EFFECTS OF DUODENAL AMINO ACID INFUSION ON SMALL INTESTINAL STARCH DIGESTION IN CATTLE

by

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B.S., The Ohio State University, 2006
M.S., Kansas State University, 2009

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

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Abstract

Previous data suggest that greater amounts of postruminal protein increase small intestinal starch digestion in cattle. Duodenally and ileally cannulated steers were used in 5 studies to measure responses in small intestinal starch digestion to amino acids (AA) or casein. Flows of starch to the ileum from the diet were small. Small intestinal starch digestibility was 34.0% when raw cornstarch was continuously infused into the duodenum. Infusion of casein linearly increased ($P \leq 0.05$) small intestinal starch digestibility, and small intestinal starch digestion adapted to infusion of casein in 6 d. Ethanol-soluble starch and unpolymerized glucose flowing to the ileum increased linearly ($P \leq 0.05$) with increasing infusion of casein. Plasma cholecystokinin was not affected by casein infusion, but circulating levels of glucose increased linearly ($P \leq 0.05$). In another study, 5 steers were fed a low-starch diet and provided continuous duodenal infusion of raw cornstarch in combination with AA or casein in order to measure response of small intestinal starch digestion. Duodenal infusion of casein increased ($P \leq 0.05$) small intestinal starch digestion. When a mixture of AA with a profile similar to casein (CASAA) was infused, small intestinal starch digestion was similar ($P = 0.30$) to casein infusion. Infusion of only non-essential AA tended to increase ($P = 0.14$) small intestinal starch digestion relative to control; however, infusion of essential AA alone did not affect ($P = 0.84$) small intestinal starch digestion. Additionally, infusion of casein or essential AA increased ileal flows of ethanol-soluble starch, but non-essential AA alone were not different than the negative control. Duodenal infusion of Glu increased ($P \leq 0.05$) small intestinal starch digestion, whereas a mixture of Phe, Trp, and Met (PTM) did not. Neither Glu nor PTM increased ileal flow of ethanol-soluble starch, but Glu and PTM provided together tended ($P = 0.07$) to increase ileal flows of ethanol-soluble starch. Our data suggest that Glu alone can increase small intestinal

starch digestion in cattle similar to casein, but increases in small intestinal starch digestion in response to Glu are not associated with an increase in ileal flows of ethanol-soluble starch.

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Major Professor
Evan C. Titgemeyer

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An idiom commonly used in reference toward the effort required for the proper care of children is that, “it takes a village to raise a child”; I contend that this phrase holds equal applicability toward the training of graduate students. Throughout my graduate training many individuals have left an indelible impression on me, and I am sincerely grateful for their efforts and contributions; without the help of so many, the successful completion of my graduate training would not have been possible.

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No one has been more instrumental in my life and toward my current accomplishments than Ashley, my wife. Ashley followed me to Kansas from Ohio shortly after I started my graduate training in 2007 and ever since I have been blessed with her love, support and companionship. The science of seeking answers related to the principles by which cattle digest and metabolize nutrients is complex, and for those who dare to pull back the mask of nature in effort to gaze upon these fundamental aspects of biology, the graduate training experience can be both challenging and demanding. Often the hardships inherent to the type of training I have pursed throughout the past five years have not been conserved to personal challenges, but have also, affected my family (i.e., my wife and son). Undoubtedly, there were many hours spent in the lab that kept me from being able to do all the things that one would like to do with family. Yet, Ashley’s continued temperance, understanding, love and devotion have served as a constant in my life and not a day goes by where I do not appreciate her efforts. Her willingness to endure

constant references to the similarities between her and the physiological and metabolic characteristics of cattle or my musings over the writings and contributions of some of the prominent scientists in my field are not likely among those things she cherishes most about our marriage; however, she apparently understands (or at least tolerates the fact) that this is now how my mind comprehends things and continues to love me all the same. Ashley continues to be a spectacular mother to our son and for this I am eternally grateful. I am very blessed to have found such a wonderful companion in life, and I continue to try and be as good a husband as she is a wife.

A little more than three years ago, about three minutes after I had arrived home from the national meetings ASAS meetings in Montreal, Ashley changed my life forever when she informed me that I was to be a father. At that time, I was simultaneously overcome with joy and fear, because I had neither received an appreciable amount of training nor studied a sufficient amount of literature on how to be a father. Nonetheless, about nine months later Liam came into our lives, and I have tried every day since to be the best father I can be. I have enjoyed nothing more than watching Liam grow and learn about the world around him. Observing his fascination with the world and his surroundings continues to remind me how wonderful it is to learn about new things and appreciate the complexities of others. Indeed, Liam has taught me a great many things and allowed me to gain new perspectives on the world around me. For instance, it was my son who allowed me to truly understand and appreciate the meaning of 'Protein Foundation' (for reference the reader is directed to the 'Dedication' page of this dissertation). Regardless of any of my present and future contributions toward society, there are none that will be greater nor that I will be prouder of than my son.

I came to Manhattan, Kansas with the hope to gain training in ruminant nutrition over five years ago. Since that time, Evan has graciously served as my mentor, and his training and advisement have allowed me to become the scientist that I am today. Isaac Newton is most commonly attributed for popularizing the phrase, "If I have seen further it is by standing on the shoulders of giants." Thus, in Newton's terms, Evan has served as my "giant", and while I have yet to make any significant contributions to my field, were it not for Evan's training I would not have the opportunity to do so. Indeed, there are very few men who have contributed so greatly to my life, and I greatly appreciate Evan's guidance and friendship. I hope that I am able to be as good a scientist and teacher as Evan, and I will undoubtedly miss his wit and brilliance as I move forward from Manhattan.

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Dedication

This work is dedicated to my wife and children.

"Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

- *Sir Winston Churchill*

Protein Foundation

How inoffensive are the feces
Of all the graminivorous species
That grind on grain and graze on grasses,
Like sheep and horses, mules and asses,
Or practiced in regurgitation,
Spend idle hours in rumination.
Such are the cows, the goats, the camels
And other ungulated mammals.
But ah, how offal they excrete
Who pry their protein needs from meat
From chops and steaks and yet, from cheeses
And pork and everything that pleases
From sulfurous eggs and oily fishes
And all the highly seasoned dishes;
Such is the odorous part of man
Devoted to his frying pan.

-Anonymous. 1963. Protein foundation. N. Engl. J. Med. 269:1254.

Chapter 1 - A Brief Review Of The Current Literature On Starch Digestion In Ruminants And A Summary Of The Known Physiology Regarding Small Intestinal Starch Digestion In Mammals

Introduction

Limitations to efficient production of animal products (i.e. meat and milk) by cattle are often associated with dietary restrictions in net energy to support physiologically productive purposes. Utilization of dietary energy from α-glucosides (starch) is potentially limited by starch assimilation from the alimentary tract in cattle, and a precise understanding of digestion of carbohydrates in the small intestine of cattle remains equivocal. Thus, most ruminant nutritionists currently adopt feeding strategies designed to increase ruminal starch fermentation to improve production efficiencies. However, increases in fermentable energy supply are positively correlated with increased instances of metabolic disorders and reductions in dry matter intake, and energy derived by cattle through ruminal fermentation is less than that derived when glucose is absorbed intestinally. Thus, improving small intestinal digestion of starch in cattle may provide great benefit to cattle production systems.

Cattle production is primarily limited by energy

Energy and protein are the 2 primary nutrients required to support growth or lactation in cattle (Poppi and McLennan, 1995). Growth is largely the sum of protein and fat deposition *in vivo* (Owens et al., 1995; NRC, 1996). The composition of body tissue is dependent on physiological maturity (i.e. body weight) in cattle, and rates of protein accretion and fat synthesis are not similar at different body weights (Anderson et al., 1988; Owens et al., 1995; NRC, 1996).

When neither protein nor energy limit growth, protein accretion increases linearly with weight (and presumably physiological maturity), but, fat synthesis and deposition increases at an exponential rate. Indeed, when Owens et al. (1995) reviewed the literature, they found that the amount of fat deposited exceeded that of protein accretion in cattle of physiological maturities similar to or slightly greater than those at the common age of weaning. Additionally, when cattle are fed sufficient levels of metabolizable protein to support protein accretion but levels of energy that do not support maximal fat deposition, protein accretion is relatively unaffected by increased energy supply; moreover, greater rates of gain observed with greater amounts of dietary protein can be explained by increased energy intake supported by supply of protein above requirements for protein accretion (Anderson et al., 1988). When diets of cattle are restricted at levels above maintenance, a greater proportion of gain is protein (Fox and Black, 1984; Abdalla et al., 1988; NRC, 1996), and when realimented to high-energy rations compensatory gains are observed that are largely explained by increased fat deposition (NRC, 1996). Therefore, a greater requirement is placed on energy for growth once cattle have reached a physiological maturity near to that typically found at weaning. Thus, when growing cattle consume typical rations, and the genetic maximum is not met for deposition of lean tissue, intake of dietary energy by cattle is often first limiting for growth. This has led to prediction equations that predict production of cattle from energy supply (Lofgreen and Garrett, 1968; Fox et al., 1992; NRC, 1996). Clearly, intake of energy and the efficiency of its use are likely to determine production rates of cattle to a greater extent than protein.

Role of starch in cattle feeding

During the finishing stage, nearly all cattle grown for beef production in the United States are fed high-energy diets, that are primarily composed of cereal grains (e.g. corn, sorghum, barley). Feeding of high-grain diets to finishing cattle is incentivized by the relative performance of cattle and associated cost of energy for growth between cereal grains and forages. The reported (NRC, 1996) net energy available for gain in cattle from flaked corn grain, flaked sorghum grain and ground wheat is 1.62, 1.50, and 1.50 Mcal/kg, respectively. Conversely, reported (NRC, 1996) values for common forages in the United States such as full bloom alfalfa hay (0.58 Mcal/kg), full bloom fescue (0.68 Mcal/kg), and prairie hay (0.35 Mcal/kg) are approximately one third that of common cereal grains. Over 300 billion kilograms of corn grain are produced annually by the United States (NASS, 2011), and this relative abundance of corn likely contributes, in part, to its role as the primary cereal grain in diets formulated by consulting ruminant nutritionists for cattle in feedlots (Vasconcelos and Galyean, 2007). Starch comprises 72% of dry matter in corn grain and is the major energy component found in corn and other cereal grains (Huntington, 1997). Typically, starch intake of finishing cattle exceeds 5,000 g/d (Theurer, 1986)! Because of starch's primary role as an energy nutrient to cattle, considerable effort has been made to understanding its digestion and metabolism in ruminants, and these works have been periodically reviewed (Orskov, 1976; Theurer, 1986; Huntington, 1997). However, due to inherent complexities found in the ruminant gastrointestinal tract, apparent limitations of starch digestion in the small intestine, and the hypothesis that postruminal responses for digestion are mitigated by a more constant supply of nutrient passage, much of this work has focused on ruminal parameters of digestion and metabolism.

Ruminal starch digestion and its limitations

Corn, like all cereal grains, stores energy in the kernel as starch, which is a heterogeneous polysaccharide of either amylose or amylopectin polymers. Amylose is the smaller of the 2 polymers (900 to 3000 glucose molecules; Kotarski et al., 1992) and is linear with α-1,4 linkages, whereas amylopectin is larger (10,000 to 500,000 glucose molecules; French, 1973), highly branched, and contains both α-1,4 and α-1,6 linkages. Three distinct components comprise the corn kernel: 1) the pericarp, 2) the germ, and 3) the endosperm. The pericarp is a protective outer coating and the germ is a highly proteinaceous layer that makes up the plant embryo; together, the pericarp and germ account for a small proportion of the kernel by weight (Kotarski et al., 1992). Nearly all of the energy contained in corn grain, as starch, is located in the endosperm, which is generally thought to consist of 4 segments. The aleurone is the outermost layer and contains enzymatic inhibitors and autolytic enzymes. The subaleurone (i.e., peripheral endosperm) and corneous layers contain starch in a dense protein matrix that is resistant to hydrolysis, and vitreousness is a term broadly used to describe proportional composition of these 2 layers in relation to the innermost floury endosperm. Starch most accessible to hydration, enzymatic degradation, and hydrolytic cleavage is located in the floury endosperm.

Because total tract starch digestion (Orskov, 1976; Owens et al., 1986) and performance in finishing cattle (Galyean et al., 1981; Huntington, 1997; Huntington et al., 2006) are reduced when ruminal fermentation of starch is limited, most feeding strategies for finishing cattle seek to increase net energy available for gain by maximizing extent and rate of ruminal starch fermentation while minimizing incidence of metabolic disorders (e.g., ruminal acidosis). Ruminal microbes are responsible for a preponderance of pregastric fermentation of starch in

cattle. Rate and extent of ruminal starch digestion is dependent on many variables, such as physical and biochemical characteristics of starch sources, adaptation of ruminal microbiota to a diet, and levels of feed intake or rates of digesta passage.

