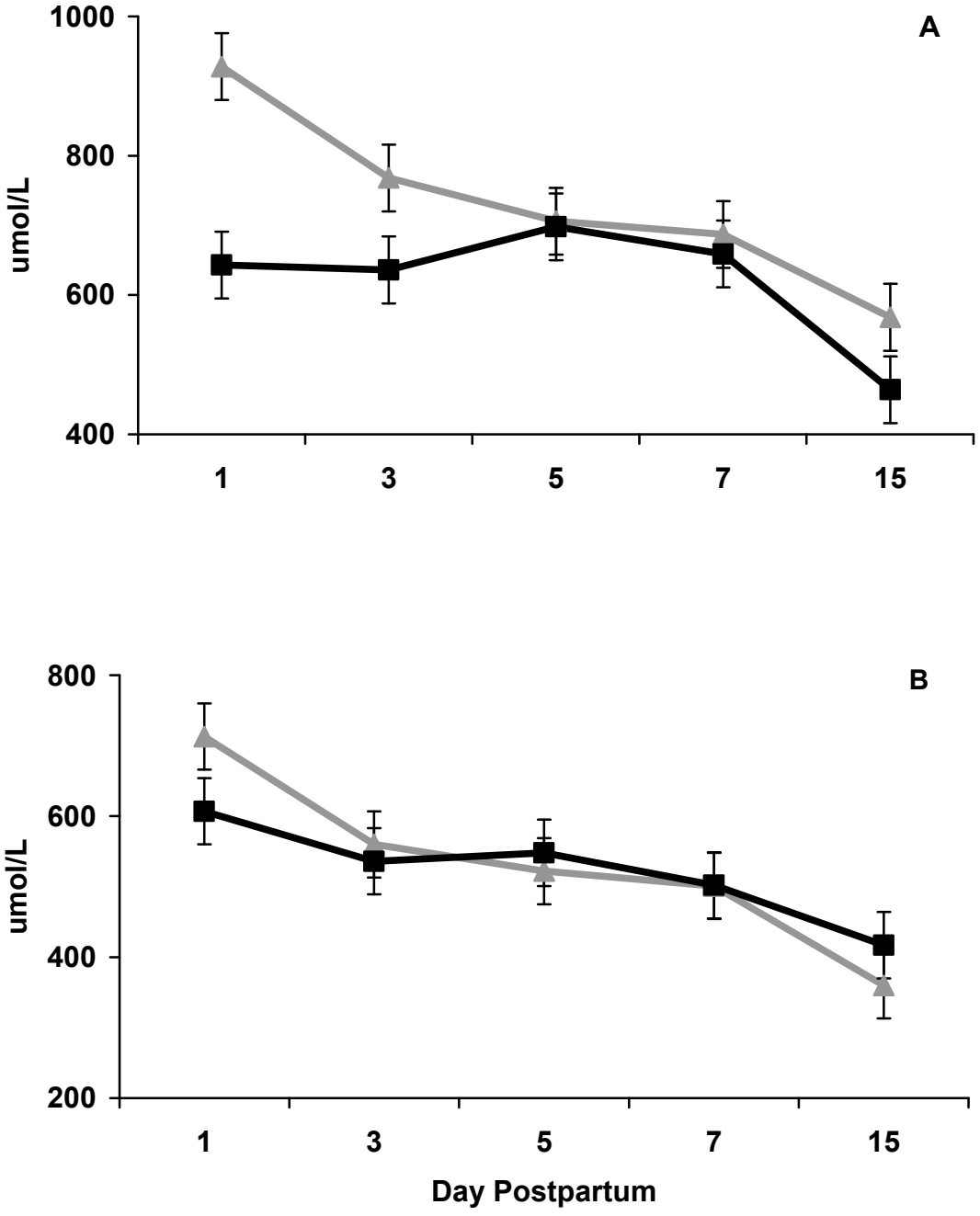

Figure 2-1. Postpartum plasma NEFA concentration for primiparous cows (Panel A; Pooled SEM=48, n=13) and multiparous cows (Panel B; Pooled SEM=47, n=14) fed control (triangles) or molasses (squares) diets during a 60 d dry period (Exp 1).

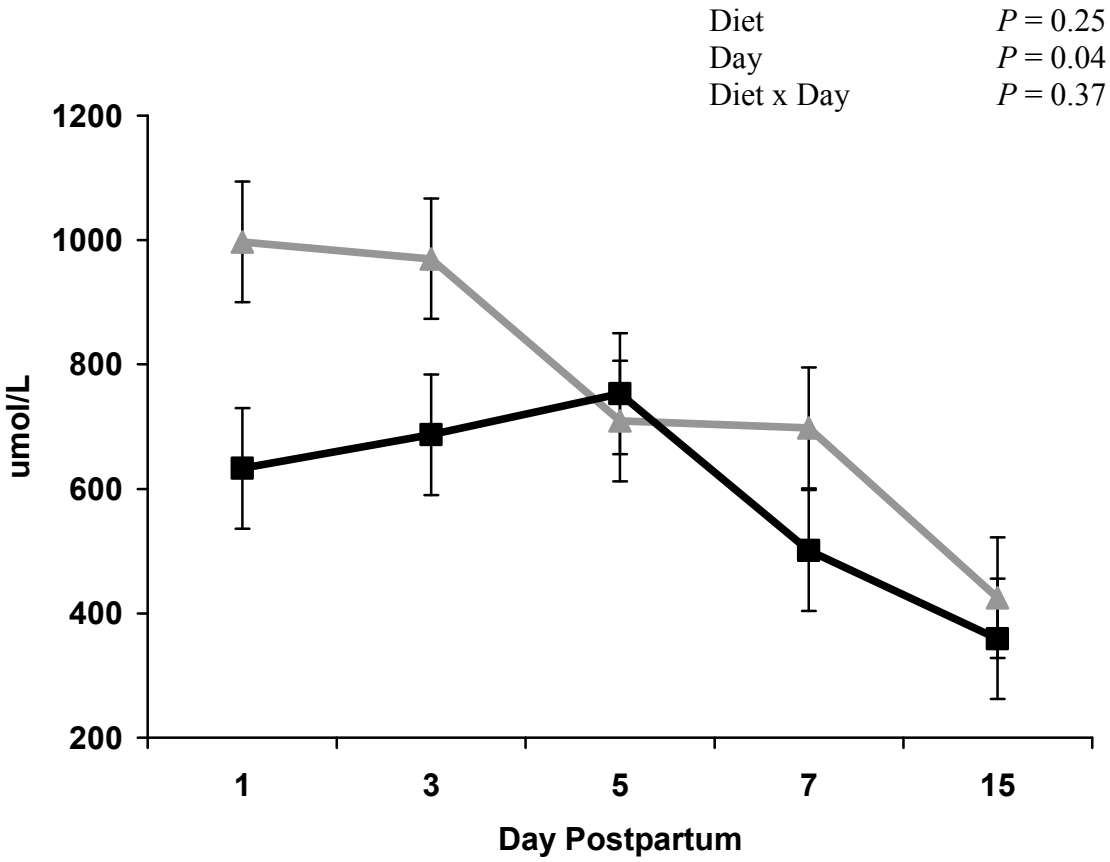

Figure 2-2. Postpartum plasma NEFA concentration for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=97; Exp. 2, n=3).

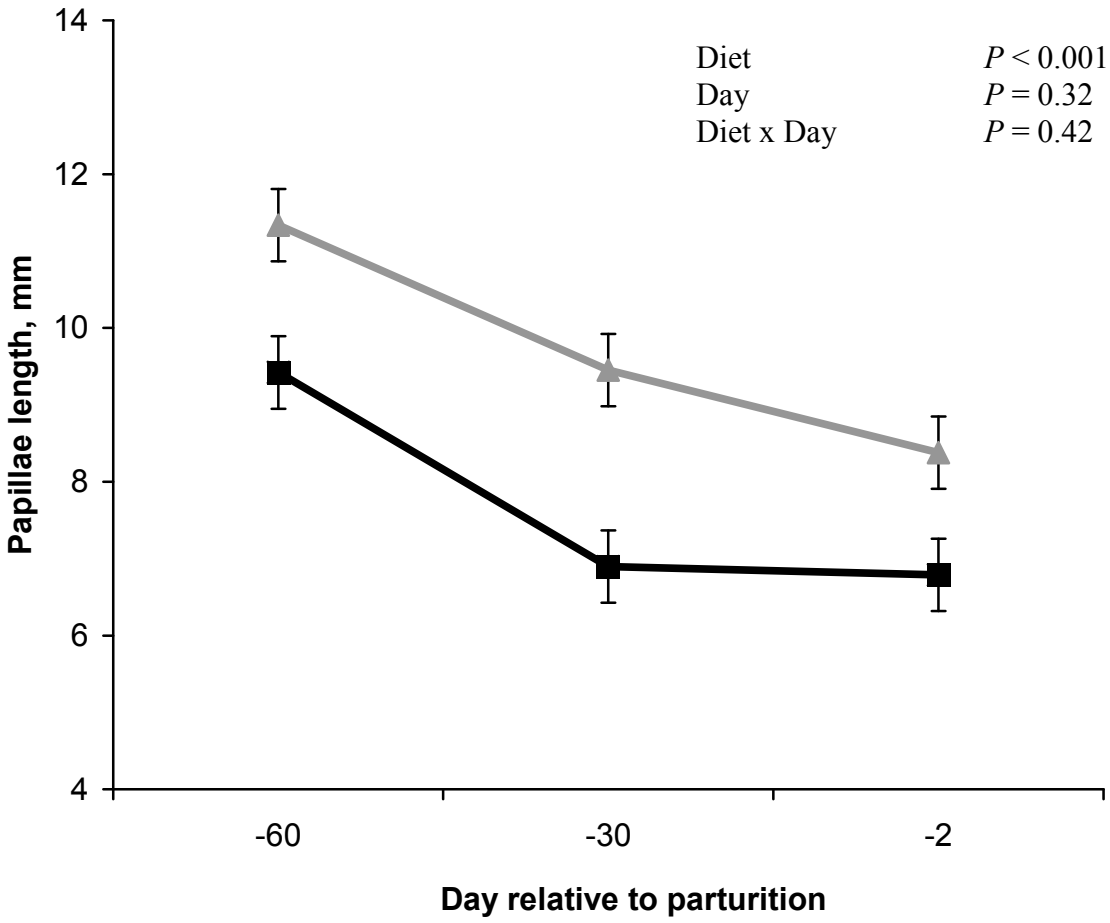

Figure 2-3. Prepartum papillae length for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=0.47, Exp. 2., n=3).

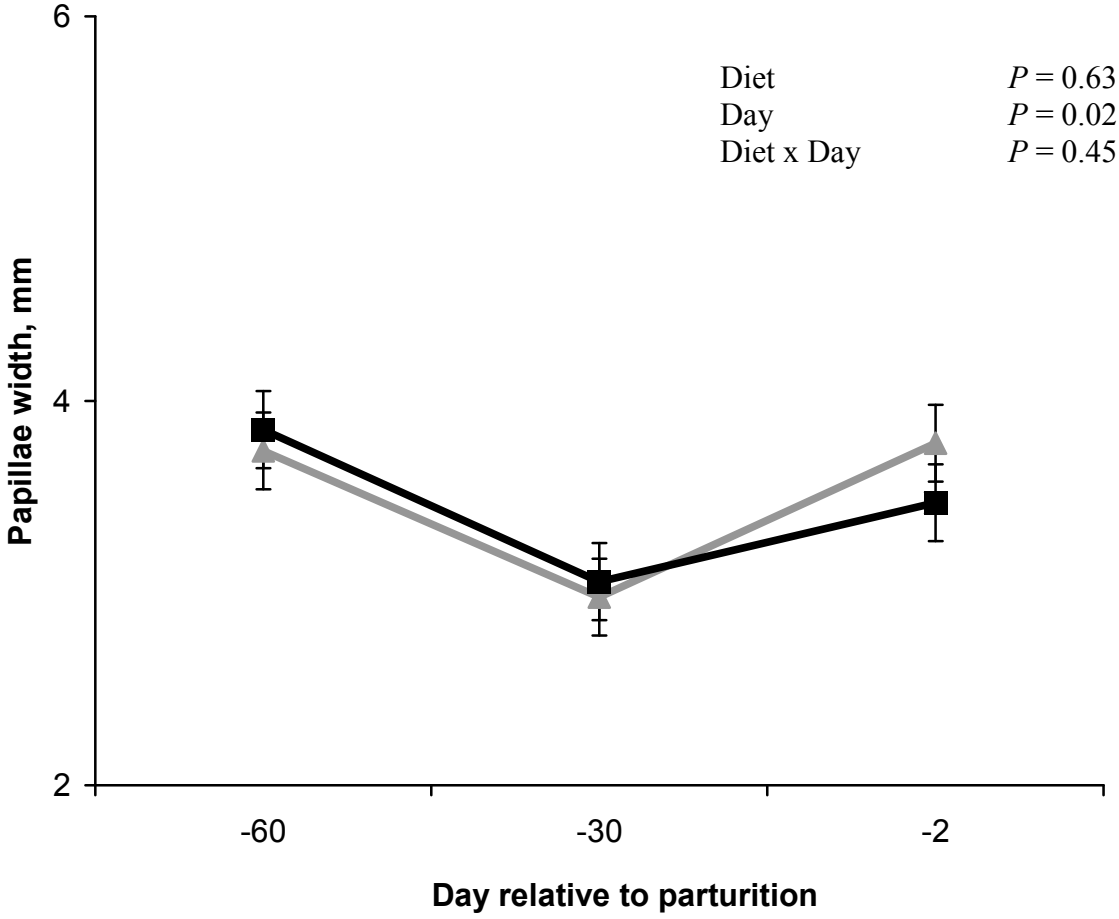

Figure 2-4. Prepartum papillae width for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=0.20, Exp. 2., n=3).

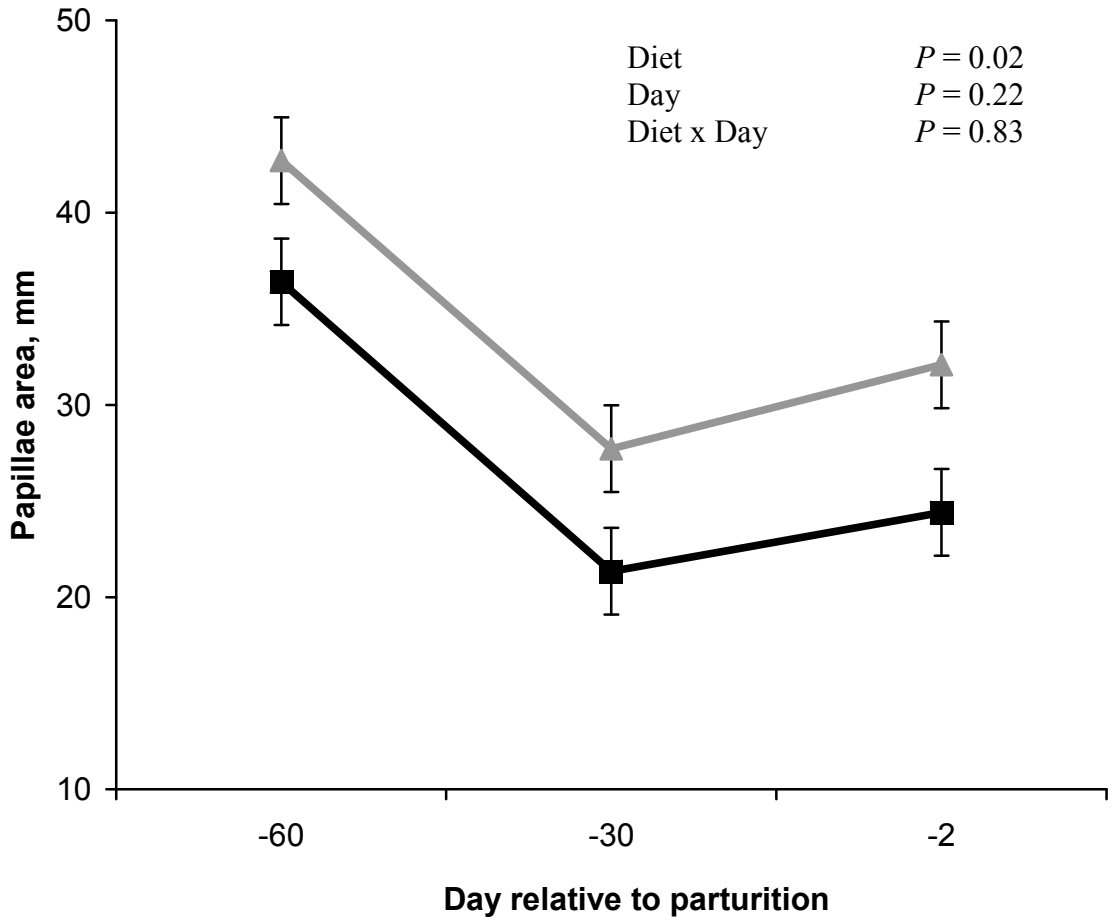

Figure 2-5. Prepartum papillae surface area for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=2.25, Exp. 2., n=3).

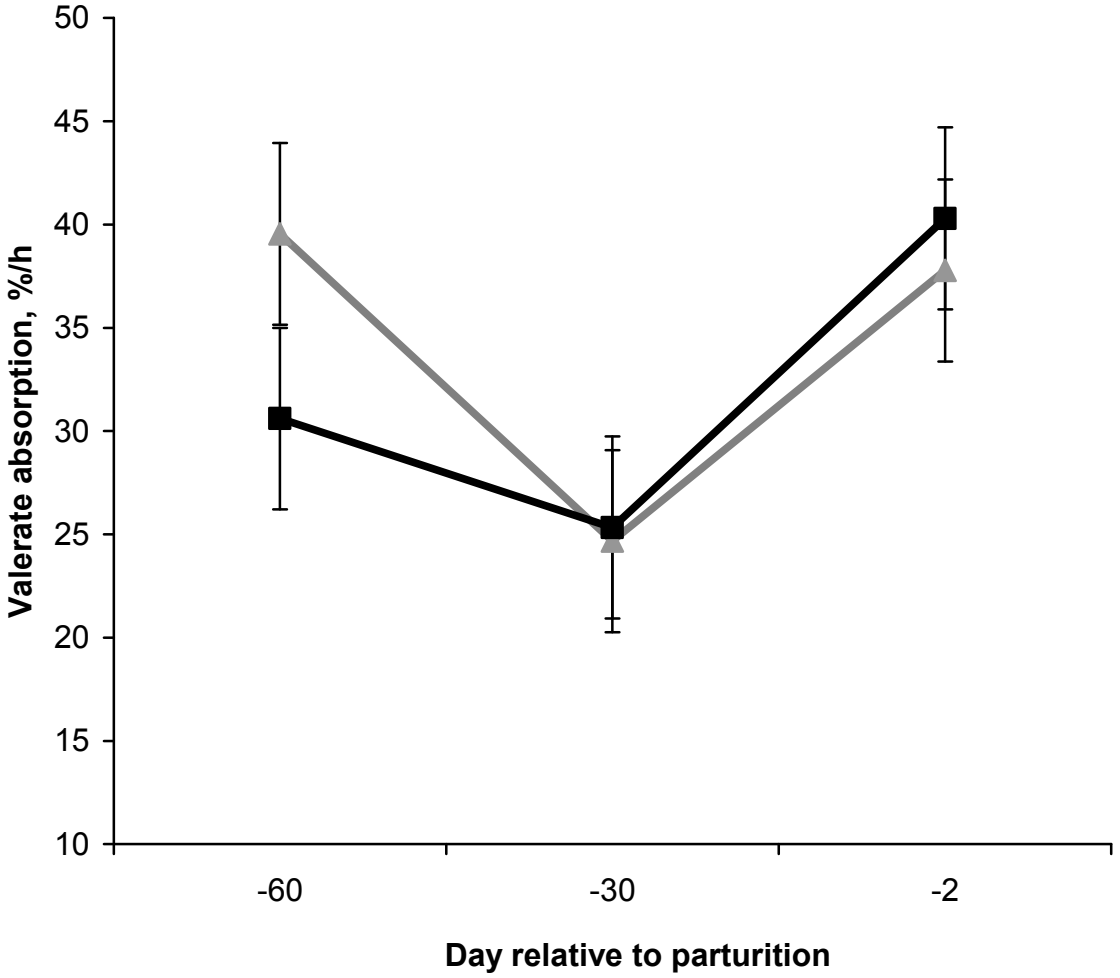

Figure 2-6. Prepartum valerate absorption for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=4.4, n=3).