The ruminal ecosystem is a dynamic environment that consists of many different species of bacteria, protozoa, and fungi capable of fermenting a wide array of substrates. Readily soluble components of ingested feed particles likely act as chemoattractants for fungal zoospores (Orpin and Bountiff, 1978) and protozoa (Orpin and Letcher, 1978), which facilitates movement of these microbiota from the fluid milieu to substrates where they subsequently attach (McAllister et al., 1994). Ruminal fungi are likely integral in disrupting the outer layers of cereal grains that inhibit bacterial attachment, thereby increasing potential surface area for microbial attack of fermentable substrates. Protozoa are capable of directly ingesting starch granules and limiting rates of microbial fermentation. However, the combined activities of both ruminal fungi and protozoa toward total starch digestion are negligible in comparison to ruminal bacteria (McAllister et al., 1994). Ruminal bacteria produce both endo-amylases (e.g., α-amylase) and exo-amylases (e.g., amyloglucosidase) that are capable of degrading starch to smaller oligomers or glucose (Kotarski et al., 1992). It is generally thought that the preponderance of microbial amylolytic capabilities are closely associated with the peripheral of the cell in the glycocalyx (McAllister et al., 1994). Thus, attachment of ruminal microbes to feed particles is an essential and potentially rate-limiting step to ruminal fermentation of feeds (McAllister et al., 1994).

Ruminal microbes rapidly attach to ingested feed particles through either specific binding proteins located on the periphery of the cell or by chemical forces (e.g., Van Der Waals forces; Kotarski et al., 1992; McAllister et al., 1994), and microbes that closely adhere to feed particles are responsible for 70% of ruminal amylolytic processes (McAllister et al., 1994). It is unlikely

that any single microbial species known to exist in the rumen is capable of producing all the requisite enzymes for complete digestion of an ingested feed particle (McAllister et al., 1994). Therefore, it has been suggested (McAllister et al., 1994) that ruminal fermentation of ingested feeds proceeds through a series of sequential attacks by microbes that are preferentially suited for attachment to the substrate. Digestion then continues until completion and attached species are released to the fluid where they are then free to associate with newly ingested feed particles (Bowman and Firkins, 1993). Differences in rate and specificity for attachment, inherent among microbial species, are likely the primary mode of action whereby ruminal adaptations occur. Those species not preferentially equipped for attachment to feed particles of a certain diet are "washed out" from the ruminal environment at greater rate. This leads to effects of diet (e.g., grain processing, fat inclusion, chemical treatments) altering ruminal digestibilities.

Apparently, since cereal grains first began to be used as a feed source for domesticated ruminants, further processing has been viewed as desirable (cited by Orskov, 1976). Indeed, a considerable amount of effort has been made toward understanding grain processing effects in ruminant and these data have been regularly reviewed (Orskov, 1976; Theurer, 1986; Huntington et al., 2006). Processing of cereal grains alters innate physical and chemical structures of kernels through additions of heat, moisture, and mechanical action that may facilitate an improved rate and extent of ruminal fermentation. Physical alterations by processing of grain act by increasing potential surface area for microbial attachment to starch in the endosperm. Chemical alterations are achieved primarily through either destruction of insoluble protein matrices in the subaleurone and corneous endosperm or gelatinization of starch. Gelatinization occurs when starch crystals become hydrated and irreversibly release amylose and amylopectin molecules (Kotarski et al., 1992). Typically, more extensively processed grains have led to greater rates and extents of

ruminal digestion with a concomitant increase in total tract digestibility (Orskov, 1976; Theurer, 1986; Huntington et al., 2006). Galyean et al. (1981) reported that corn grain that was either harvested and ensiled with a high moisture content or steam-flaked had greater ruminal starch digestion than corn processed to a lesser extent (i.e., dry-rolled). Also, more extensively processed grains incubated with ruminal bacteria result in greater end-products of fermentation (Trei et al., 1970). Theurer (1986) summarized the available literature and found that, when incidences of ruminal acidosis were averted, steam-flaking increased efficiencies of gain in cattle nearly 10% when compared to dry-rolled corn. However, it is unlikely that further significant advancements in starch digestion are possible through some unknown novel processing technique, because "many of the significant advances possible in physical and chemical processing of feed have likely already been made" (McAllister et al., 1994).

Unfortunately, limitations in ruminal digestion and risks of increased animal morbidity and mortality are associated with feeding rapidly fermented starch to cattle. Acidosis is a prevalent metabolic disorder in cattle consuming energy dense diets, occurring when cattle consume, "too much of a good thing" (Huntington, 1997), and it is commonly associated with rapid ruminal fermentation of energy substrates. Typically, acidosis in ruminants is classified as either acute or subclinical (Owens et al., 1998; Nagaraja and Titgemeyer, 2007). Cattle with acute acidosis exhibit clinical signs of illness, while those suffering from a subclinical condition may not express obvious signs of morbidity but will have reduced performance and feed intake. Cattle consuming energy-dense rations are most susceptible to ruminal acidosis after consuming large quantities of easily fermentable substrates. This commonly occurs when cattle are adapted from a diet that limits nutrient intake by bulk-fill mechanisms (i.e., forage-based diet) to a diet where intake is largely controlled by chemostatic mechanisms (e.g., corn-based diets), but it may

also occur when feeding intervals are increased or during short-term environmental change (Owens et al., 1998). Ruminal acidosis is initiated by the rapid accumulation of volatile fatty acids and D-lactate in the rumen (Nagaraja and Titgemeyer, 2007). The excessive accumulation of these fermentative end products overcomes the buffering capacity of the rumen and results in a decrease in ruminal pH (Owens et al., 1998; Nagaraja and Titgemeyer, 2007). Greater proton concentrations in the ruminal milieu decrease rate and extent of digestion by reducing microbial enzyme activity and increasing microbial cell lyses (Owens et al., 1998; Nagaraja and Titgemeyer, 2007). Additionally, a lower pH in the ruminal environment leads to increased amounts of protonated volatile fatty acids leading to increased diffusion of volatile fatty acids across ruminal epithelium, increased osmolality of ruminal contents, and a rapid influx of water. Histological studies have reported that increased osmotic pressures associated with ruminal acidosis leads to sloughing of ruminal papillae and necrosis of ruminal epithelium (Eadie and Mann, 1970). Damage to ruminal epithelia then allows for transit to the liver of 2 resident ruminal microflora, *Arcanobacterium pyogenes* and *Fusobacterium necrophorum*, which are the primary species responsible for development of liver abscesses in cattle.

Negative effects associated with ruminal fermentation of rapidly fermented energy substrates are long lasting and likely limit performance for years (Krehbiel et al., 1995). Thus, considerable effort has been made to moderate rates of ruminal starch digestion. Generally, as intake increases a concomitant increase in digesta passage from the rumen occurs. Altering feed intake through management technique (Murphy et al., 1994a,b; Clark et al., 2007) or chemical additives (Spears, 1990; McAllister et al., 1992) seems to alter ruminal digestion of starch. Further, efforts to limit ruminal starch digestion by addition of fat (Bock et al., 1991; Kucuk et al., 2004; Montgomery et al., 2008) or including cereal grains with differing physiochemical

properties (Corona et al., 2006; Plascencia et al., 2011) have been made. However, attempts to alter site of ruminal fermentation through additions of chemicals or fat have yielded inconsistent results. Attempts to alter the site of starch digestion from the rumen to a more distal region of the gastrointestinal tract of cattle create a quagmire for most ruminant nutritionists. Typically, as ruminal starch digestion is reduced, so too is total tract starch digestion and presumably digestible dietary energy (Orskov, 1976; Owens et al., 1986; Harmon, 1992; Huntington et al., 2006). Reductions in total tract starch digestion are likely due, in part, to limited small intestinal starch digestion (Orskov, 1976; Owens et al., 1986; Harmon, 1992; Huntington et al., 2006). A potential solution for nutritionists seeking to mitigate deleterious occurrences of acidosis, but to maximize total tract starch digestion and digestible dietary energy may be to optimize small intestinal starch digestion.

Small intestinal starch digestion in ruminants

Starch digestion occurs by similar means in both ruminants and nonruminants, and its digestion can be separated into 3 independent segments (Huntington, 1997): 1) hydrolysis by α-amylase to smaller oligosaccharides, 2) release of glucose from oligosaccharides by oligosaccharidases closely associated with intestinal epithelium, and 3) transport of glucose from the lumen by transmembrane glucose transporters of intestinal epithelia. Digestion of starch appearing in the lumen of the small intestine is initiated by α-amylase that is secreted from acinar cells in the pancreas as part of its exocrine function (Brannon, 1990). α-Amylase (EC 3.2.1.1) is secreted in its active form into the lumen where it attacks 5 contiguous α-1,4-linked glucose molecules in starch and releases smaller oligosaccharides (i.e., maltose, maltotriose and branched limit-dextrins). Released oligosaccharides are subsequently hydrolyzed to glucose by

brush border enzymes (i.e., isomaltase, maltase, glucoamylase) that are produced by and remain closely associated with enterocytes. Ultimately, glucose molecules released from starch are actively transported across the brush border membrane or absorbed directly via the solvent drag phenomenon.

Typically, ruminants digest only 5 to 20% of dietary starch postruminally (Harmon, 1992), despite digestion of starch being theoretically more energetically efficient in the small intestine than in the rumen. Owens et al. (1986) reviewed the available literature and reported that small intestinally digested starch provides 42% more energy than starch fermented in the rumen. However, it is known that small intestinal starch digestion in cattle is limited (Orskov, 1976; Owens et al., 1986; Theurer, 1986; Harmon, 1992; Huntington, 1997), and this limitation is proportional to small intestinal starch appearance rather than there being an absolute maximal value beyond which no further starch can be digested (Orskov, 1976; Theurer, 1986). Orskov et al. (1969) were first to suggest that limitations in small intestinal starch digestion could be expressed as a proportion of starch flowing to the small intestine. Orskov (1976) calculated that in early weaned lambs ileal starch appearance was equal to 0.56 × abomasal starch minus 0.6. This model suggests that the amount of small intestinal starch digestion is linearly related to starch flow to the abomasum, but with an efficiency of only 56%. However, digestion of starch is typically greater in sheep than cattle when these species are fed similar diets (Orskov, 1976). Therefore, it may be that small intestinal starch digestion is limited to an even greater extent in cattle than sheep. When Huntington (1997) summarized data from reviews on limitations to small intestinal starch digestion in cattle, he estimated an average small intestinal digestibility of 55%. A proportional limitation in small intestinal starch digestion in ruminants suggests that digestion is responsive to nutrient intake, flow to the abomasum, or energy intake. However,

maximum limits for the small intestinal hydrolytic capacity of starch have been demonstrated (Orskov et al., 1970), but this is yet to be observed with levels of starch flows that could be achieved naturally by the animal. Regardless, small intestinal starch digestion in ruminants is limited in comparison to nonruminants who are typically capable of digesting nearly all starch appearing luminally in the small intestine (Brannon, 1990).

Potential limitations of small intestinal starch digestion in ruminants

Differences between ruminants and nonruminants for small intestinal starch digestibility have received considerable attention by many researchers; however, these specie differences remain a biological enigma and further study is likely necessary before significantly greater starch digestion by ruminants can be obtained. Owens et al. (1986) described some potential physiological factors that may explain the reduced ability of ruminants to digest starch in the small intestine: 1) inadequate time for sufficient digestion, 2) reduced capabilities in glucose absorption from the lumen, and 3) deficient activities of small intestinal carbohydrases (i.e., α-amylase and brush border oligosaccharidases).

Digesta can flow through the ruminant small intestine in less than 3 hours (Owens et al., 1986). Under normal feeding situations flow of digesta to the small intestine is constant (Merchen, 1988), but alterations in dry matter intake can affect the rate of digesta passage (Merchen, 1988). Additionally, flow of digesta through the small intestine is closely regulated and responsive to nutrient composition. Greater appearance of nutrients capable of enzymatic degradation at distal locations in the gastrointestinal tract leads to increased secretion of hormonal and neural regulators that act to slow digesta passage and increase digestion of nutrients in more proximal locations (Croom et al., 1992). If digestion is limited by rates of

digesta passage in the small intestine of ruminants, then response to these regulators must be minimal or their secretion must be too insignificant to elicit a response. Even if rates of digesta passage limit digestion, this factor is not independent of either activity or amounts of small intestinal carbohydrases, and limitations in small intestinal starch digestion associated with rates of passage would certainly be mitigated with increasing amounts and activities of carbohydrases.

Transit of glucose from the lumen of the small intestine to the cytosol of the enterocyte is thought to be dominated by the activity of the Na^+/glucose cotransporter (SGLT1; Wright, 1993). Expression of SGLT1 has been reported in sheep (Shirazi-Beechey et al., 1991) and cattle (Rodriguez et al., 2004; Guimaraes et al., 2007). Reports (Mayes and Orskov, 1974; Kreikemeier and Harmon, 1995) of free glucose concentrations in the terminal ileum of ruminants greater than the apparent affinity of SGLT1 for glucose have led to speculation that starch digestion may be first limited by transport from the lumen. However, Shirazi-Beechy et al. (1991) observed that SGLT1 in sheep is upregulated between 50- to 80-fold in response to increases in luminal free glucose. Several researchers (Bauer et al., 2001; Guimaraes et al., 2007) have studied the relationship of glucose uptake capacity with capabilities for hydrolysis of oligosaccharides along the small intestine of cattle. These authors reported that glucose uptake capacities exceed capabilities for hydrolytic cleavage of oligosaccharides along all locations of the small intestine except the ileum. Bauer et al. (2001) speculated that reports of free glucose at the terminal ileum were likely a result of lower levels of SGLT1 in that region relative to oligosaccharidases. Secretion of pancreatic α-amylase occurs via the pancreatic duct located at the terminal duodenum (St-Jean et al., 1992), and it is likely that hydrolytic cleavage of starch occurs at its greatest rates near that location (Bauer et al., 2001). Apparently, the digestive physiology of ruminants is well suited to maximize absorption of free glucose liberated from the hydrolytic

activity of α-amylase and the oligosaccharidases, because the greatest levels of SGLT1 are located in the jejunum (Bauer et al., 2001; Guimaraes et al., 2007), and it would seem unlikely that transport of free glucose is first limiting to starch assimilation in the small intestine of ruminants. Indeed, when Huntington and Reynolds (1986) postruminally infused cattle with unpolymerized glucose or starch approximately 65% of the unpolymerized glucose was recovered in the portal blood supply while only 35% of glucose from the starch appeared. Additionally, when hydrolyzed starch is postruminally infused in cattle, glucose uptake by SGLT1 remains unaffected (Bauer et al., 2001; Guimaraes et al., 2007). If the ability for SGLT1 regulation by free glucose is conserved between ruminants, this would suggest that other factors, such as hydrolytic cleavage of polysaccharides and oligosaccharides is more limiting to small intestinal starch digestion.

Potential limitations of small intestinal glycohydrolases in ruminants

It is likely that the primary factor limiting small intestinal digestion of starch by ruminants is suboptimal capabilities for hydrolytic cleavage of polysaccharides and oligosaccharides. Early investigations concerning limitations of small intestinal α-glycohydrolases using sheep led Mayes and Orskov (1974) to conclude that α-amylase was not limiting to small intestinal starch digestion. However, sheep are more efficient digesters of starch than cattle (Orskov, 1976) with greater postruminal digestibilities of starch (Tucker et al., 1968), and it is known that small intestinal digestion is significantly greater in sheep than cattle (Theurer, 1986). Thus, responses in sheep may not translate to equivalent responses in cattle. Kreikemeier and Harmon (1995) measured small intestinal disappearance and portal flux of glucose when they abomasally infused glucose, corn dextrins, or corn starch. These authors

reported that both starch and ethanol-insoluble reducing sugars (i.e., oligosaccharides) were present in digesta of the terminal ileum when cattle were infused with either corn starch or corn dextrins. Even though observed means for portal glucose flux were not statistically different, due to a relatively large coefficient of variation (28%), those cattle infused with corn dextrins had 63% more glucose appearing in the portal blood supply than those infused with corn starch. Kreikemeier and Harmon (1995) speculated that either α-amylase or oligosaccharidases may be first limiting to small intestinal starch digestion in cattle. Kreikemeier et al. (1990) reported that both pancreatic α-amylase and a small intestinal oligosaccharidase (i.e., glucoamylase) in calves are affected by energy intake and cereal grain content of the diet. Kreikemeier et al. (1990) fed calves either a forage- or grain-based diet and restricted intakes to either one or two times the level required for maintenance. They observed that both α-amylase and glucoamylase activities were increased (140% and 56%, respectively) when energy intake was doubled. However, when starch content was increased, activities of both carbohydrases were paradoxically decreased. Interestingly, as energy content was increased in diets with grain, the disparity between grain to forage fed cattle in activity for glucoamylase was reduced (27 to 15%), but for activity of α-amylase it was increased (40 to 46%). Similarly, several others have reported reductions in α-amylase activity (Swanson et al., 2002) or starch digestion (Taniguchi et al., 1995; Branco et al., 1999) when levels of starch flowing to the small intestine increase. Guimaraes et al. (2007) reported values for brush border maltase activity that were increased approximately 79% when starch was infused. Further, Guimaraes et al. (2007) observed an equivalent increase in maltase activity when casein was infused. Additionally, Le Huerou et al. (1992) reported that activities of small intestinal oligosaccharidases continually increased with age in weaned calves. Taken together, these data may suggest that, for finishing cattle fed typical grain-based diets, α-amylase

may be more limiting to small intestinal starch digestion than brush border α-glycohydrolases. Indeed, when Huntington (1997) developed models to simulate small intestinal starch digestion in cattle, he concluded that rate and extent of starch assimilation were most limited by the hydrolytic capabilities of pancreatic α-amylase rather than brush border α-glycohydrolases activities or glucose transport. Ostensibly, limitations in small intestinal starch digestion in cattle are likely a combination of deficiencies of both pancreatic α-amylase and the brush border α-glycohydrolases; however, it is likely that when ruminants are fed typical rations to levels similar to those currently consumed by rapidly growing finishing cattle, α-amylase activity first limits small intestinal starch digestion.

Adaptation of pancreatic α-amylase secretions to dietary protein and amino acids

Researchers have suggested for over a century that enzymatic secretions of the pancreas in nonruminants are responsive to dietary components flowing to the small intestine (Pavlov, 1898; Snook, 1971; Merritt and Karn, 1977; Brannon, 1990). An interdependence between carbohydrate and nutritionally essential amino acid content flowing to the duodenum has been characterized in nonruminants for optimal pancreatic α-amylase secretions (Snook, 1971; Johnson et al., 1977; Riepl et al., 1996). Snook et al. (1971) reported that pancreatic α-amylase secretions were increased 114% when up to 30% of the diet was replaced with casein in rats. Johnson et al. (1977) provided rats with diets with either zein or casein; they observed greater pancreatic enzyme secretions when casein was fed. Additionally, Johnson et al. (1977) noted that when zein was reconstituted with amino acids, to resemble casein, responses in enzymatic secretions from pancreata were similar to when casein was provided. Thus, Johnson et al. (1977)

concluded that high-quality protein flow to the small intestine was essential for optimal enzymatic secretions from pancreata. Schick et al. (1984) replaced dietary carbohydrate with casein in rat diets and observed that responses in α-amylase secretion were quadratic and greatest when casein composed 22% of the diet. Therefore, Schick et al. (1984) concluded that a proportional relationship was required between small intestinal carbohydrate and nutritionally essential amino acid flow for optimal starch digestion, and they speculated that this response was likely conserved across many species. Indeed, similar responses have been reported in man (Riepl et al., 1996). Yet, Croom et al. (1992) have speculated that pancreatic enzymatic secretions of ruminants are dissimilar to those of nonruminants and are not responsive to composition of nutrients flowing to the small intestine, because postprandial flow of digesta is more constant.

We now know that enzymatic secretions of ruminant pancreata are responsive to changes in composition of digesta flowing to the lumen of the small intestine (Harmon, 1992; Huntington, 1997; Swanson et al., 2004). A preponderance of the published data suggests that response in pancreatic α-amylase secretion to casein (i.e., amino acids) is conserved between ruminants and nonruminants. Taniguchi et al. (1995) studied nutrient fluxes across splanchnic tissues with either ruminal or postruminal supply of casein when cornstarch was provided abomasally. They observed that as postruminal protein supply increased a concomitant increase occurred for glucose release across both the portal drained viscera and total splanchnic tissues leading to increased circulating glucose levels. These improvements in circulating glucose were related to a nearly 50% improvement in N retention by these cattle. Wang and Taniguchi (1998) reported that increased postruminal starch depressed α-amylase secretion, but additions of postruminal casein restored α-amylase secretions to that of the control. Richards et al. (2002)

directly measured small intestinal cornstarch digestibility with titrated levels of casein provided abomasally, and reported linear increases in small intestinal OM and starch digestibility with increasing abomasal casein. Direct measures of rate and content of pancreatic secretions by surgically modified steers in response to increasing abomasal infusions of casein were reported by Richards et al. (2003). They observed linear increases in concentration, specific activity and rate of secretion of pancreatic α-amylase with greater amounts of casein. Swanson et al. (2008) fed increasing levels of ruminal-escape soybean meal and reported responses similar to Richards et al. (2003) in removed pancreata, and they speculated that greater amounts of postruminal protein (i.e., amino acids) led to greater rates and amounts of enzymatic secretions. Recent reports (Swanson et al., 2004; Liao et al., 2009) suggest that interactions between supplemental proteins (i.e., amino acids) and α-amylase might be dynamic and that requirements for nutritionally essential amino acids to optimize pancreatic secretions may be proportional to starch appearing in the lumen of the small intestine. Ostensibly, postruminal protein and likely amino acids increase α-amylase secretions of pancreata in cattle. However, a significant paucity in the available data exists for direct effects of essential amino acids on pancreatic α-amylase secretions, and data are necessary in this area.

Regulation of glycohydrolase production in the small intestine of ruminants

Relatively little is known of the nutritional physiology occurring postruminally and its subsequent regulation in comparison to the understanding of processes that occur in the reticulorumen of ruminants or post-gastric compartments of their nonruminant counterparts. In fact, Van Soest (1994) remarked on this disparate emphasis of study in the available literature and concluded that, “Judging from the emphasis, one would think ruminants had no lower tract.”

A current consequence of this paucity in the data leads investigators to make inferences about postruminal nutritional physiology from studies of the post-gastric splanchnic tissues of nonruminants. Unfortunately, for both ruminant and nonruminant nutritionists the usefulness of this technique is limited, because, "Studies of the lower digestive tract have been dominated by physiologists with limited appreciation for nutrition, and the available literature deals more with nervous control and gut motility relative to flow than with any quantitative assessment of the contribution of digestive capacity and absorption in the respective compartments" (Van Soest, 1994). Nonetheless, a discussion of the potential nutritional physiology occurring in the small intestine of ruminants in relation to starch digestion from the lumen is warranted so that a clearer understanding of small intestinal starch assimilation in ruminants may be achieved; however, the reader is advised to review data from nonruminants in relation to ruminants critically. Clearly, a great need exists for further study of postruminal nutritional physiology, particularly with emphasis toward small intestinal starch digestion.

The greatest amounts and nutritionally important (with the exclusion of lactose in regards to neonates) carbohydrates in nature are of plant origin (Van Beers et al., 1995). Photosynthetic processes, innate to nearly all plants, are capable of producing glucose from sunlight and CO_2 (Voet et al., 2008). Plants utilize synthesized glucose as both a cellular structural moiety (i.e., cellulose) and glucose produced in excess of plant maintenance requirements is largely stored as densely packed starch in seeds for the purpose of providing energy to sustain developmental growth of the next generation before photosynthesis is possible (Voet et al., 2008). As a result of plant biochemical processes, cellulose is the most abundant biomolecule in nature. Yet, very few mammalian digestive enzymes are capable of hydrolyzing the β-glycosidic bonds (e.g., lactase). Thus, in many mammals, ingestion of cellulose provides little dietary energy; however, many

microbial species produce enzymes capable of hydrolyzing β-glycosidic linkages in cellulose. Symbiotic relationships among mammals and commensal bacteria in various locations of the alimentary tract provide opportunity for mammals to capture dietary energy from microbial fermentation end-products (e.g., volatile fatty acids, ethanol) of cellulose. Indeed, the development of nonsecretory tissues of the abomasum by ruminants into the reticulorumen and omasum (Moir, 1968; Langer, 1988; Van Soest, 1994) serve as an example of a specie evolutionary development (Darwin, 1859) that facilitated the capture of significant amounts of dietary energy from cellulose and other plant structural carbohydrates (e.g., hemicellulose, pectin). Nonetheless, fermentation of feed for dietary energy has several limitations, which inhibit the efficiency of energy utilization from ingested material in comparison to aerobic respiration. Fermentation of substrates has an inherent heat loss (i.e., energy loss) associated with this biochemical process and some end-products of fermentation do not serve metabolically useful purposes (e.g., CH_4). Further, while some species have the capability to capture a significant amount of dietary energy from cellulose (e.g., ruminants, camelids), cellulose commonly serves a structural moiety in plants and is often less densely concentrated in feeds in comparison to starch. Typically, in order for a sufficient amount of energy to be digested from cellulose a relatively greater amount of biomass must be consumed. Clearly, fermentation of feed can provide great benefit to those species well adapted to capture of energy from cellulose when non-fiber nutrient sources are scarce, but when non-fiber carbohydrate sources are abundant fermentation provides a less efficient method by which to process ingested energy.