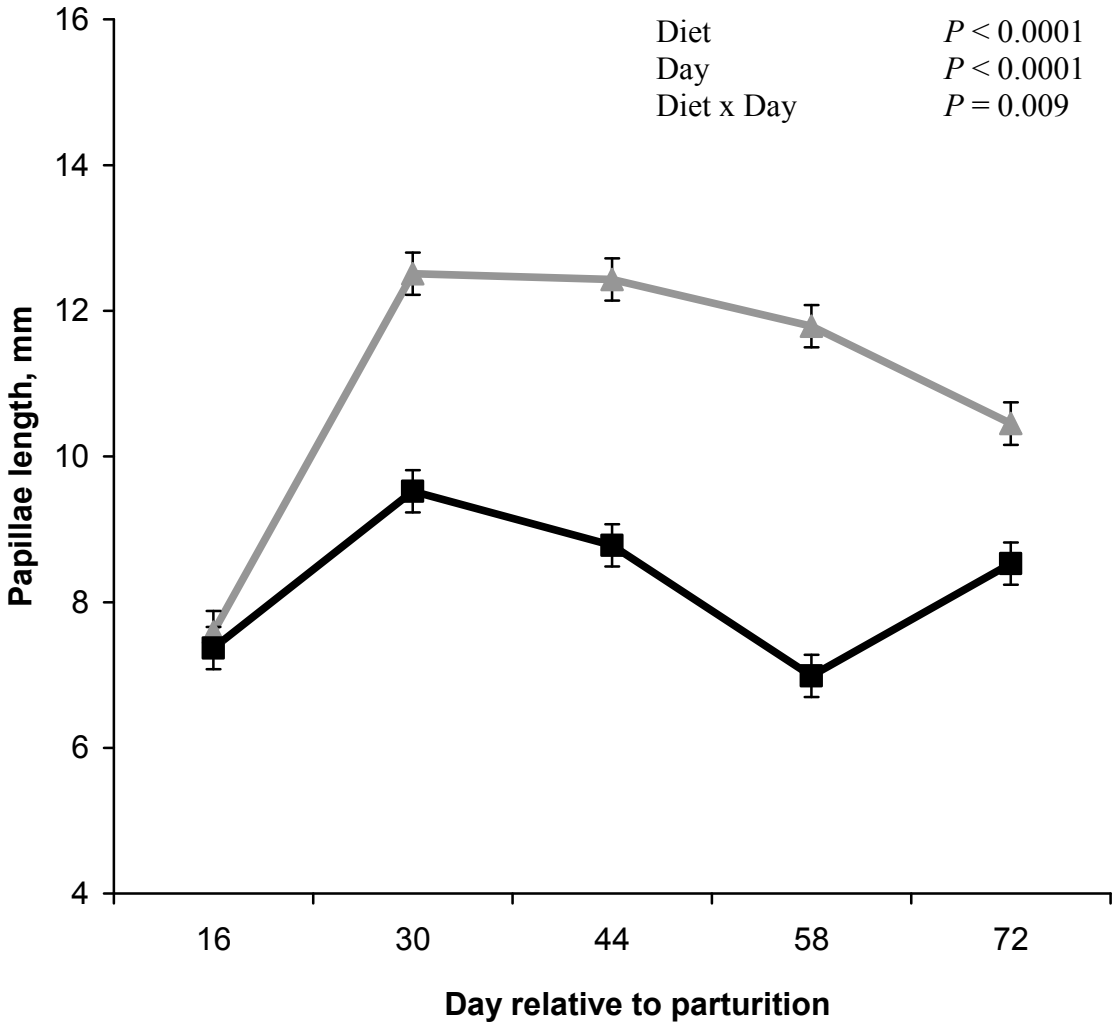

Figure 2-7. Postpartum rumen papillae length for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=0.29, Exp. 2, n=3).

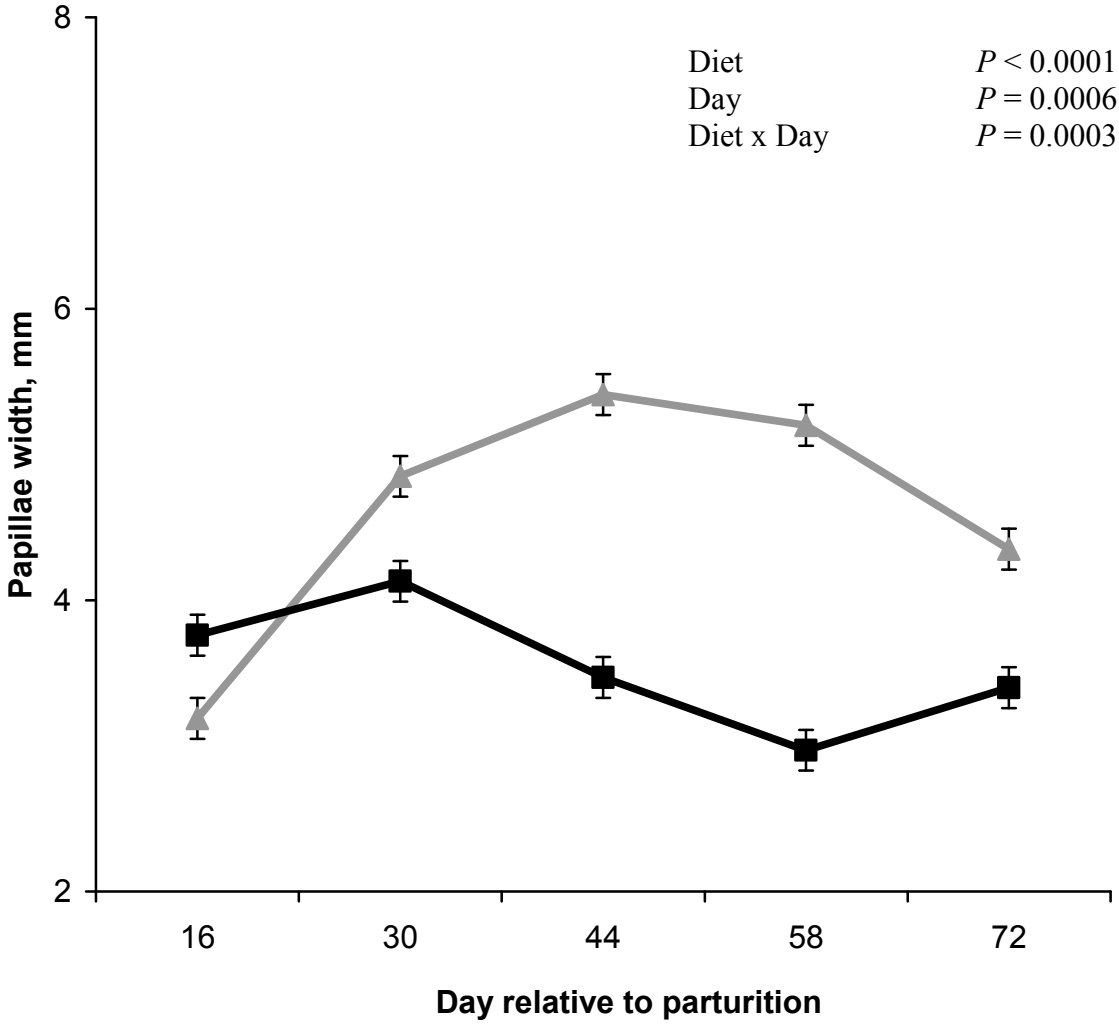

Figure 2-8. Postpartum rumen papillae width for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=0.14, Exp. 2, n=3).

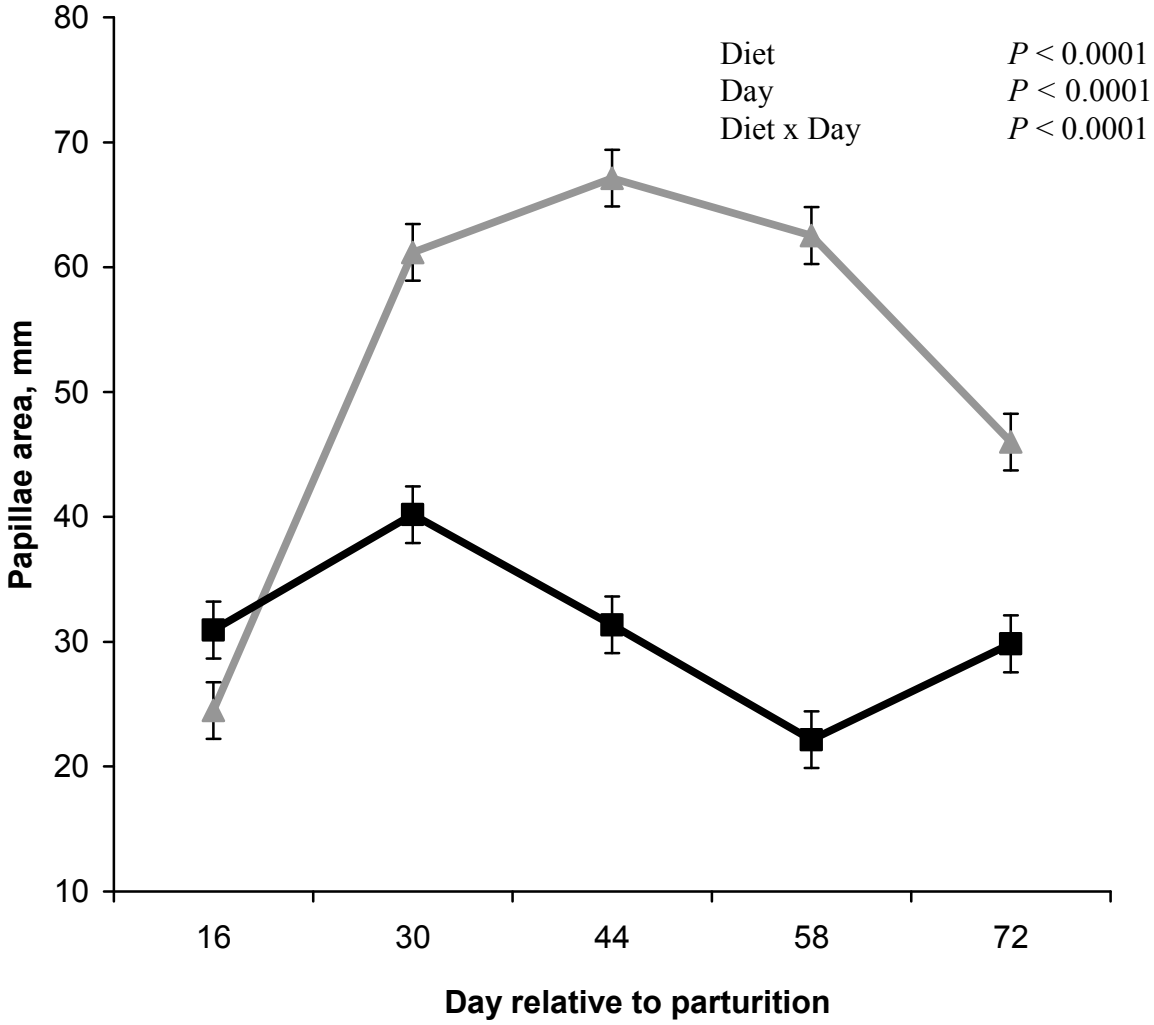

Figure 2-9. Postpartum rumen papillae surface area for cows fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=2.2, Exp. 2, n=3).