Because the mammalian alimentary tract acts as an impervious barrier to di-, oligo-, and polysaccharides, dietary starch must be hydrolyzed before glucose can be assimilated *in vivo* and provide benefit to metabolic processes. Small intestinal starch digestion occurs in three distinct

steps: 1) hydrolysis of starch to small-chain (i.e., consisting of less than five glucose subunits) oligosaccharides, 2) hydrolysis of small-chain oligosaccharides to glucose, and 3) absorption of glucose from the small intestinal lumen. This tripartite digestion of starch in the small intestine is facilitated by secreted α-glycohydrolases, which are any of a number of enzymes that hydrolyze saccharides and whose hydrolytic products have an α-optical isomer configuration. The α-glycohydrolase responsible for the initial hydrolysis of starch in the small intestine is α-amylase (E. C. 3.2.1.1) secreted by the pancreas into the proximal duodenum.

It is well known that the pancreas participates in both exocrine and endocrine functions that are integral for nutrient digestion and metabolism. The islets of Langerhans and acini are the two cell types that comprise the pancreatic parenchyma; the former consists of cells that account entirely for the pancreas' endocrine function while the latter is the primary contributor toward exocrine secretions. Generally, the islets of Langerhans account for a relatively small portion of the organ composition (approximately 2 to 3%) and are unevenly dispersed amongst the acinar cells which account for the majority (90 to 95%) of the pancreas (Brannon, 1990). Considerable effort has focused on elucidating the form, function, and regulation of the mammalian pancreas over the previous 150 years, and this is not surprising given the pancreas' complexity and central role in digestion and metabolism of ingested nutrients.

Early studies of pancreatic regulation in mammals (Pavlov, 1876) reported that the autonomic nervous system (i.e., 'nervism') was integral to regulation of the exocrine pancreas and other digestive organs. This led Pavlov (1876) to speculate that neuronal control was the exclusive regulator of pancreatic exocrine functions as well as the exclusive regulator for secretion of all other digestive enzymes. Nearly 7 decades later, however, Harper and Raper (1943) reported that cholecystokinin (i.e., 'pancreozymin') found in porcine duodenal extracts

was a potent stimulator of pancreatic enzyme secretion. The combined early data from Pavlov (1876) and Harper and Raper (1943) led to the common assumptions that mammalian pancreatic exocrine functions were regulated by both neural and endocrine functions (Konturek et al., 2003).

Since the early works of Pavlov (1873) and Harper and Raper (1943), considerable efforts have been made to elucidate the precise mode of action by which pancreatic exocrine secretions are regulated. This has led to the discovery of several biologically active proteins and peptides released by the gut that apparently influence pancreatic exocrine secretions; however, many of these apparently exert their influence by either mitigating or potentiating pancreatic responses to cholecystokinin (CCK; Chey, 2001; Dufresne et al., 2006; Dockray, 2012). A preponderance of data indicates that pancreatic exocrine secretions are responsive to luminal nutrient flows, and pancreatic secretions are predominately regulated via luminal nutrient sensing during the intestinal contributing phase of postprandial response or the interdigestive phase of nutrient digestion (Konturek et al., 2003).

Postprandial regulation of pancreatic secretions can be characterized to occur in 3 phases in nonruminants (Konturek et al., 2003). These phases have been characterized as the cephalic, gastric, and intestinal contributing phases, and their contributions to pancreatic exocrine secretions have been estimated to account for 20, 10 and 70% of total secretions, respectively. Based on observations of digesta flow from the abomasum (Merchen, 1988) and measurements of pancreatic secretions in relation to the duodenal migrating myoelectric complex in cattle (Zabielski et al., 1997), it appears that functional ruminants are in a continuous state of digestion that is analogous to the intestinal contributing phase in nonruminants. Regulation of pancreatic secretions during the intestinal contributing phase has been attributed almost entirely to CCK

secretion by the gut (Konturek et al., 2003), and while this accounts for a preponderance of pancreatic response in nonruminants (~70%) it may be suggested that CCK may mediate the entire pancreatic response in ruminants.

Cholecystokinin is produced by enteroendocrine I cells in small intestinal mucosa (Wang et al., 2002; Zabielski, 2003; Jaworek et al., 2010; Nilaweera et al., 2010). The apical membrane of enteroendocrine I cells contacts the small intestinal lumen, and sensing of luminal nutrients via apically located protein receptors and transporters facilitate increased secretion of CCK. Considerable effort has focused on elucidating the precise method by which enteroendocrine I cells sense luminal nutrients, and which nutrients act as CCK secretagogues. Much of this work has been facilitated by use of STC-1 cells, which are isolated from intestinal enteroendocrine tumors that develop in double-transgenic mice that express an insulin promoter linked to specific antigens (Young et al., 2010).

Protein, peptides, amino acids, fatty acids and carbohydrates have all been indicated to increase CCK gene expression and/or CCK secretion by enteroendocrine I cells (Nilaweera et al., 2010). Each class of nutrient (i.e., proteins, fats and carbohydrates) apparently acts on specific receptors, transporters, and ion-channels that modulate their signals via intracellular downstream effectors (Nilaweera et al., 2010). A thorough discussion of the functional aspects in which fatty acids and carbohydrates affect enteroendocrine I cell production of CCK is beyond the scope of this review, because this review's primary focus is aimed at discussion of increased small intestinal starch digestion in ruminants in response to greater luminal protein and amino acid content. Yet, several interesting aspects concerning the overall effects of fatty acids and carbohydrates on production of CCK are worth noting. Fatty acids have been identified as stimulators of CCK release in cattle (Choi and Palmquist, 1996; Bradford et al., 2008) and from

STC-1 cells (Nilaweera et al., 2010). Indeed, several free fatty acid receptors that are responsive to volatile (i.e., short-chain), medium-chain (C8 to C10), and long-chain fatty acids have been identified in STC-1 cells (Nilaweera et al., 2010). Recent evidence, however, suggests that only ingestion of long-chain fatty acids produced significant increases in CCK release from enteroendocrine I cells, and that medium-chain fatty acids only had slight effects while stimulation of CCK release from volatile fatty acids was not quantifiable (Raybould, 1999). The relative influence that fatty acids and protein contribute to overall CCK release remains a matter of conjecture (Nilaweera et al., 2010). Indeed, Hand et al. (2010) reported that long-chain fatty acids contribute to nearly 16% of the release of intracellular stores of CCK from STC-1 cells as well as 0.8- to 1.2-fold concurrent increases of CCK gene expression; however, contributions of long-chain fatty acids to portal blood supplies of CCK in cattle have been questioned by others (Benson and Reynolds, 2001).

Increases in plasma CCK in response to duodenal infusions of glucose in man (Pilichiewicz et al., 2007; Chaikomin et al., 2008) and to abomasal infusions of starch hydrolysates in cattle (Swanson et al., 2004) have been reported; however, no research has indicated that increases in CCK release in response to glucose or starch hydrolysates are related to increased gene expression (Nilaweera et al., 2010). Small intestinal starch digestion in cattle is considerably less than that of their nonruminant counterparts (see 'Small intestinal starch digestion in ruminants' of this review). It is plausible that increases in glucose absorption from the small intestinal lumen in cattle may increase CCK release from enteroendocrine I cells; however, the ability of luminal glucose absorption to increase CCK release is clearly limited. Rather, these data seem to suggest that when small intestinal starch digestion in cattle is first

limited by pancreatic α-amylase secretion that limitations are likely related to insufficiencies in CCK gene expression instead of CCK secretion.

Increased dietary protein has been associated with increased plasma CCK in both nonruminants (Konturek et al., 1973; Nishi et al., 2003; Veldhorst et al., 2008) and ruminants (Furuse et al., 1992). Indeed, several G-protein transmembrane receptors have been identified in STC-1 cells that are responsive to protein hydrolysates and L-amino acids. Also, the peptide transporter PEPT1 has been suggested to effect CCK secretion by the enteroendocrine I cells.

Overexpression of the G-protein receptor GPR93 (also referred to as GPR92) in STC-1 cells has been reported to increase CCK gene expression about 2.5-fold in response to protein hydrolysates (Choi et al., 2007). The mechanism by which GPR93 increases the genetic expression of CCK has been defined (Nilaweera et al., 2010). Apparently, after activation by protein hydrolysates, GPR93 activates adenylate cyclase and stimulates phospholipase C. Activated adenylate cyclase is known to increase intracellular concentrations of cyclic adenosine monophosphate which activates protein kinase A. Phospholipase C leads to increased intracellular concentrations of Ca^{2+} by creating diacylglycerol and inositol triphosphate through lipolysis of membrane phospholipids. Inositol triphosphate then acts on endoplasmic reticulum leading to release of endoplasmic reticulum Ca^{2+} stores into the cytosol. Greater intracellular Ca^{2+} concentrations then leads to increased interaction of Ca^{2+} with calmodulin, which can activate the Ca^{2+}/calmodulin-dependent protein kinase (CaMK). Activated CaMK is known to interact with the mitogen-activated protein RAF, which can then lead to the sequential activation of mitogen-activated or extracellular signal-regulated protein kinase (MEK) and extracellular signal regulated protein kinase. Protein kinase A, the downstream effector of the mitogen-activated protein kinase pathway (i.e., extracellular signal regulated protein kinase) and CaMK

are all capable of activating the transcription factor cyclic adenosine monophosphate response element binding protein that has been shown to be involved in increasing CCK gene expression in STC-1 cells (Bernard et al., 2001; Akrouh et al., 2009).

The other G-protein receptor implicated in luminal nutrient sensing of amino acids is the Ca^{2+} sensing receptor (CaR). Typically, CaR is associated with controlling extracellular Ca^{2+} concentrations by modulating the release of parathyroid hormone (Brown, 2007). Yet, work on taste receptors in the alimentary tract has clearly indicated that CaR is responsive to L-amino acids (Conigrave and Brown, 2006). It is currently thought that apically located CaR on enteroendocrine I cells can be activated by amino acids (Nilaweera et al., 2010). This activation leads to increased intracellular inositol triphosphate by phospholipase C and ultimately increased CCK expression. Interestingly, CaR is activated by aromatic amino acids (i.e., tryptophan and phenylalanine) and in the presence of extracellular Ca^{2+} interacts with the transient receptor potential channel which facilitates an influx of Ca^{2+} into the cytosol (Rey et al., 2005). The multiple actions of CaR in response to aromatic amino acids may explain why aromatic amino acids, particularly phenylalanine, have long been known to be potent stimulators of CCK release *in vivo* (Owyang et al., 1986a; Owyang et al., 1986b; Mangel et al., 1995).

The di- and tri-peptide transporter PEPT1 has been discovered in both the nonruminant (Fei et al, 1994; Liang et al., 1995; Miyamoto et al., 1996) and ruminant gut (Chen et al., 1999). Darcel et al. (2005) recently discovered that inhibition of PEPT1 by a non-translocated competitive inhibitor mitigated the effect of protein hydrolysates on CCK sensitive vagal afferent fibers in the rat duodenum. Further, STC-1 cells transfected with PEPT1 have increased intracellular Ca^{2+} in response to di-peptides which correlates to greater CCK release (Matsumura et al., 2005; Raybould, 2008). Apparently, PEPT1 increases intracellular Ca^{2+} by opening of L-

type voltage operated Ca^{2+} channels (Nilaweera et al., 2010), and Nilaweera et al. (2010) suggested that this transporter's activities could also increase secretion of enteroendocrine I cells.

Downstream effectors of apically located G-protein receptors, peptide transporters, and Ca^{2+} channels located on I cells that are activated by luminal proteins and amino acids have also been suggested to not only increase CCK gene expression but to also increase CCK secretion (Nilaweera et al., 2010). Indeed, Nilaweera et al. (2010) summarized the literature related to enteroendocrine I cell release of CCK and reported that Ca^{2+}, protein kinase A, and CaMK apparently increase exocytosis of CCK by direct signaling on intracellular CCK-containing secretory vesicles. Nilaweera et al. (2010) also reported that protein kinase C, which is activated by increased intracellular concentrations of diacylglycerol, may also directly signal increases in exocytosis of CCK by secretory vesicles.

Intestinal release of CCK is also apparently regulated by 2 CCK releasing peptides, monitor peptide and luminal CCK releasing factor, which are produced by the pancreas and enterocytes, respectively (Miyasaka and Funakoshi, 1998). Green and Lyman (1972) first suggested that pancreatic proteases were involved in feedback regulation of pancreatic juice secretions in rats. Typically, diversion of pancreatic secretions from the small intestinal lumen resulted in hypersecretion of pancreatic juices (Green and Lyman, 1972; Davicco et al., 1979; Corring et al., 1985). Indeed, Green and Lyman's (1972) work in rats and work by others in pigs (Corring et al., 1985), milk fed calves (Davicco et al., 1979), and hamsters (Andren-Sandberg and Ihse, 1983) have drawn the conclusion that pancreatic juices in these species were involved in feedback regulation mechanisms of pancreatic protein secretions in response to diversion of pancreatic juices from the small intestinal lumen.

Monitor peptide was first purified from rat pancreatic secretions and found to elicit CCK release (Iwai et al., 1987). Since its discovery, monitor peptide has been completely characterized and has been reported to be identical to protein secretory trypsin inhibitor-61, which is one of several trypsin inhibitor proteins inherent to pancreatic juice (Iwai et al., 1989). The presence of trypsin inhibitor proteins in pancreatic secretions is generally thought to be related to prevention of premature activation of pancreatic enzymes by trypsin (i.e., activation prior to pancreatic juice entering the duodenal lumen); however, protein secretory trypsin inhibitor-61 is the only trypsin inhibiting protein that directly impacts intestinal CCK release (Miyasaka and Funakoshi, 1998).

Luminal CCK releasing factor has been isolated from the pig (Herzig et al., 1996) and rat intestine (Lu et al., 1989; Miyasaka and Funakoshi, 1992; Spannagel et al., 1996). Initial comparisons of the relative bioactivities of monitor peptide and luminal CCK releasing factor reported that luminal CCK releasing factor elicited CCK secretory responses that were approximately 60% that of monitor peptide; however, when compared on an equal molar basis luminal CCK releasing factor has been reported to provoke maximal responses at nearly 100-fold less concentration than monitor peptide (Miyasaka and Funakoshi, 1998). Indeed, the equimolar comparisons of monitor peptide and luminal CCK releasing factor apparently support early work in multiple species that observed pancreatic hypersecretion in response to diversion of pancreatic juice from the small intestinal lumen, because diversion of pancreatic juice also removes secretion of monitor peptide (Miyasaka and Funakoshi, 1998).

Typically, in nonruminants it is generally considered that basal secretions of monitor peptide and luminal CCK releasing factor do not influence release of CCK from the alimentary tract (Sale et al., 1977; Konturek et al., 2003), because sufficient levels of trypsin are available

for digestion of luminal CCK releasing factor and adsorption to monitor peptide (Konturek et al., 2003). Presently, I am unaware of any data that has directly measured the effects of monitor peptide and/or luminal CCK releasing factor in ruminants. Direct extrapolation of conclusions from nonruminant data might lead one to conclude that these peptides do not exert influence on release of CCK from the ruminant small intestine, because ruminants apparently do not experience postprandial increases in pancreatic secretions (Kato et al., 1984; Zabielski et al., 1997). An alternative hypothesis may be that monitor peptide and luminal CCK releasing factor may potentiate significant responses in CCK release from the small intestine in ruminants; particularly in response to greater amounts of protein flowing to the duodenum. This suggestion may be considered polemic; however, flow of digesta to the small intestine in ruminants is apparently continuous (Merchen, 1988; Zabielski et al., 1997) and typically contains considerable amounts of protein (Titgemeyer, 1997). Also, the pH of digesta in the proximal duodenum of cattle is low (pH~3; Russell et al., 1981; Hibberd et al., 1985; Hill et al., 1991; chapter 2 of this dissertation), and monitor peptide binds trypsin in a 1:1 ratio at pH 3 to 7 (Konturek et al., 2003). Thus, a continuous flow of protein and a low pH of duodenal digesta in cattle may obviate digestion of luminal CCK releasing factor by trypsin in the regions of the small intestine most densely populated with enteroendocrine I cells (Moran and Kinzig, 2004; Cummings and Overduin, 2007); however, small intestinal digestion of protein in cattle is generally considered to be extensive (NRC, 1996), and at least one report in cattle failed to measure increases in plasma CCK in response to greater luminal protein supplies (Swanson et al., 2004).

Since its discovery, CCK was generally thought to act as humoral stimulant on target organs (e.g., pancreas, abomasum, intestine; Konturek et al., 2003), and many authors have

reported increases in circulating CCK in response to various CCK secretagogues in a variety of species. Rosewicz et al. (1989) reported that gene expression in rat pancreata is correlated with circulating levels of CCK. Also, Swanson et al. (2003) observed direct responses of cattle pancreatic tissues when incubated with a CCK analog (i.e., caerulein) provided at levels thought to elicit a maximal response. Interestingly, Swanson et al. (2003) reported that responses of cattle pancreatic tissue to a CCK analog was dynamic and only occurred in tissues harvested from cattle that had greater postruminal protein supplies. Nonetheless, some authors (Morisset et al., 2003; Konturek et al., 2003; Swanson et al., 2003; Zabielski, 2003) have questioned classic hormone theory associated with CCK, and a preponderance of recent findings suggests that the parasympathetic nervous system is often involved in mediation of CCK's stimulatory effect.

Considerable effort has been made to elucidate the precise method in which CCK impacts responses in the pancreas and other organs. This work has led to the discovery of 2 G-protein receptors present in various tissues amongst a variety of species that are responsive to CCK (Dufresne et al., 2006). Initially, a single CCK receptor was first discovered to exist in rat pancreatic acini (Christophe et al., 1978; Deschodt-Lanckman et al., 1978; Robberecht et al., 1978; Jensen et al., 1980; Sankaran et al., 1980), and shortly after these reports others (Innis and Snyder, 1980; Saito et al., 1980) identified a second independent receptor sensitive to CCK first characterized from brain tissues. Both receptors have since been identified in multiple tissues from a variety of different species (Dufresne et al., 2006).

Because of the location from which the CCK receptors were first identified, they were initially termed CCK receptor type A ("alimentary") and type B ("brain"); however, based on recommendations of the International Union of Pharmacology committee, these receptors have been renamed as CCK1 and CCK2, respectively (Dufresne et al., 2006). Apparently, CCK1

binds sulfated CCK (a common posttranslational modification of the biologically active peptide) with an affinity that is about 500- to 1000-fold stronger than non-sulfated CCK or gastrin (Dufresne et al., 2006; gastrin is a biologically active peptide released from the alimentary tract very similar in structure to CCK). Conversely, CCK2 responds to gastrin and non-sulfated CCK similarly. Both CCK1 and CCK2 have low- and high-affinity sites for CCK (Jensen et al., 1980; Jensen et al., 1989; Langer et al., 2005). Typically, the dissociation constant of CCK for the low- and high- affinity sites of CCK1 range from 50 to 200 n*M* and 50 to 300 p*M*, respectively; however, high-affinity binding sites for CCK are much less abundant in comparison low-affinity sites (Dufresne et al., 2006). The dissociation constant values of CCK for low- and high-affinity binding sites on CCK2 are approximately 2 to 5 n*M* and 100 to 300 p*M*. Differences among the low- and high-affinity binding sites between CCK1 and CCK2 have led some authors to refer to CCK2 as the "high-affinity receptor" (Morisset et al., 2003); however, intracellular downstream effectors of CCK1 and CCK2 activation have been characterized, and both low- and high-affinity binding sites in each receptor can have biologically important and potentially divergent roles (Dufresne et al., 2006). Indeed, it would appear more appropriate to consider CCK2 to be the "gastrin receptor" rather than the "high-affinity" CCK receptor, because physiological levels of circulating gastrin typically exceed that of CCK by 5- to 10-fold (Dufresne et al., 2006).

Because CCK1 and CCK2 are both G-protein receptors, intracellular signaling cascades are not dissimilar to those occurring during signal transduction of responses to luminal proteins and amino acids in enteroendocrine I cells (see previous discussion of this chapter). Yet, an apparent methylation event is required for the appropriate function of CCK receptors, as ethionine (an antagonist of methionine metabolism) has been demonstrated to obviate α-amylase secretion in vivo and in vitro (Capdevila et al., 1997).

Data indicate that expression of the 2 CCK receptors is variable among various tissues and species. Further, expression of the CCK receptors is affected by ontogenic factors in some species. CCK1 is the most abundant CCK receptor in the rat pancreas, which either does not or at least poorly expresses CCK2 (Monstein et al., 1996). Similarly, dogs (Fourmy et al., 1987) and preruminant calves (Desbois et al., 1998; Morisset et al., 2003) predominately express CCK1 in pancreatic tissues; however, as calves age and develop pregastric fermentation CCK1 expression diminishes to undetectable levels (Desbois et al., 1998; Morisset et al., 2002). Apparently, CCK2 is the only CCK receptor found in the pancreas of ruminant cattle (Desbois et al., 1998; Morisset et al., 2003), and typically CCK receptors in ruminating cattle have not been associated with pancreatic acini (Morisset et al., 2003). Thus, CCK receptor expression in adult cattle is similar to that found in both pigs (Philippe et al., 1997; Lhoste et al., 2002; Evilevitch et al., 2004) and man (Ji et al., 2001). The precise role of CCK2 in the mammalian pancreas is incompletely understood; however, in dogs (Fourmy et al., 1987) CCK2 in pancreatic tissues is not linked to any physiological event. It remains to be determined if CCK2 acts alternatively in other species (e.g., cattle, pigs, man), but if the role of CCK2 in the pancreas is conserved across species then clearly others signaling mechanisms are required to elicit CCK's effect on pancreatic α-amylase secretion.

Cholecystokinin's role, strictly as a classical hormone, on pancreatic enzyme secretions has been equivocal since reports that a cholinergic antagonist (i.e., atropine) depressed responses of pancreatic secretions to low doses of CCK and nutritional stimulants of CCK release in dogs (i.e., Phe and Leu; Konturek, 1993; Niebergall-Roth and Singer, 2001). Abdominal branches of the vagus nerve in rabbits (Lin and Miller, 1992) and vagal afferent fibers in rats (Broberger et al., 2001) that innervate the muscularis mucosa have been reported to express CCK1. Li et al.

(1997) concluded that CCK binding to vagal CCK1 mediated pancreatic enzyme secretions in rats. Similarly, low concentrations of CCK (i.e., increases in CCK but still near physiologic norms) excited vagal afferent fibers in the proximal duodenum of sheep (Cottrell and Iggo, 1984) and ferrets (Blackshaw and Grundy, 1990). Richards et al. (1996) reported that vagal afferent fibers were stimulated in a dose dependent manner by CCK, and that administration of devazepide (a CCK1 antagonist) eliminated this response. Interestingly, responses in ferrets were not affected by increases in intraduodenal glucose or tryptophan (Blackshaw and Grundy, 1990).

Data suggest that even in species with CCK1 expression on pancreatic acini (Owyang and Logsdon, 2004) the primary effects of CCK on pancreatic enzyme secretions are mediated by neuronal fibers (Dufresne et al., 2006). Zabielski (2003) questioned the ability of circulating CCK to deliver a precise chemical message in vivo because data from rats have indicated that plasma (Deschodt-Lanckman et al., 1983) and hepatic tissue contain enzymes capable of deactivating CCK (Gores et al., 1986). Further, the capability for hepatic clearance of CCK in rats is apparently conserved in cattle (Benson and Reynolds, 2001). Yet, a number of reports have indicated that CCK directly increases enzyme secretions from the rat pancreas; however, Li and Owyang (1993) reported that atropine and hexamethonium (2 cholinergic antagonists) deleted the pancreatic effects of physiological but not supraphysiological levels of CCK. It is possible that CCK functions to increase pancreatic enzymes through both humoral and neuroendocrine methods, but a preponderance of data suggests that the majority of CCK stimulation of pancreatic enzyme secretions is mediated via a neuroendocrine signal transduction method rather than through direct stimulation (Konturek et al., 2003; Zabielski, 2003; Dufresne et al., 2006).