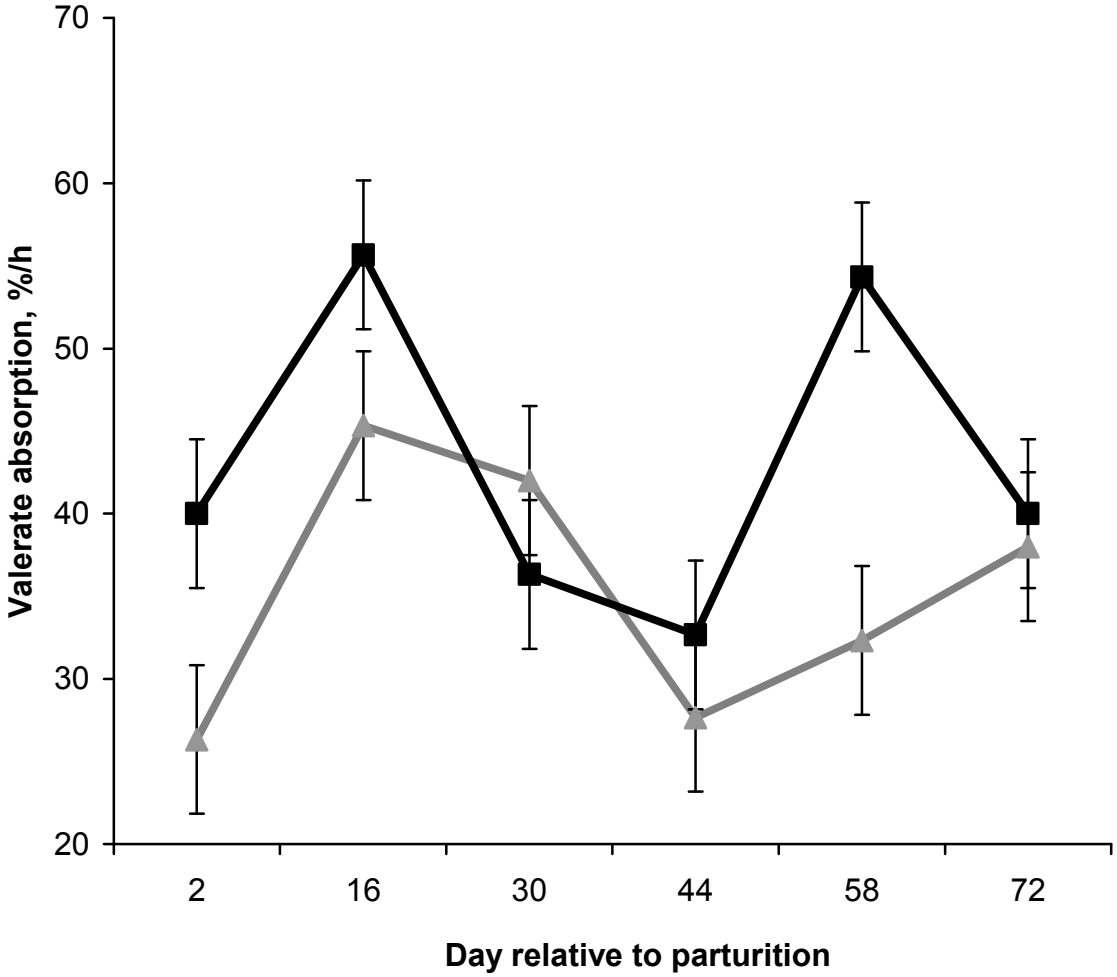

Figure 2-10. Postpartum valerate absorption for cow fed control (triangles) or molasses (squares) diets during a 60-d dry period (Pooled SEM=4.5, Exp. 2, n=3).

Chapter 3. Expression of glucagon-like peptide-2 receptor in bovine rumen epithelial and gastrointestinal tissue.

Abstract

This investigation was conducted to confirm the presence of glucagon-like peptide-2 receptor (GLP-2R) in the bovine rumen epithelium and gastrointestinal tract tissue obtained from 4 steers. Results from quantitative RT-PCR indicate the presence of GLP-2R mRNA in various tissues of the bovine including the rumen epithelium, omasum, abomasum, duodenum, jejunum, ileum, large intestine, and pancreas. Expression of mRNA for GLP-2R in pregastric bovine tissues is numerically less than in tissues from the lower gastrointestinal tract. The greatest expression of mRNA for GLP-2R is in the regions of the small intestine and large intestine. Within the small intestine, the jejunum and ileum have numerically greater concentrations of GLP-2R mRNA than the duodenum. In contrast, rumen epithelium and the abomasum demonstrated the lowest levels of GLP-2R mRNA expression. Among tissues sampled, Western blot analysis revealed the greatest density of GLP-2R protein in rumen epithelium obtained from the ventral sac. Results of this investigation demonstrate the presence of the glucagon-like peptide-2 receptor in bovine tissue which is responsible for mediating the actions of the hormone GLP-2. Further investigations are warranted to determine the actions and potential implications of GLP-2 in the bovine.

Introduction

Glucagon-like peptide-2 receptor is a 550-amino acid trans-membrane G protein-coupled receptor that mediates the response of the 33-amino acid hormone, glucagon-like peptide-2

(Drucker, 1996). Expression of mRNA for GLP-2R has been detected in tissues of the stomach, small and large intestine, and the central nervous system of monogastric species such as rats, mice, humans, and pigs (Munroe et al., 1999; Yusta et al., 2000), but investigations with ruminant tissues is lacking. The hormone GLP-2, acting through the receptor GLP-2R, is a potent stimulator of small intestine epithelial proliferation in the rat (Drucker, 1996) as histological findings upon GLP-2 administration demonstrate cell proliferation in crypts leading to elongation of the villous epithelium (Ghatei et al., 2001) and increased microvilli length (Benjemin et al., 2000). Additionally, responses to GLP-2 include increases in mucosal surface area (Drucker et al., 1997; Tsai et al., 1997), reduction in injury (Boushey et al., 1999), restored mucosal integrity (Ramsananhie et al., 2002), and enhanced intestinal absorptive function (Brubaker et al., 1997; Cheeseman, 1997) in rodents. Because the actions of this hormone are mediated through its receptor, our investigation was conducted to determine receptor presence in various bovine tissues.

Materials and Methods

Tissue Samples

Samples from steers were obtained from local abattoirs in Clay Center, KS and Manhattan, KS. Immediately following euthanasia of the animal, tissue samples were obtained from the rumen ventral sac and dorsal sac, duodenum, jejunum, ileum, and large intestine. Harvested tissues were rinsed in 0.90% saline solution, then frozen by submerging in liquid N_2 and stored at -80º C.

Protein Extraction

Total protein extraction from tissue was completed in the lab by homogenizing ~500 mg of sample for 30 s in 2.5 mL M-PER (78501; Pierce Biotechnology, Rockford, IL) with 22.0 mg NaCl added. Homogenate was then centrifuged at 10,000 x *g* for 15 min to pellet debris.

Aliquots of supernatant were transferred to 2 tubes, flash frozen in liquid N_2, and stored at -80ºC until removed for analysis. Concentration of protein was later determined at a wavelength of 260/280 nm using a ND-1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE).

Western Blot Analysis

Sixty micrograms of total protein was separated by gel electrophoresis using SDS-PAGE with precast Pierce Precise Protein 4-20% Gradient Gels (Pierce Biotechnology, Rockford, IL), and separated proteins were transferred onto a nitrocellulose membrane using a Trans-Blot Semi Dry Electrophoretic Transfer system (Bio-Rad, Hercules, CA). Nitrocellulose membranes were blocked using a blocking buffer (Starting Block Buffer, Pierce Biotechnology, Rockford, IL) for 15 min at 37ºC. Polyclonal rabbit primary antibody against GLP-2R (LS-A1312; Lifespan Biosciences, Seattle, WA) in 5 mL blocking buffer was added to the membrane and incubated overnight at 4ºC. The nitrocellulose membrane was then washed 3x for 15 min each with PBST. Secondary antibody, goat anti-rabbit-HRP (sc-2004; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), was added in 1 mL of blocking buffer to the membrane and incubated for 1 h. Detection of GLP-2R was completed using chemi-luminescence and a Fluorchem 8800 Imaging System (AlphaInnotech, San Leandro, CA).

Sample Preparation and RNA Isolation

Total RNA was isolated from 100 mg of tissue samples using sterile steel mortar bowls cooled by liquid N_2. Tissue samples were homogenized using a sterile pestle in liquid N_2. Then, 3 mL TRI Reagent (Sigma, St. Louis, MO) was added to the ground tissue sample, and 1 mL of tissue in TRI Reagent was incubated at room temperature for 5 min. Following incubation, chloroform (Sigma, St. Louis, MO) was added and samples were centrifuged for 15 min at 12,000 x *g* at room temperature. Following centrifugation, the top layer was removed and transferred to a new microcentrifuge tube. Isopropanol (Sigma, St. Louis, MO) was added, and

samples were centrifuged for 10 min at 12,000 x *g* to isolate the RNA pellet. The RNA pellet was then treated to remove any contaminating genomic DNA using the DNA-*free* kit (Ambion, Austin, TX). The RNA concentration was determined by absorbance at 260 nm and integrity of RNA was determined by gel electrophoresis. Total RNA with ethidium bromide was loaded onto a 1% agarose gel to separate and visualize 28S and 18S rRNA bands.