It is likely CCK stimulates pancreatic enzyme secretions in species such as cattle, pigs and man almost entirely through vagal stimulation. Yet, lack of a correlation between jugular concentrations of CCK and pancreatic α-amylase in cattle has obfuscated some researchers' interpretation of data and led to suggestions that either circulating metabolites or other biologically active peptides regulate α-amylase secretions in cattle (Swanson et al., 2004). These investigators (Swanson et al., 2004) cited data (Hara et al., 2001) in which rats with chronic pancreatic diversions hypersecreted CCK but pancreatic α-amylase was decreased; however, chronic elevation of CCK in rats down-regulates pancreatic CCK1 expression (Ohlsson et al., 2000) and is commonly known to induce pancreatitis, which makes interpretation of relationships among CCK and pancreatic α-amylase secretion in this model difficult to interpret. Nonetheless, potential for CCK to function as both a classical hormone and vagal stimulator in vivo cannot be entirely discounted, and it may be that the method in which CCK stimulates pancreatic enzyme secretion is proportionate to CCK1 associated acini. Swanson et al. (2003) reported that a CCK mimic (i.e., caerulein) only stimulated α-amylase release from pancreatic tissues collected from cattle receiving postruminal infusions of casein. Swanson et al. (2003) also observed increased α-amylase release from pancreatic tissues incubated with amino acids but not glucose or volatile fatty acids in response to caerulein. Further, postruminal infusions of partially hydrolyzed starch and glucose have been reported to decrease pancreatic α-amylase secretions in cattle (Swanson et al., 2002), which is in contrast to Harmon's conclusions that increases in dietary energy increase α-amylase secretions (Harmon, 1992). Initial interpretations of these data may suggest that pancreatic secretions of α-amylase in cattle are sensitive to circulating metabolites; however, abomasal infusions of partially hydrolyzed starch have been shown to increase fat associated with the omentum (McLeod et al., 2007), and observations from mice

have shown that CCK and 5-hydroxytryptamine (i.e., serotonin) stimulation of vagal afferent fibers that innervate the small intestine are mitigated by obesity (Daly et al., 2011). Additionally, carbachol (an analog of acetylcholine) stimulated α-amylase release from tissues regardless of increased postruminal flows of protein or partially hydrolyzed starch (Swanson et al., 2003). In toto, these data provide strong evidence that pancreatic α-amylase secretion in cattle is most likely mediated via neuroendocrine signaling mechanisms. Further, increases in omental adiposity in cattle may cause a refractory response to CCK, and greater amounts of CCK release may be required in fatter cattle to provide improvements to small intestinal starch digestion limited by pancreatic α-amylase secretions.

Pancreatic stimuli other than cholecystokinin

Regulation of pancreatic enzyme secretion is complex and responsive to stimuli other than CCK (Chey, 2001; Swanson et al., 2004; Jaworek et al., 2010). It is beyond the scope of this review to discuss all mammalian humoral peptides the exert effects on pancreatic exocrine secretions, because many of these peptides may participate in only a minor regulatory role of bovine pancreatic exocrine secretions. Discussion of humoral peptides other than CCK released by the gut that influence pancreatic exocrine secretions will be limited to those considered more likely to impact bovine pancreatic secretions based on observations and inferences of the unique characteristics of the ruminant digestive system (e.g., an apparent continuous intestinal contributing phase, pregastric modification of ingested nutrients). Gut humoral peptides beyond CCK that affect pancreatic exocrine secretions have been thoroughly reviewed by others (Chey, 2001; Dufresne et al., 2006; Dockray, 2012), and the reader is referred to these excellent reviews

for a greater understanding of all humoral peptides that have been observed to affect pancreatic exocrine secretions in nonruminant digestive systems.

Serotonin

Tryptophan and other aromatic amino acids are commonly considered to be potent stimulators of CCK and pancreatic exocrine secretions. Serotonin or 5-hydroxytryptamine is a neurotransmitter primarily produced by enterochromaffin cells in the alimentary tract from tryptophan (Chojnacki et al., 2011). Administration of L364,718 (a CCK1 antagonist) reduced about 50% of pancreatic enzyme secretions increased by intragastric infusion of rodent feed in rats, but when both the CCK1 antagonist and a serotonin antagonist were provided elevations in pancreatic enzyme secretion were almost completely eliminated (Li et al., 2000). Also, when Chey and Cheng (2001) reviewed the available literature they concluded that pancreatic enzyme secretions were stimulated by CCK and serotonin. Little is known of serotonin's effects on small intestinal nutrient digestion, and to my knowledge no data are reported on serotonin's effects on pancreatic secretions of cattle.

Melatonin

Melatonin is commonly associated with regulation of circadian rhythms in mammals and is released in significant quantities by the pineal gland during times of sleep. Yet, enterochromaffin cells readily produce melatonin (Bubenik, 2001; Messner et al., 2001; Chojnacki et al., 2011). In fact, melatonin secretions by the alimentary tract have been reported to exceed pineal secretion by 10- to 100-fold (Bubenik, 2001)! Classically, melatonin has received considerable research interest because it is a powerful antioxidant (Bubenik, 2008; Jaworek et al., 2010; Chojnacki et al., 2011). These efforts have led, in part, to discoveries that melatonin affects pancreatic exocrine secretions. Melatonin receptors have been discovered to

exist on rat pancreata (Stebelova et al., 2010) and some have observed that melatonin is capable of preventing acute damage to pancreatic tissues (Jaworek et al., 2003, 2004a). Further, rats receiving intraperotineal infusions of melatonin had increased pancreatic enzyme secretions, but α-amylase was not affected (Jaworek et al., 2004b, 2007; Leja-Szpak et al., 2004). Nonetheless, intraduodenal infusions of melatonin in rats have significantly increased pancreatic α-amylase secretions which correlated with increasing plasma CCK concentrations (Nawrot-Porabka et al., 2007). Similarly, Swanson et al. (2012) observed increases in pancreatic mass and α-amylase activity in gestating ewes supplemented with melatonin. Increases in pancreatic secretions of α-amylase in rats receiving intraduodenal melatonin were eliminated when rats received a CCK1 antagonist (Li, 2007). Thus, Jaworek et al. (2010) concluded that melatonin may act directly on pancreatic tissues as an innate modulator of inflammatory damage, and indirectly as a CCK secretagogue.

Leptin and Ghrelin

Leptin is a recently discovered biologically active peptide primarily produced by adipocytes (Zhang et al., 1994), but has also been detected in the gastrointestinal tract (Bado et al., 1998). Ghrelin was initially discovered in abomasal tissues shortly after leptin (Kojima et al., 1999), and has since been isolated from several segments of the small intestine (i.e., duodenum and ileum; Date et al., 2000). Both of these peptides are present in cattle (Bradford et al., 2006; Bradford and Allen, 2008; Bradford et al., 2008; Schoenberg et al., 2011) and sheep (Sugino et al., 2003, 2010), and they have been studied with respect to feed intake. Leptin and ghrelin have been commonly linked to regulation of feed intake, energy metabolism and body weight homeostasis (Jaworek et al., 2010) and these biologically active peptides have been respectively referred to as a "satiety" and "hunger" hormone (Konturek et al., 2003). Initial reports have

indicated that both leptin and ghrelin exert influence on pancreatic functions, perhaps as a biological first messenger to elicit responses in anticipation of luminal nutrient flows (Konturek et al., 2003). Initial data from intravenous infusion of leptin in rats suggested that leptin mitigated pancreatic exocrine secretions (Matyjek et al., 2003), but further investigations revealed that intraduodenal leptin significantly augmented pancreatic enzyme secretions (Jaworek et al., 2003a). Increases in pancreatic α-amylase secretion in response to leptin was concurrent with greater levels of circulating CCK, and deactivation of vagal afferent fibers by either capsaicin or bilateral subdiaphragmatic vagotomy completely eliminated intraduodenal leptin's effects on pancreatic α-amylase secretions (Nawrot-Porabka et al., 2004). The relationship between CCK, leptin and ghrelin remains unclear. Leptin and CCK both apparently potentiate the release of the other (Dufresne et al., 2006; Jaworek et al., 2010). Dockray (2012) concluded that leptin and CCK act cooperatively on vagal afferent fibers whereby leptin potentiates CCK's stimulation by upregulation of the abundance of a downstream transmitter (i.e., early growth transcription factor 1) of the CCK stimulatory signal. Often ghrelin is thought to invoke opposite biological responses in comparison to leptin; however, controversy remains as to whether ghrelin mitigates neuronal responses to CCK (Dockray, 2012). Sugino et al. (2010) reported that abomasal infusions of starch and casein in sheep rapidly increased circulating concentrations of ghrelin which corresponded to greater glucose and insulin concentrations. Clearly, the biological role of leptin and ghrelin on pancreatic enzyme secretions and small intestinal starch digestion warrants futher investigation.

Insulin

Insulin is secreted by the endocrine pancreas and is a hormone that tightly regulates blood glucose levels. Typically, pancreatic β-cell secretion is considered to be the result of greater

energy metabolite availability and increased circulating levels of incretins. A considerable amount of data in nonruminants and some data in ruminants (Mineo et al., 1995, 1997) has reported that CCK is involved in regulation of blood glucose levels by stimulating insulin secretion. Swanson et al., (2004) concluded that the role of insulin in regulating α-amylase secretions in ruminants is not clear. In nonruminants, however, exogenous insulin increases pancreatic secretion of α-amylase by augmenting responses to CCK and acetylcholine (Kanno and Saito, 1976; Saito et al., 1980b, 1980c), and immunoneutralization of circulating insulin obviated pancreatic responses to physiological doses of CCK (Lee et al., 1990). Nonetheless, pancreatic α-amylase secretions were inhibited by administration of exogenous insulin in diabetic sheep (Pierzynowski and Barej, 1984), and Swanson et al. (2004) did not observe a correlation between plasma insulin concentrations, CCK and α-amylase secretion in response to greater luminal flows of hydrolyzed starch and/or casein. It may be that the biological relationship among pancreatic exocrine secretions and insulin is different in ruminants compared to non-ruminants, but further data are required before this hypothesis can be confirmed.

Nitric oxide

Since its initial discovery in pancreatic acini of rats, a great body of literature has been focused on elucidating the precise methods in which CCK affects cellular responses, and this work has been promoted further by more recent discoveries of CCK receptors on certain carcinomas along with CCK's apparent role in potentiating chronic pancreatitis. In addition to those intracellular cascades previously discussed in enteroendocrine I cells, data have indicated that CCK receptors increase intracellular nitric oxide formation via induction of nitric oxide synthase, and may induce cell proliferation (Cordelier et al., 1997; Cordelier et al., 1999). Cholecystokinin has been observed to have a putative role in pancreatic hypertrophy and

proliferation in Otsuka Long-Evans Tokushima fatty rats (Miyasaka et al., 1997; Miyasaka et al., 1998) that are deficient in CCK receptors. Others have reported that CCK is not a required growth factor for the mouse (Lacourse et al., 1999; Takiguchi et al., 2002) or guinea pig (Herrington et al., 1995) pancreas. Nonetheless, pancreatic weight has been suggested to be an important regulator of α-amylase production and secretion in cattle (Wang et al., 1998; Swanson et al., 2002) and sheep (Swanson et al., 2000). Swanson et al. (2002) reported that increases in pancreatic protein amounts were greater because of increases in pancreatic mass rather than increases in protein concentration, and this correlates well with another report (Swanson et al., 2004) that measured increases in α-amylase secretions in cattle under similar experimental conditions. Apparently, cattle pancreatic secretions of α-amylase may be related to nutrients flowing to the pancreas (Swanson et al., 2003; Swanson et al., 2004). Nitric oxide's role as a vasodilator and neurotransmitter may play a role in regulating the volume of blood that perfuses the pancreas (Yago et al., 2001). This might be related to the observed involvement of CCK2 in regulation of pancreatic blood flow (Griesbacher et al., 2006).

Regulation of brush border glycohydrolases and the sodium-dependent glucose transporter

The penultimate and final processes required before dietary energy from carbohydrates may be assimilated from the intestinal lumen is hydrolysis of small chain di-, tri- and oligosaccharides to glucose and glucose's subsequent absorption from the lumen. Small intestinal α-glycohydrolases (i.e., maltase-glucoamylase, sucrase-isomaltase, lactase and trehalase) anchored to the apical membrane of enterocytes ('brush border membrane') are responsible for hydrolytic cleavage of small chain oligosaccharides in vivo (Van Beers et al.,

1995). Considerable study has focused on the precise regulatory mechanisms in nonruminants, but I am aware of no studies that have reported mechanisms regulating brush border α-glycohydrolase production by enterocytes in ruminants. Yet, there are data which have reported effects of luminal nutrient flows on brush border α-glycohydrolase activity in ruminants. Monosaccharides (e.g., glucose, fructose, galactose) can be absorbed from the small intestinal lumen through both energy-dependent (facilitated by the energy-dependent production of a Na^+ gradient) or energy-independent routes (Van Beers et al., 1995). The Na^+-glucose transporter 1 (SGLT1) is specific to glucose and galactose; this transporter utilizes the energy dependent Na^+ gradient (Van Beers et al., 1995). Energy-independent assimilation of monosaccharides is mediated either by the broad transport of glucose, galactose and fructose by glucose transporter 2, glucose transporter 5 or via paracellular absorption (Van Beers et al., 1995). Energy-independent absorption, however, requires relatively high concentrations of luminal monosaccharides and/or increased fluid flow capable of penetrating tight-junctions of enterocytes (Pappenheimer, 1993; Wright et al., 1994). Additionally, glucose is usually released in much greater concentrations relative to other monosaccharides (Van Beers et al., 1995). Thus, much of the regulator work concerning monosaccharide transport has focused on regulation of SGLT1 (Shirazi-Beechey et al., 2011). Similar to the study of regulatory mechanisms of brush border α-glycohydrolases, a preponderance of work has been done in nonruminants; however, some initial reports on ruminant regulation of monosaccharide absorption have been recently published.

Regulation of brush border α-glycohydrolases

Regulation of brush border α-glycohydrolases is apparently far less complex than regulation of pancreatic α-amylase secretion. Sucrase-isomaltase is expressed to a much greater extent than maltase-glucoamylase in nonruminants and is thought to be the primary enzyme that hydrolyzes small chain oligosaccharides in these species (Van Beers et al., 1995). Ruminants, however, have no measurable sucrase activity in the small intestine (Siddons, 1968) and this is strong evidence that the evolutionary divergence of ruminants has led to some level of altered small chain oligosaccharide digestion (Harmon, 1992). In fact, ruminants commonly express greater activity of maltase-glucoamylase throughout the small intestine (Russell et al., 1981; Janes et al., 1985; Kreikemeier et al., 1990), and it may be that small chain oligosaccharide digestion is primarily controlled by this enzyme in ruminant small intestines.

Basal levels of expression of all the α-glycohydrolases is developmentally imprinted early on in fetal development, and is relatively insensitive to effects of hormones (Van Beers et al., 1995). Despite the fact that levels of α-glycohydrolase expression are developmentally imprinted there is strong evidence that general regulatory mechanisms can influence α-glycohydrolase expression in nonruminants (Van Beers et al., 1995). Goda et al. (1983) reported that decreased starch intake by rats led to rapid decreases in maltase-glucoamylase and other α-glycohydrolase activities. Bustamente et al. (1986) and Morrill et al. (1989) observed significant increases in α-glycohydrolase activities that corresponded to increased starch intake in rats. Interestingly, starvation increased expression of jejunal sucrase-isomaltase, but not lactase in rats (Nsi-Emvo et al., 1994). Further, when rats were re-fed a band of enterocytes migrating up the intestinal villi with upregulated sucrase-isomaltase existed (Nsi-Emvo et al., 1994). These data have been interpreted to suggest that enterocyte regulation of α-glycohydrolases is only capable

of occurring in developing stem cells located in the intestinal crypt, and that mature enterocytes are incapable of differentially expressing α-glycohydrolases. It is yet to be clearly defined whether these apparent mechanisms for regulation α-glycohydrolase expression in nonruminant enterocytes are controlled at the transcriptional level.

To date there are no reports that indicate that cattle are capable of increasing small intestinal α-glycohydrolase activity in response to greater luminal flows of small chain oligosaccharides; however, several authors (Russell et al., 1981; Khatim and Osman, 1983; Kreikemeier et al., 1990; Bauer et al. 2001) have reported the relative activities of small intestinal α-glycohydrolases along the small intestine. All of these reports (Russell et al., 1981; Khatim and Osman, 1983; Kreikemeier et al., 1990; Bauer et al. 2001) indicated that α-glycohydrolase activity was greatest in the jejunum and significantly less in the proximal and distal portions of the small intestine. Russell et al. (1981) fed cattle increasing amounts of energy as either a forage- or corn-based diet and observed no effects on intestinal maltase activity. Yet, Janes et al. (1985) observed an increased hydrolytic capacity of sheep fed a corn- versus a grass-based diet. Maltase activity was not affected by treatment in this study, but increases in isomaltase activity mirrored increases in hydrolytic capacity. Additionally, the report of Janes et al. (1985) may indicate an increase in the functional area in the small intestine in which small chain oligosaccharides may be hydrolyzed, because isomaltase activity was greatest in the proximal one-third of the small intestine for corn-fed sheep rather than in the middle third. Kreikemeier et al. (1990) fed cattle a forage- or corn-based diet at 1 or 2 times maintenance energy requirements and reported that α-glycohydrolase activities were unaffected; however, maltase and isomaltase activities were greater over the entire small intestine as a result of a longer small intestine when more energy was fed. When Harmon (1992) reviewed these data he

concluded that small intestinal α-glycohydrolase activity of cattle was responsive to dietary energy rather than substrate, and that responses of cattle were less than their nonruminant counterparts.

Regulation of the energy-dependent Na^+-glucose transporter 1

Regulation of SGLT1 has been defined, and is apparently related to the sweet taste receptor expressed in lingual and endocrine cells in intestinal tissues (Shirazi-Beechey et al., 2011). The sweet taste receptor is a heterodimer of taste receptor 1, members 2 and 3 (T1R2/T1R3) which are capable of binding the gustatory G-protein receptor gustducin (Shirazi-Beechey et al., 2011). When available sugars in the small intestinal lumen are above some unknown threshold, they are apparently capable of upregulating the expression of SGLT1 via activation of T1R2/T1R3 and gustducin on endocrine cells in the intestine. Shirazi-Beechey et al. (2011) postulated that downstream effectors in intestinal endocrine cells increase secretion of neurotransmitters (likely glucagon like peptide 1) that result in neural stimuli that signal increased SGLT1 expression in enterocytes. Indeed, Shirazi-Beechey et al. (1991) reported that glucose absorption in sheep could be increased by 50- to 80-fold. Also, this proposed mode of SGLT1 regulation might explain why some (Guimaraes et al., 2007; Lohrenz et al., 2011) have failed to observe increases in SGLT1 expression in ruminants if basal flows of available sugars were already beyond those required to stimulate T1R2/T1R3 in intestinal endocrine cells. Also, a growing body of literature supports that small intestinal starch digestion in ruminants is not limited by glucose absorption (Shirazi-Beechey et al., 2011).

Conclusions

In summary, growing cattle are often most limited in growth by energy. Because small intestinal starch digestion is poor, most feeding strategies seek to optimize ruminal fermentation of starch. Yet, small intestinal digestion of starch is more energetically efficient than ruminal fermentation. Clearly, many factors may regulate the small intestinal hydrolytic capacity of starch in ruminants; however, these factors seem to be responsive to postruminal nutrient flows. Thus, increased knowledge of effects of postruminal nutrients on the small intestinal hydrolytic capacity of starch in cattle may allow for improvements in feeding strategies that could facilitate a greater efficiency of energy utilization.

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Chapter 2 - Effects Of Postruminal Protein And Amino Acids On Small Intestinal Starch Digestion in Beef Cattle: I. Small Intestinal Digestion Of Raw Cornstarch In Cattle Consuming A Soybean Hull-Based Diet Is Rapidly Improved By Duodenal Casein Infusion

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Abstract

Six duodenally and ileally cannulated steers were used in 3 sequential studies to measure basal nutrient flows from a soybean hull-based diet, small intestinal digestibility of raw cornstarch continuously infused in the duodenum, and responses of small intestinal starch digestion to duodenal infusion of 200 or 400 g/d casein. Our objective was to evaluate responses in small intestinal starch digestion in cattle over time, and to measure responses in small intestinal starch digestion to increasing amounts of metabolizable protein. On average, cattle consumed 3.7 kg/d DM, 68.1 g/d dietary N, and 69.6 g/d dietary starch. Starch flow to the duodenum was small (37.9 g/d) and N flow was 91.3 g/d. Small intestinal digestibility of duodenal N was 57.2%, and small intestinal digestion of duodenal starch flows was extensive (92.1%). Small intestinal starch digestibility was 34.0% when raw cornstarch (1.5 kg/d) was continuously infused into the duodenum. Subsequently, cattle were placed in 1 of 2 replicated Latin squares that were balanced for carryover effects to determine response to casein infusions and length of adaptation required. Infusion of casein linearly increased ($P \leq 0.05$) small intestinal starch digestibility, and small intestinal starch digestion adapted to infusion of casein in 6 d. Ethanol-soluble starch and unpolymerized glucose flowing to the ileum increased linearly ($P \leq 0.05$) with increasing infusion of casein. Plasma cholecystokinin was not affected by casein infusion, but circulating levels of glucose increased linearly ($P \leq 0.05$). Apparently, responses in small intestinal starch digestion in cattle adapt to casein within 6 d, and increases in duodenal supply of casein up to 400 g/d increases small intestinal starch digestion in cattle.

Introduction

Improving the efficiency with which cattle utilize dietary energy may allow significant opportunity for improvement in efficiency of cattle production and cattle health and for reduction in environmentally harmful emissions by cattle production systems. Small intestinal digestion of starch (Owens et al., 1986; Harmon, 1992; Huntington, 1997) and glucose (Harmon and McLeod, 2001) is energetically more efficient than ruminal fermentation and does not yield methane; however, small intestinal starch digestion in cattle is often poor (Owens et al., 1986; Harmon, 1992; Huntington, 1997). Total tract digestion of starch and dietary energy is often improved by increasing ruminal fermentation via more extensive processing of cereal grains, which may result, in part, from limitations in small intestinal starch digestion in cattle. Thus, improvements in ruminally fermentable starch by extensive processing of grains is often viewed as beneficial to cattle production systems. Unfortunately, extensive processing of cereal grains can be associated with increased incidences of metabolic disorders (e.g., acidosis), which can have long-term detrimental effects on cattle (Krehbiel et al., 1995). Also, the potential for improving ruminal starch digestion among cattle by some unknown or novel processing technique has been questioned because, "many of the significant advances possible in physical and chemical processing of feed have likely already been made" (McAllister et al., 1994). Improving small intestinal digestion of starch in cattle may be an avenue by which significant advancements in the efficiency with which cattle utilize dietary energy can be made.

Typically, greater postruminal flows of α-linked glucose (i.e., starch and starch hydrolysates) are associated with greater amounts of starch digested in the small intestine, but with a decreasing digestibility (Kreikemeier et al., 1991; Nocek and Tamminga, 1991; Branco et al., 1999). Reductions in small intestinal starch digestibility have been associated with decreases

in pancreatic α-amylase secretion (Walker and Harmon, 1995; Richards et al., 2003; Swanson et al., 2004); however, small intestinal starch digestion (Richards et al., 2002), portal glucose absorption (Taniguchi et al., 1995), and pancreatic α-amylase secretion (Richards et al., 2003) can be augmented by greater postruminal flows of high-quality proteins (i.e., casein) in cattle. Nonetheless, Swanson et al. (2002) did not observe increases in bovine pancreatic expression of α-amylase when they abomasally infused casein and partially hydrolyzed starch. Several factors may have contributed to the apparently disparate results of Swanson et al. (2002; e.g., initial BW of cattle, type of α-linked glucose infused, amounts of casein and α-linked glucose infused, length of adaptation to treatment). Swanson et al. (2002) adapted cattle to dietary treatment for 7 d, which was identical to that used by Richards et al. (2003) where pancreatic α-amylase was increased by casein, but less than the 12-d adaptation used by Richards et al. (2002) to measure increases in small intestinal starch digestion and the 9-d adaptations used by Taniguchi et al. (1995) to measure increases in portal glucose absorption.

Our experiments were designed to measure amounts of casein required to improve small intestinal starch digestion in cattle and to evaluate the length of adaptation required. We hypothesized that duodenal infusions of casein would increase small intestinal starch digestion in cattle but that adaptation times required to demonstrate the response might be greater than 7 d.

Materials and Methods

Animals, Husbandry and Diet

The Kansas State University Institutional Animal Care and Use Committee approved all surgical, post-operative, experimental and animal husbandry procedures.

Six crossbred (predominately Hereford and Black Angus) steers were surgically fitted with double-L shaped (Streeter et al., 1991) duodenal (approximately 10-cm distal to the pylorus and proximal to the common pancreatic-bile duct) and ileal (approximately 10-cm proximal to the ileocecal junction) cannulas 30 d prior to experimentation. Cattle were fitted with neck collars and tethered to tie-stalls (1.7 × 1.2 m) in a temperature-controlled room (20°C) under 16 h of light (0500 to 2100) and 8 h dark.