Real-time Quantitative-PCR

Real-time quantitative-PCR was used to measure the quantity of glucagon-like peptide 2 receptor (GLP2-R) gene expression relative to the quantity of 18S ribosomal RNA (**rRNA**) in total RNA isolated from tissue. Measurement of the relative quantity of cDNA was performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 900 n*M* of the appropriate forward and reverse primers, 200 n*M* of appropriate TaqMan detection probe, and 1 µL of the cDNA mixture. The bovine specific GLP2-R forward (GTGAGACAGAGTGGCTGTCCTATG) and reverse (TGCCCACAAAGTAGTGCAAGC) primers and TaqMan (6FAM-TTGCTGCCTCCTGCCGCTCA-TAMRA) detection probes were synthesized using published GenBank sequences. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; Genbank Accession #X03205). The ABI Prism 7000 detection system (Applied Biosystems, Foster City, CA) was used to perform the assay utilizing the recommended thermal cycling variables by the manufacturer (50 cycles of 15 s at 95ºC and 1 min at 60ºC). The 18S rRNA endogenous control was used to normalize the expression of GLP2-R.

Results

We conducted quantitative RT-PCR to determine the presence and quantity of GLP2-R mRNA in selected tissues of the bovine. Our results confirm the presence of GLP2-R in the rumen ventral sac epithelium, omasum, abomasum, duodenum, jejunum, ileum, large intestine,

and pancreas of the bovine. Statistical differences were not detected among tissues in mRNA expression because only 4 steers were used for tissue collection. Quantitative distribution of GLP2-R mRNA results from RT-PCR for tissue samples obtained from steers A and B are provided in Figure 3-1 and from steers C and D in Figure 3-2. The mRNA for GLP-2R was present in all tissues sampled and appears to be numerically greater in small and large intestine (Figure 3-1) for steer B and in the small intestine of steer D (Figure 3-2). With regard to preintestinal tissues for steers C and D, GLP-2R mRNA was numerically more concentrated in the omasum than of the abomasum and rumen ventral sac (Figure 3-2).

Western blot analysis was conducted to investigate the presence of the protein GLP-2R. Western blot analysis was not as conclusive as the RT-PCR results, nonetheless, it revealed the tissue with the greatest density of GLP-2R to be the epithelium of the rumen ventral sac (Figures 3-3 and 3-4). Other tissues were elusive in providing definite results using Western blot analysis with only the duodenum of steer D expressing a signal at 76kDa, indicative of the GLP-2 receptor.

Discussion

To our knowledge, no previous investigations have been conducted with regard to GLP2-R in bovine tissue. Our results using RT-PCR indicate the presence of mRNA for GLP-2R in the bovine tissues sampled. Expression of mRNA for the receptor is consistent with previous results from Munroe et al. (1999) where rat tissues dissected from the jejunum of the small intestine expressed the greatest concentration of mRNA for GLP-2R. Tissue samples from the duodenum, ileum, colon, and stomach of the rat followed in order regarding the concentration of mRNA for GLP-2R (Munroe et al., 1999). Although some variation exists between steers in our results (Figures 3-1 and 3-2) for mRNA concentration in sampled tissues, similarities exist between rat tissues and the sampled bovine tissues.

Concentration of mRNA for GLP-2R can vary with age as abundance was highest in piglets at birth and significantly less for weaned piglets (Petersen et al., 2003). The feeding state of our steers may have played a role in mRNA abundance as enteral food intake in piglets decreased mRNA concentration for GLP-2R in tissues of the small intestine (Petersen et al., 2003). Results from Western blot analysis (Figures 3-3 and 3-4) were intriguing in that consistent results were difficult to obtain for tissues other than the rumen ventral sac. Expression of GLP-2R at or near 76 kDa is common for the receptor in a glycosylated form (Guan et al., 2006). Lack of signal for tissues other than the rumen ventral sac may be related to total receptor present in the tissue. Results utilizing identical primary and secondary antibody have been less than desirable in piglets as well and is likely reflective of tissue receptor content (personal communication Doug G. Burrin, February 2007). Nevertheless, presence of the GLP-2 receptor in the rumen ventral sac was clear in our investigations with indication of receptor presence in the duodenum.

Conclusions

The mRNA for GLP-2R was present in the bovine tissues sampled. The GLP-2R protein was present in the rumen ventral sac, but GLP-2R protein in other tissues could not be definitively verified. It can be postulated that GLP-2 plays a role in the development of rumen epithelium and tissues of the lower gastrointestinal tract mediated through its receptor GLP-2R. Improved health of the rumen epithelium and intestinal mucosa could potentially improve animal performance and reduce morbidity during the transition period. Additional research regarding transition cow diets, GLP-2 secretion, and GLP-2R are essential to clarify the importance this mechanism may have in promoting uneventful transition periods.

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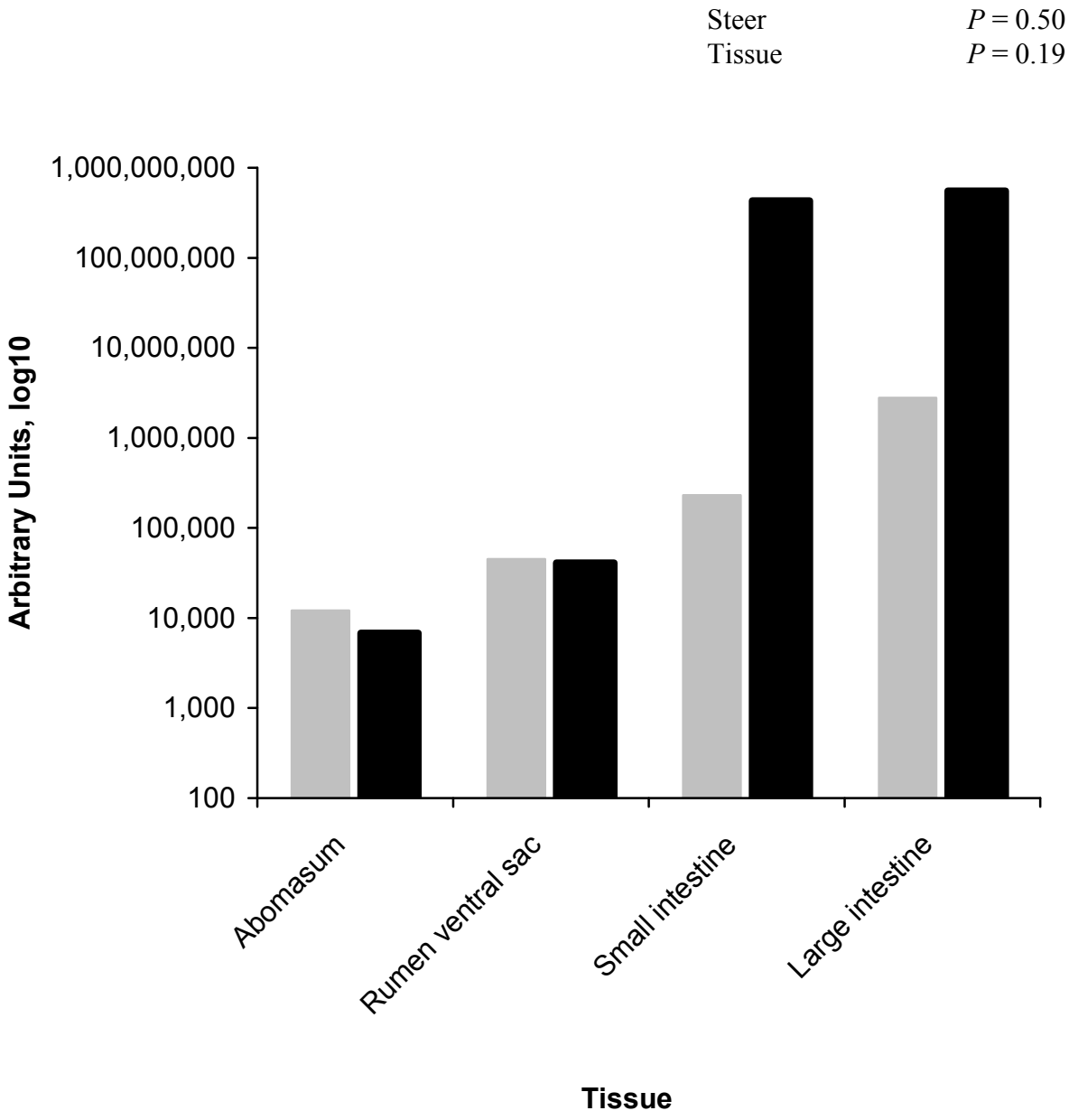

Figure 3-1. Glucagon-like peptide-2 receptor mRNA expression in tissues from steer A (grey bars) and steer B (black bars) (Pooled SEM = 1.44×10^8, n=2.

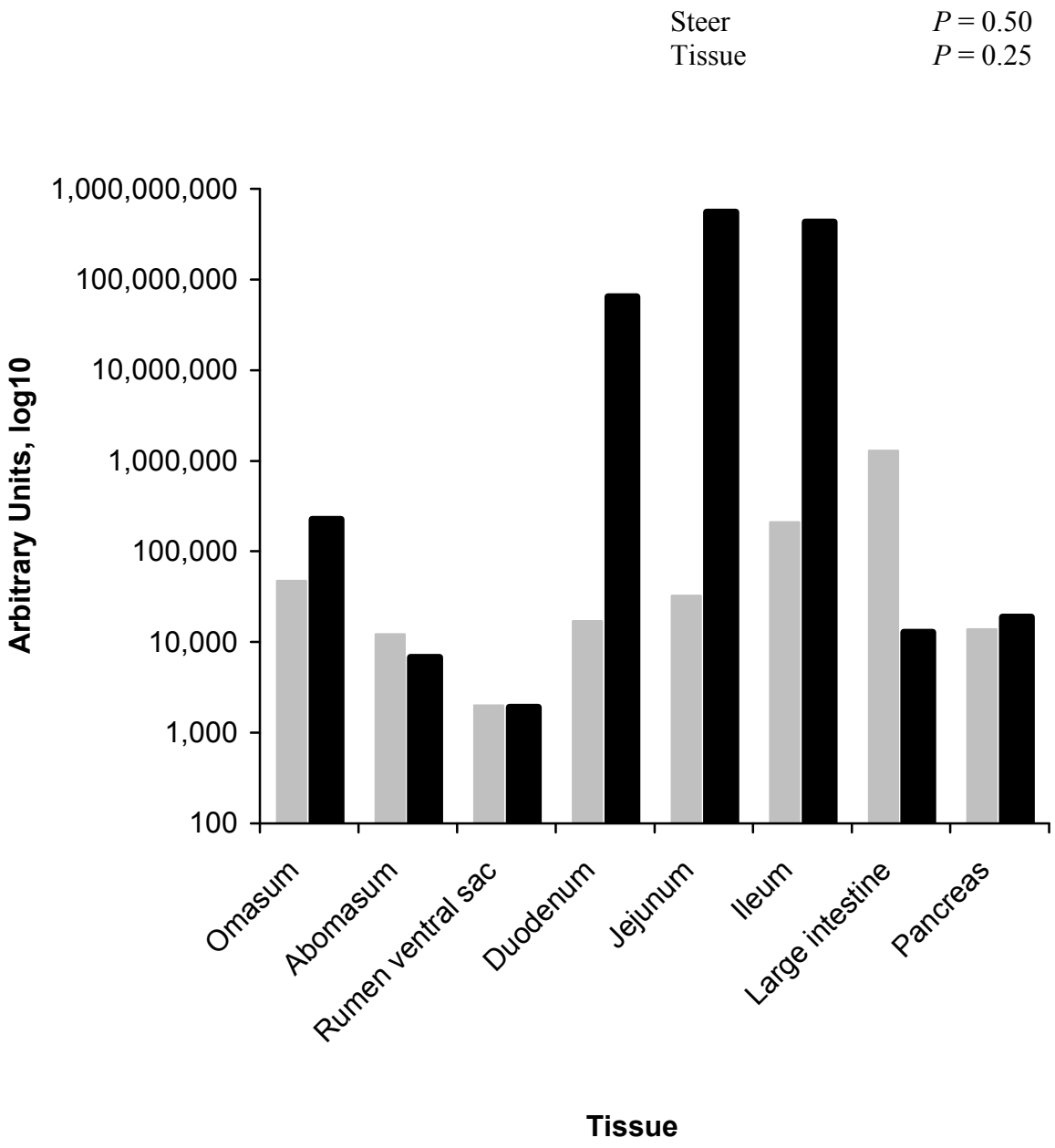

Figure 3-2. Glucagon-like peptide-2 receptor mRNA expression in tissues from steer C (grey bars) and steer D (black bars) (Pooled SEM = 74729634, n=2).

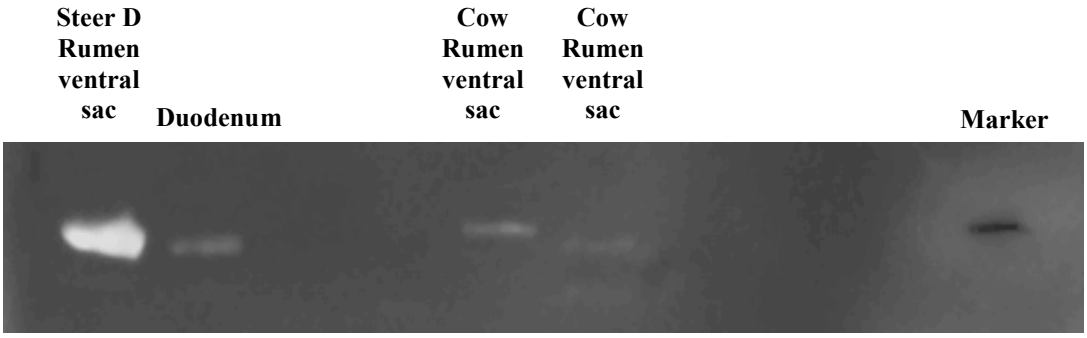

Figure 3-3. Representative Western blot for steer D from Manhattan, KS.

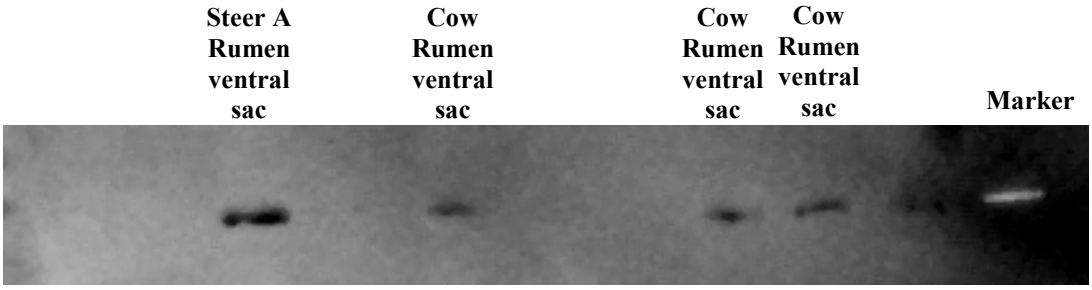

Figure 3-4. Representative Western blot for steer A from Clay Center, KS.

Chapter 4. Expression of glucagon-like peptide 2 receptor in rumen epithelium during the periparturient period in Holstein cows fed cane molasses

Abstract

Six multiparous Holstein cows fitted with rumen cannulas were utilized in a randomized complete block design to investigate the change in glucagon-like peptide-2 receptor mRNA and protein levels in rumen epithelium during the periparturient period. Cows were selected and blocked based on previous 305-d mature equivalent milk yield and projected calving date then randomly assigned to treatment. Treatments were a control diet and a diet supplemented with molasses fed during the far-off period (d -60 to -30) and the close-up period (d -29 to 0) relative to projected calving date. Cane molasses was added at 3.3% of DM during the far-off period and 3.7% of DM during the close-up period. Upon parturition a common lactation diet was fed for 77 d. Rumen epithelium was biopsied on d -60, -30, and -2 relative to projected calving date and on d 16, 30, 44, 58, and 72 postpartum for PCR and Western blot procedures. Prepartum glucagon-like peptide-2 receptor (GLP-2R) mRNA expression from rumen epithelium tended to be greater for cows fed molasses, whereas postpartum mRNA expression tended to be greater for cows previously fed control. Expression of mRNA for GLP-2R was increased on d 30 prepartum. A significant interaction was observed for diet x day for mRNA expression prepartum as expression increased from d 60 to d 2 prepartum for molasses diet while decreasing for control diet. Glucagon like peptide-2 receptor protein density was not affected by diet during the prepartum period in this study. During the postpartum period, GLP-2R density was greater on d 30, 58, and 72. Postpartum GLP-2R protein density in rumen epithelium was greater for

cows fed control diet during the prepartum period. Results from these data indicate that diet can affect mRNA expression of GLP-2R. Data indicate that GLP-2R is influenced in the bovine by stage of gestation and lactation.

Key words: dairy cow, periparturient, rumen epithelium, glucagon like peptide-2

Introduction

The rumen is a dynamic region of the digestive tract responsible for absorption of VFA from microbial fermentation of feedstuffs. Maintenance of intake during the prepartum period has been reported to be imperative to the success of the approaching lactation (Bertics et al., 1992). Thus adaptive changes undertaken by rumen epithelium to promote VFA absorption and minimize VFA accumulation likely will result in a positive response in intake. Rumen epithelial tissue has been reported to undergo a state of involution and proliferation in relationship to diet, as rumen papillae growth and VFA absorption was markedly improved when cows were fed high-concentrate diets (Dirksen et al., 1985). Infusions of butyrate into the rumen of sheep demonstrated stimulation of cellular proliferaton in rumen epithelial tissue (Sakata and Tamate, 1978). However, no studies have investigated mechanisms associated with rumen epithelial adaption with regard to a periparturient dairy cow.

Glucagon-like peptide-2 is a 33 amino acid hormone co-secreted with GLP-1 from the enteroendocrine L cells of the small intestine (Drucker et al., 1996). Cellular actions of GLP-2 are mediated through its receptor, GLP-2R, which is a 7-domain transmembrane protein linked to the G-protein-coupled receptor superfamily. Drucker et al. (1996) first reported that GLP-2 had trophic effects in the murine intestine. Studies with GLP-2 have demonstrated increases in mucosal surface area (Drucker et al., 1997, Tsai et al., 1997), reduction in injury (Boushey et al., 1999), restored mucosal integrity (Ramsananhie, et al., 2002), and enhanced intestinal absorptive function (Brubaker et al.; 1997, Cheeseman, 1997) in rodents. Increases in butyrate levels (9 or

60 mmol/L) supplemented to total parenteral nutrition (TPN) in neonatal piglets increased plasma GLP-2 concentrations and villus height in the jejunum and ileum. In light of the GLP-2 response to butyrate and the proliferative effects demonstrated in the lower gastrointestinal tract, our hypothesis was that a similar mechanism may be associated with papillae development in cattle. The objective of this research was to investigate the presence and response of GLP-2R in the rumen epithelium during the periparturient period of the dairy cow.

Materials and Methods

All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Six cows with rumen cannulas were housed in a tie-stall barn beginning 14 d prior to parturition to allow for adaptation. Diets (Table 15) were fed during the prepartum far-off period (-60 to -30 d) and the prepartum close-up period (-29 to 0 d). The postpartum diet (Table 15) was a common lactation diet fed to all cows. Rumen epithelium was biopsied on d -60, -30, and -2 relative to projected calving date and on d 16, 30, 44, 58, and 72 postpartum by removing the rumen contents and exteriorizing a portion of the rumen ventral sac. Upon exteriorization a small section of the epithelium was rinsed with sterile saline to remove debris. Mayo scissors were used to excise a sample of approximately 4.5 cm x 1.5 cm of rumen epithelium without invading the underlying musculature. Biopsy samples were immediately rinsed in a chilled saline solution, snap frozen in liquid N_2 and stored at -80ºC. The area of excision was rinsed with sterile saline and sutured closed using 2-0 vicryl absorbable suture material.

Protein Extraction

Total protein extraction from tissue was completed in the lab by homogenizing approximately 500 mg of biopsied tissue for 30 s in 2.5 mL M-PER (78501; Pierce Biotechnology, Rockford, IL) with 22.0 mg NaCl added. Homogenates were then centrifuged at

10,000 *g* for 15 min to pellet debris. Aliquots of supernatant were transferred to 2 tubes, frozen in liquid N_2, and stored at -80ºC until removed for analysis. Concentration of protein was later determined using a ND-1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE).

Western Blot Analysis

Total protein was separated by gel electrophoresis using SDS-PAGE with precast Pierce Precise Protein 4-20% Gradient Gels (Pierce Biotechnology, Rockford, IL). Separated proteins were transferred onto a nitrocellulose membrane using a Trans-Blot Semi Dry Electrophoretic Transfer system (Bio-Rad, Hercules, CA). Nitrocellulose membranes were blocked using a blocking buffer (Starting Block Buffer, Pierce Biotechnology, Rockford, IL) for 15 min at 37°C. Polyclonal rabbit primary antibody against GLP-2R (LS-A1312; Lifespan Biosciences, Seattle, WA) in 5 mLof blocking buffer to create a 1:20,000 dilution was added to the membrane and incubated overnight at 4°C. The nitrocellulose membrane was then washed 3x for 15 min each with PBST. Secondary antibody, goat anti-rabbit-HRP (sc-2004; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), was added at a dilution of 1:5000 in 1 mL of blocking buffer to the membrane and incubated for 1 h. Detection and quantification of GLP-2R was completed using chemi-luminescence and a Fluorchem 8800 Imaging System (AlphaInnotech, San Leandro, CA).

Sample Preparation and RNA Isolation

Total RNA was isolated from 100 mg rumen epithelial tissue using sterile steel mortar bowls cooled by liquid nitrogen. Tissue samples were homogenized using a sterile pestle in liquid nitrogen. TRI Reagent (Sigma, St. Louis, MO; 3 mL) was added to the ground tissue sample, and 1 mL of tissue in TRI Reagent was incubated at room temperature for 5 min. Following incubation, chloroform (Sigma, St. Louis, MO) was added, and samples were centrifuged for 15 min at 12,000 x *g* at room temperature. Following centrifugation, the top layer was removed and transferred to a new microcentrifuge tube. Isopropanol (Sigma, St.

Louis, MO) was added and samples were centrifuged for 10 min at 12,000 x *g* to isolate the RNA pellet. The RNA pellet was then treated to remove any contaminating genomic DNA using the DNA-*free* kit (Ambion, Austin, TX). The RNA concentration was determined by absorbance at 260nm. Total RNA with ethidium bromide was loaded onto a 1% agarose gel to separate and visualize 28S and 18S rRNA bands to determine RNA integrity.

Real-time Quantitative-PCR

The bovine specific GLP2-R forward (GTGAGACAGAGTGGCTGTCCTATG) and reverse (TGCCCACAAAGTAGTGCAAGC) primers and TaqMan (6FAM-TTGCTGCCTCCTGCCGCTCA-TAMRA) detection probes were synthesized using published GenBank sequences. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; Catalog #X03205). The ABI Prism 7000 detection system (Applied Biosystems, Foster City, CA) was used to perform the assay utilizing the recommended thermal cycling variables by the manufacturer (50 cycles of 15 s at 95ºC and 1 min at 60ºC). The 18S rRNA endogenous control was used to normalize GLP2-R mRNA concentration. Real-time quantitative-PCR was used to measure the quantity of glucagon-like peptide 2 receptor (GLP2-R) gene expression relative to the quantity of 18S ribosomal RNA (**rRNA**) in total RNA isolated from rumen epithelial tissue. Measurement of the relative quantity of cDNA was performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 900 n*M* of the appropriate forward and reverse primers, 200 n*M* of appropriate TaqMan detection probe, and 1 µL of the cDNA mixture. Data were presented as arbitrary units.

Statistical Analysis

Prepartum samples and postpartum results were analyzed separately using PROC MIXED of SAS version 8.01 (SAS Inst., Inc., Cary, NC) as repeated measures over time. The

model included diet, period, and diet x period interactions as fixed effects. Random effects included cow, block, and cow x block. Statistical significance was declared at $P < 0.05$ and trends noted at $P > 0.05$ to $P < 0.15$. Least square mean separation for treatment effects were conducted using the PDIFF statement in SAS.

Results

Primary antibody was polyclonal rabbit antibody raised against human antigen; because of the conserved nature of GLP-2R, its use is likely acceptable. A query using BLAST in Pub Med returned a homology of > 93% for human and bovine GLP-2R. Representative Western blots are presented in Figure 4-3 and 4-4. The glycosylated form of GLP-2R is 73 kd in size.

A diet x day interaction ($P = 0.05$) during the prepartum period was observed for expression of mRNA for GLP-2R (Figure 4-1). Initial rumen epithelial tissue samples taken on d -60 before offering experimental diets were similar for expression of mRNA (Figure 4-1). After the initial 30 d of feeding control or molasses diets and immediately prior to the close-up period, mRNA expression was elevated for cows fed the molasses diet. Messenger RNA expression encoding GLP-2R declined as parturition approached on d -2 (Figure 4-1). The mRNA expression for GLP-2R tended ($P = 0.14$) to differ between diets during the prepartum period (Figure 4-1). A significant day effect ($P = 0.03$) was present for mRNA expression for GLP-2R due to the increase observed at -30 d prior to parturition for cows fed molasses (Figure 4-1).

Postpartum expression of mRNA for GLP-2R is represented in Figure 4-2. Day tended ($P = 0.08$) to differ during the postpartum period for expression of mRNA encoding GLP-2R, whereas no effect of diet was observed ($P = 0.13$).

Representative Western blots of prepartum and postpartum GLP-2R are shown in panel A of Figure 4-3 and Figure 4-4. Prepartum GLP-2R Western blot density was unaffected by diet or day (Figure 4-3, Panel B). Blot density for GLP-2R during lactation is represented in

Figure 4-4. Cows previously offered the control diet during the prepartum period had greater ($P < 0.01$) receptor density during the postpartum period (Figure 4-4, Panel B).

Discussion

Information on the presence or response of GLP-2R to diet or the transition period has been lacking for dairy cattle. Previous research has focused on effects of IGF-I on rumen epithelium growth and development (Shen et al., 2004; Baldwin, 1999). Recently glucagon-like peptide-1 (GLP-1), a proglucagon derived peptide, has been reported to increase in plasma concentration as DMI increases during early lactation for Holstein cows (Relling and Reynolds, 2007). Small intestinal secretion of gastrointestinal tract hormones is a response to the presence of nutrients in the lumen (Hansen et al., 2004). As lactation begins, DMI for lactating dairy cattle increases. This increase in DMI should stimulate the secretion of gut peptides from the small intestine and consequently circulating concentrations of those hormones responsible for metabolic and physiological adaptations to aid nutrient absorption. Actions of these circulating gastrointestinal hormones are mediated through the expression of specific receptors such as GLP-2R. In our experiment, circulating GLP-2 was not evaluated, but the receptor responsible for a response by GLP-2 hormone was. Results are consistent with our previous investigation using steers (Chapter 3) and reconfirm the presence of the GLP-2R in bovine rumen epithelium. Significant interaction and day effects for mRNA expression of GLP-2R during the prepartum period is driven by the increase observed at d -30 for cows that received the molasses diet. The increase was only observed in cows fed molasses diets. DMI during this time was similar for cows fed diets with or without molasses (Chapter 2) which indicates that total nutrient supply was unlikely responsible for the difference. Propionate and butyrate concentrations in rumen fluid were numerically greater for cows fed molasses during the prepartum period (Chapter 2) which may have contributed to rumen epithelial metabolism during this time.

Diminishing expression of mRNA for GLP-2R (Figure 4-2) over the initial 72 d of lactation may reflect an adaptation in gut epithelium to intake and nutrient absorption. The initial increase in mRNA during early lactation for cows offered the control diet prepartum may suggest a delayed response by gut tissue of these cows as mRNA expression peaked at d 30 prepartum for cows fed molasses.

Although the presence of GLP-2R in the rumen suggests that GLP-2 may play an important role in rumen development, the changes in GLP-2R over time or in response to dietary treatment are unlikely to tell the complete story. Clearly, the concentrations of GLP-2 also need to be considered. It is possible that GLP-2R is regulated either directly or indirectly by changes in blood concentrations of GLP-2. Moreover, the tissue responsiveness to GLP-2 will be dependent upon the concentrations of the receptor and of the agonist.

Conclusion

The existence of GLP-2R in rumen epithelial tissue was demonstrated, implying a potential mechanism for anabolic effects on rumen epithelial proliferation. Glucagon-like peptide-2 receptor demonstrated no response to diet or ongoing gestation during the prepartum period, whereas receptor density increased as lactation proceeded. We believe that our study is the first to investigate glucagon-like peptide-2 receptor in cattle, and more extensive research is warranted to elucidate the importance of this hormone receptor in regard to rumen epithelial tissue adaptation.

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Table 13. Ingredient and nutrient composition of control and cane molasses diets

	Far-off		Close-up		
Ingredient	Control	Molasses	Control	Molasses	Lactation
	----------% DM ----------				
Prairie hay	57.5	56.6	37.3	37.3	-
Corn silage	12.6	12.4	12.2	12.2	20.5
Wet corn gluten feed	9.8	9.6	11.9	11.9	20.6
Corn, ground	1.3	-	16.4	12.9	21.7
Alfalfa hay	6.8	6.7	9.3	9.3	15.5
Whole cottonseed	3.4	3.0	5.1	5.1	8.4
Soybean meal, solvent	7.1	7.0	5.1	5.1	-
Soybean meal, expeller	-	-	-	-	7.5
Molasses, sugarcane	-	3.3	-	3.7	0.90
Sodium bicarbonate	-	-	0.48	0.46	0.80
Magnesium oxide	0.14	0.13	0.40	0.40	0.20
Fishmeal, menhaden	-	-	1.25	1.23	2.08
Limestone	0.54	0.53	0.14	0.14	1.36
Trace mineralized salt[a]	0.53	0.52	0.20	0.19	0.33
Vitamin ADE premix[b]	0.14	0.14	0.17	0.16	0.12
Sodium selenite[c]	0.03	0.03	0.02	0.02	0.008
Nutrient					
DM, % as fed	73.2	73.3	70.9	71.6	62.5
CP	12.4	12.2	14.0	13.9	18.8
NDF	53.7	52.4	46.9	45.8	35.8
ADF	33.4	32.6	26.5	25.9	19.1
NE_L, Mcal/kg	1.25	1.29	1.41	1.42	1.63
Calcium	0.51	0.52	0.43	0.46	0.94
Phosphorus	0.34	0.35	0.39	0.39	0.51
Magnesium	0.39	0.29	0.44	0.45	0.42
Postassium	1.67	1.73	1.42	1.55	1.19
Sodium	0.25	0.30	0.27	0.27	0.45

[a]Composition not less than: 95.5% NaCl, 0.24% Mn, 0.24% Fe, 0.05% Mg, 0.032% Cu, 0.032% Zn, 0.007% I, 0.004% Co.
[b]Contributed 4,400 IU Vitamin A; 2,200 IU Vitamin D; and 16 IU Vitamin E per kg of dietary DM.
[c]Provided 0.06 mg Se per kg of diet DM.

Figure 4-1. Glucagon-like peptide-2 receptor mRNA expression in rumen epithelial tissue from cows fed control (triangles) and molasses (boxes) diets during the prepartum period. (pooled SEM = 161, n=3)

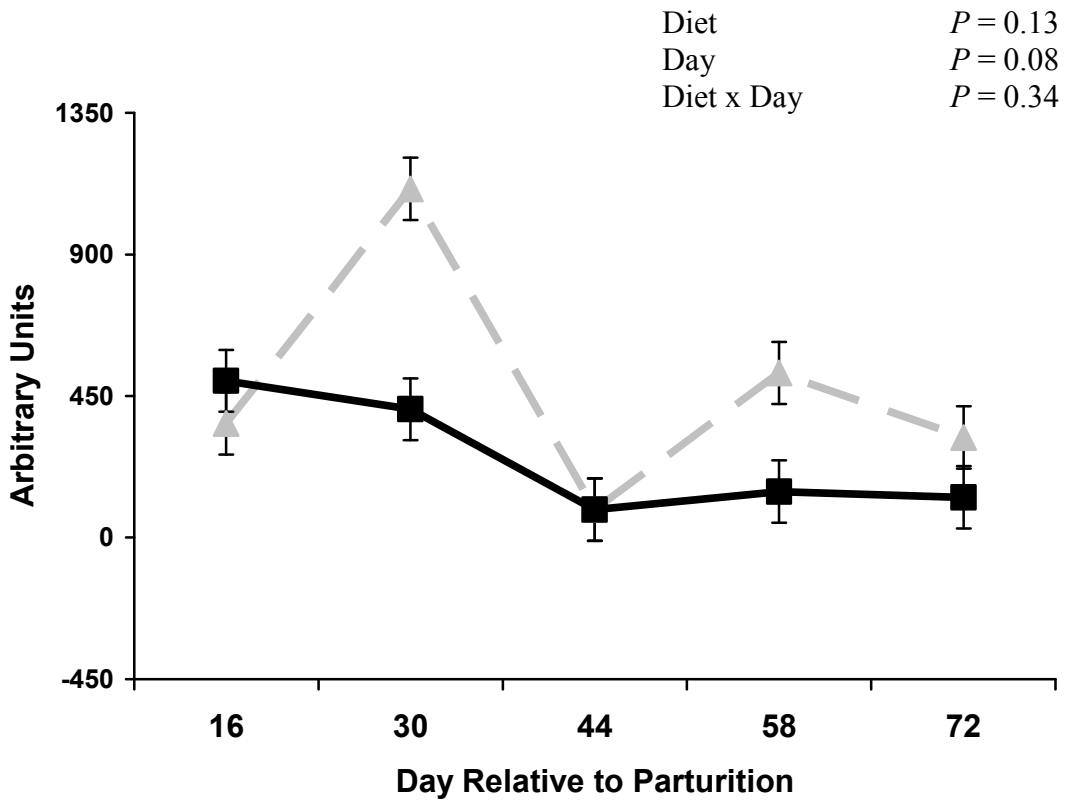

Figure 4-2. Glucagon-like peptide-2 receptor mRNA expression in rumen epithelial tissue from cows fed control (triangles) and molasses (boxes) diets during the postpartum period. (pooled SEM = 99, n=3)

A.

76 kDa

B.

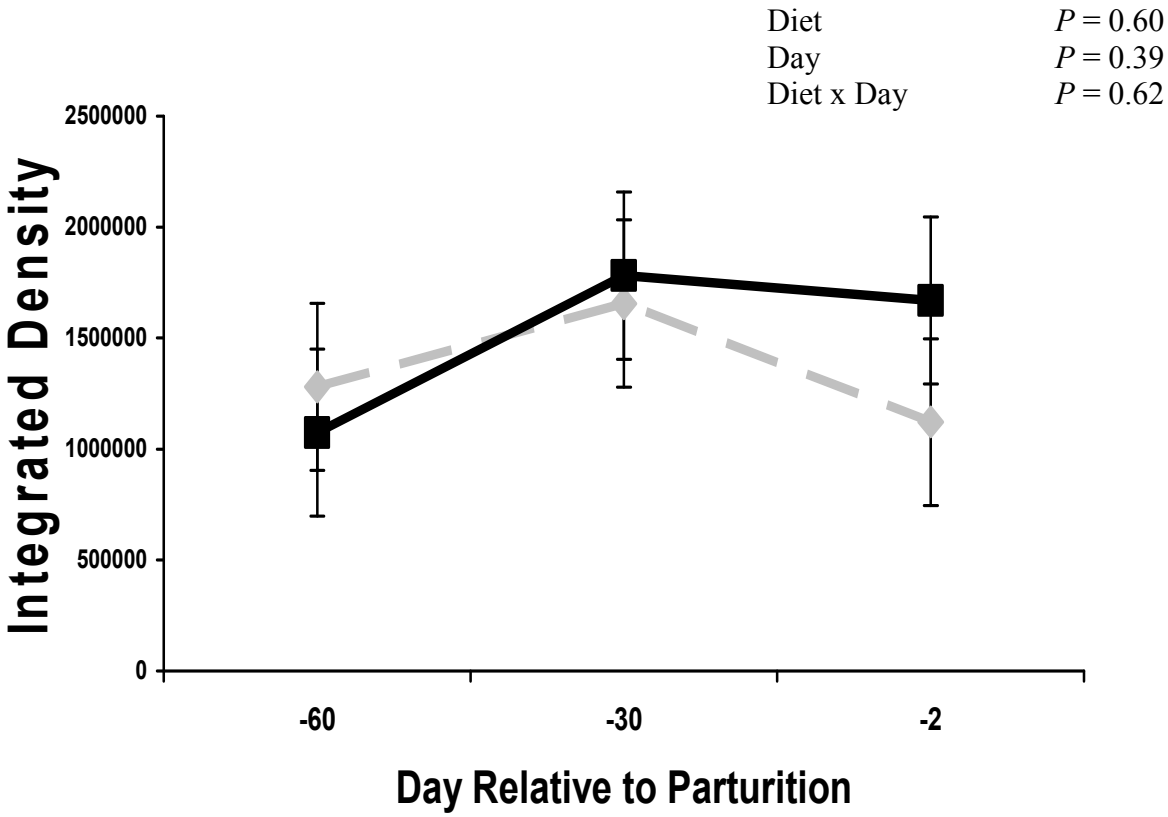

Figure 4-3. Representative Western blot analysis of glucagon-like peptide-2 receptor from rumen epithelium during the prepartum period panel A. Glucagon-like peptide-2 receptor density in rumen epithelial tissue from cows fed control (triangles) and molasses (boxes) diets during the prepartum period. (pooled SEM = 235331, n=3) Panel B

A.

76 kDa

B.

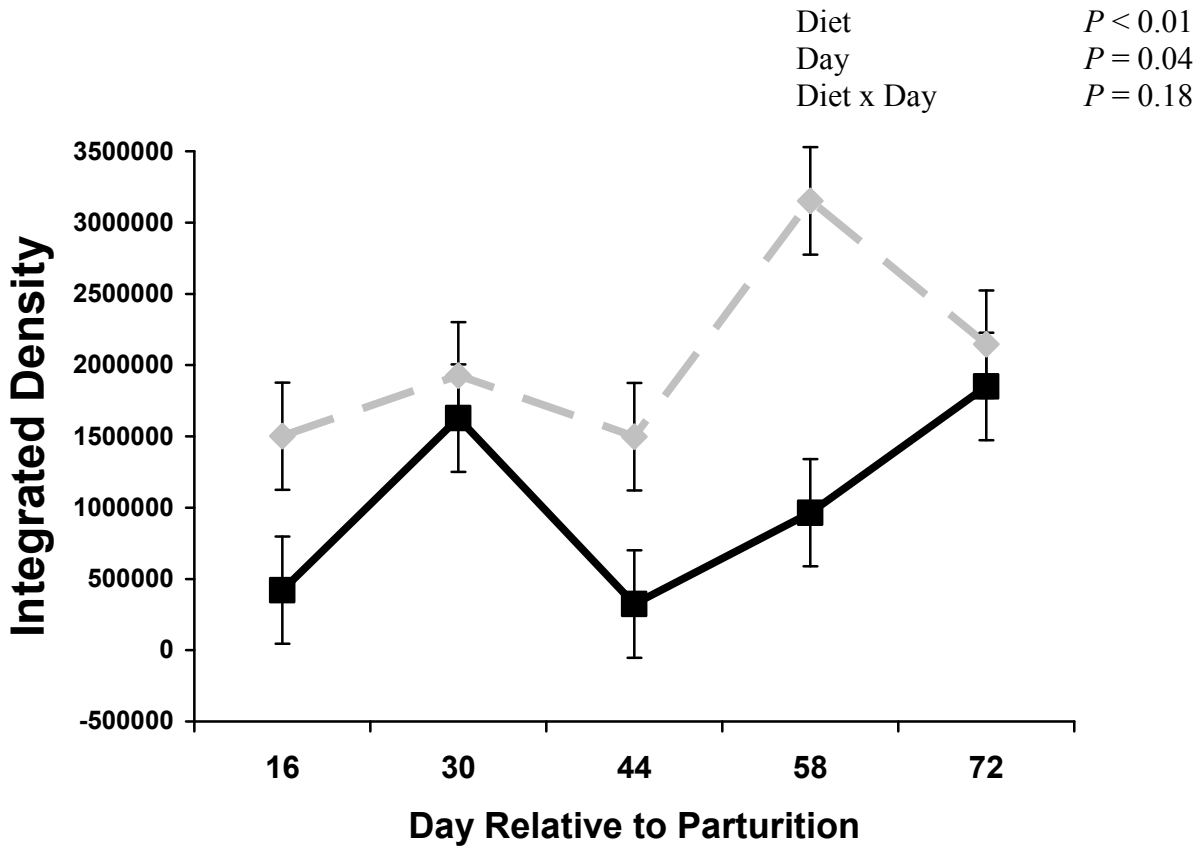

Figure 4-4. Representative Western blot analysis of glucagon-like peptide-2 receptor from rumen epithelium during postpartum period panel A. Glucagon-like peptide-2 receptor density in rumen epithelial tissue from cows fed control (triangles) and molasses (boxes) diets during the postpartum period (pooled SEM = 504,376, n=3) Panel B