Prior to and during experimentation, cattle were fed a common soybean hull-based diet (Table 1) twice daily (0700 and 1900 h) and allowed ad libitum access to fresh water. The diet was formulated to provide adequate ruminally available N (NRC, 1996).

Experimental Design, Treatments and Sampling

Experiment 1

The initial experiment determined basal nutrient flows. Six duodenally and ileally cannulated cattle (initial average BW = 207 ± 7.8) were fed 3.7 kg (DM basis) of a soybean-hull based diet (Table 1; about 1.5 × maintenance energy requirement; NRC, 1996). A sample of the

diet was collected at the beginning of the experiment, stored at room temperature (22°C) and later analyzed for DM, OM, starch, and N.

Cattle were adapted to diet for 7 d. Five grams of TiO_2 was thoroughly hand-mixed into diets at each feeding (10 g/d) starting 5 d prior to sampling, and served as an indigestible marker of nutrient flow (Titgemeyer et al., 2001). Spot samples of duodenal and ileal digesta and feces were collected 2, 4, 6, 8, 10, and 12 h after feeding on d 8. Samples of duodenal (250 g) and ileal digesta (200 g) were collected by attaching a plastic bag (140 × 229 mm) to the cannula, and feces (200 g) was collected after cattle defecated in response to stimulation. Immediately after collection, pH of duodenal and ileal samples were measured with a pH meter (Portable Meter Model 250A, Orion Research, Beverley, MA). The pH of feces was measured after vigorously mixing an aliquot (~5 g) of feces with 15 mL of deionized H_2O. To preserve starch content of digesta and feces, sample pH was increased to near 11 via addition of 40% (wt/wt) NaOH (2.1 mL added to ileal and fecal samples, and 4.2 mL added to duodenal samples). Samples were composited and frozen (-20°C) between collections. Composite digesta and feces were thawed at room temperature (22°C), and a subsample was refrozen, lyophilized, and subsequently analyzed for DM, OM, TiO_2, N, starch, unpolymerized glucose and NDF. Feed and freeze-dried digesta samples were ground to pass a 1-mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific USA, Swedesboro, NJ). Dry matter content was determined by drying samples at 105°C for 24 h in a forced-air oven, and OM was subsequently determined by ashing in a muffle furnace for 8 h at 450^{o}C. Samples were analyzed for TiO_2 as described by Short et al. (1996). The wet chemistry techniques of Van Soest et al. (1991) were used to quantify NDF (with α-amylase and sodium sulfite). Nitrogen content was determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation, St. Joseph, MI), and CP was calculated as 6.25 ×

N. Starch and free glucose was analyzed using techniques described by Herrera-Saldana and Huber (1989) with glucose determined with a glucose oxidase linked assay (Gochmann and Schmitz, 1972). Lactate and VFA were measured from wet ileal digesta and feces with methods described by DeFrain et al. (2002).

Nutrient flows and digestibilities associated with diet were calculated from TiO_2 concentrations using methods described by Merchen (1988).

At 11.5 h after feeding (30 min before final digesta sampling), jugular blood (20 mL) was collected by venipuncture into glass vacuum tubes (16 × 100 mm; Monoject Blood Collection Tubes, Sherwood Medical, St. Louis, MO) containing sodium heparin isolated from porcine mucosa (143 USP) to measure concentrations of glucose, cholecystokinin (CCK), α-amino-N, and urea-N. Blood was immediately placed on ice and plasma was harvested by centrifugation (2,200 × *g*; 15 min; 4°C). Plasma was transferred to plastic tubes (Fisherbrand Premium 2.0 mL MCT Graduated Natural, Fisher Scientific), and aprotinin (500 KIU per mL plasma) was added to a portion of plasma (3 mL). Plasma samples were immediately frozen (-20°C), and plasma containing aprotinin was vortexed for 10 sec prior to freezing (-80°C). Plasma CCK was analyzed with the double antibody radioimmunoassay described by Benson and Reynolds (2001). Plasma urea (Marsh et al., 1965), plasma glucose (Gochman and Schmitz, 1972), plasma α-amino-N (Palmer and Peters, 1969) were measured with an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL).

Averages across steers for nutrient flows and digestibilities were calculated using the MEANS procedure of SAS (SAS Inst. Inc., Cary, NC). For pH, data were averaged within day by steer, and averages across steer were then calculated.

Experiment 2

Immediately after *Exp. 1*, we conducted an experiment to measure capacity of postruminal starch digestion in cattle consuming our soybean hull-based diet. Infusion lines were fitted in the duodenal cannulas of the same 6 steers from *Exp. 1*, and cattle were adapted to infusions of starch by providing sequential duodenal infusions of raw cornstarch for 48 h each, which delivered on average 363 ± 7.6, 687 ± 13.8, 1,130 ± 8.6, and 1,502 ± 9.9 g/d. During adaption to duodenal starch infusions, rectal temperature and fecal pH were measured immediately after feeding and served as indices of health in addition to daily feed intake.

Infusions were made through Tygon tubing (i.d. = 2.38 mm; Saint-Gobain North America, Valley Forge, PA) with a peristaltic pump (Model CP-78002-10; Cole-Parmer Instrument Company, Vernon Hills, IL) with 9 L/d of a cornstarch suspension. Suspensions (2 containers of 5 L) were prepared daily for each steer immediately prior to infusion. Cornstarch was maintained in suspension with an automatic stirrer (Arrow 6000, Arrow Engineering Company, Hillsdale, NJ) during infusion, and the pumping rate was 375 mL/h to allow for continuous infusion of suspensions. Each infusate was prepared daily by weight and the exact amount infused was determined by recording the weight of residual infusate after each 12 h infusion period. Cornstarch suspensions contained CrEDTA (1.4 g Cr/10 L; Binnerts et al., 1968) which served as an indigestible marker, the appropriate amount of cornstarch, and deionized H_2O. Infusate containers, the peristaltic pump, and infusion lines were above the cattle, and containers were exchanged twice daily (0700 and 1900 h). Additionally, at the end of each infusion (48 h), 100 mL of water was infused to prevent accumulation of residual infusate in infusion lines.

During the final 12 h of the final cornstarch infusion period (1,502 g/d), ileal digesta, feces, and blood were collected using methods identical to those described for *Exp. 1*.

Prior to analyses, pH of an aliquot of previously alkalized wet digesta (75 g of ileal digesta) or feces (50 g with addition of 25 mL H_2O) was neutralized with 1.0 to 1.5 mL of 6 *M* HCl. Dry matter was determined by drying at 105°C for 24 h. Starch concentrations were measured in the wet samples using the glucogenic assay described by Herrera-Saldana and Huber (1989) with glucose quantified using a glucose oxidase linked assay (Gochmann and Schmitz, 1972). Unpolymerized glucose was determined from assay tubes to which no enzyme was added. Ethanol-soluble starch was determined using techniques similar to those described by Kreikemeier and Harmon (1995). Briefly, a portion of wet digesta or feces (with added H_2O) was centrifuged (20,000 × *g*; 15 min; 4°C). Following centrifugation, supernatant was collected and 0.5 mL transferred to a microcentrifuge tube containing 1.25 mL of anhydrous ethanol. Samples were kept overnight (about 16 h) at 4°C and then centrifuged (17,000 × *g*; 10 min; 4°C), and supernatant was decanted into another microcentrifuge tube. The precipitate was subsequently resuspended in 1 mL of ethanol and centrifuged. This rinsing procedure was conducted a total of 3 times and the resulting supernatant from each rinsing was pooled with supernatant collected from the overnight incubation. Ethanol was then evaporated from the pooled supernatant and starch content measured using procedures previously described.

Concentration of Cr in ileal digesta and feces (with added H_2O) was determined by atomic absorption spectrophotometry from supernatant collected after centrifugation (20,000 × *g*; 15 min; 4°C). Also, a portion of supernatant was retained, mixed with meta-phosphoric acid and analyzed for VFA and lactic acid by GLC as described by DeFrain et al. (2002).

Digesta flows at the ileum and in feces were calculated as described by Kreikemeier and Harmon (1995) as follows:

$$\text{Ileal or fecal fluid flow (g/d)} = \frac{duodenal\ infusion\ rate\ of\ Cr\ (mg/d)}{Cr\ concentration\ in\ ileal\ or\ fecal\ fluid\ (mg/g)}$$

$$\text{Ileal or fecal total digesta flow (g/d)} = \frac{fluid\ flow\ (g/d)}{(1-digesta\ DM\ concentration)}$$

$$\text{Ileal or fecal DM flow} = total\ digesta\ flow - fluid\ flow$$

Flows of ethanol-soluble starch and organic acids were calculated as the product of fluid flow and nutrient concentration in the supernatant. Starch and free glucose flows were calculated as the product of DM flow and starch (or free glucose) concentration (DM basis). Small intestinal starch digestibility was calculated as:

$$\text{Small intestinal starch digestibility} = 1 - \left(\frac{ileal\ starch\ flow\ (g/d)}{starch\ infused\ on\ the\ day\ of\ sampling\ (g/d)}\right)$$

Averages across steers for nutrient flows and digestibilities were calculated using the MEANS procedure of SAS (SAS Inst. Inc., Cary, NC). For pH, data were analyzed as described for Exp. 1.

Experiment 3

Temporal effects of casein on starch digestion were quantified. The same 6 steers were placed in 1 of 2 replicated 3 × 3 Latin squares with treatment sequences balanced for carryover

effects; each period was 11 d. Cattle were fed the same soybean hull-based diet (3.7 kg DM/d) and provided infusions identical to the final cornstarch infusion in *Exp. 2* (1.5 kg/d), but with a portion of deionized H_2O replaced with a 10% (wt/wt) casein solution designed to deliver either 0, 200 or 400 g/d casein. On average, cattle were received 1,485 ± 7.0 g/d cornstarch and either 0, 207.4 ± 1.7, or 405.3 ± 3.4 g/d casein. Samples of ileal digesta and feces were collected on d 2, 3, 4, 6, 8 and 10 of each period; blood was collected on d 10. Collections were made over a 12-h period as described for *Exp. 1* and samples were analyzed with the methods described in *Exp. 2*.

Data from a steer receiving no casein and a steer receiving 200 g/d casein on d 6 of period 1, and a steer receiving 200 g/d casein on d 8 of period 3 were missed because of failed infusions prior to sampling on that day. In period 3, a steer receiving 400 g/d casein developed complications associated with its ileal cannula, which prevented collection of data on d 8 through the end of that period.

Data were analyzed using the MIXED procedure of SAS. For analysis of responses over time, fixed effects were treatment, period, day, and treatment × day, and steer was included as a random effect. The repeated term was day, with steer × period serving as the subject. The covariance structure was spatial power. Least squares means were calculated. Linear and quadratic effects of treatment within each day were evaluated to assess the time needed for adaptation.

Because analysis of responses over time indicated that responses in small intestinal starch digestion adapted to treatment by d 6, main effects of treatment were evaluated using data from d 6, 8 and 10 of each period; the analyses were the same as described for the full data set. Effect of

treatment on pH were analyzed by calculating the average within steer for each period; data were then analyzed using the same procedure for the full data set.

Significance among treatments was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

Results and Discussion

Experiment 1

As expected, dietary intake of starch was small (70 g/d). Relatively low amounts of dietary starch (Table 2.1) contributed to only minor amounts of starch flowing to the duodenum, ileum, and feces (Table 2.2). Apparent ruminal digestion of dietary starch was 46%, and 92% starch flowing to the duodenum was apparently digested in the small intestine. Additionally, jugular concentrations of CCK were low when cattle were provided the diet alone (Table 2.3).

Typically, α-linked glucose entering the small intestine may be comprised of ruminally undigested dietary starch, microbial polysaccharides, and secreted mucopolysaccharides (McAllan and Smith, 1974; Branco et al., 1999). Indeed, microbial polysaccharides and secreted mucopolysaccharides likely contributed to the negative large intestinal starch digestion in this study. Branco et al. (1999) reported appreciable amounts of α-linked glucose (188 g/d) flowing to the duodenum of steers limit-fed an endophyte-free fescue hay. It is possible that a significant portion of starch flowing to the duodenum was representative of microbial polysaccharides (McAllan and Smith, 1974; Branco et al., 1999). Interestingly, Branco et al. (1999) reported small intestinal digestibilities of duodenal starch related to diet (32%) that were considerably less than our observations. Nonetheless, data from this experiment indicates that contributions of starch reaching the ileum from microbial polysaccharides, secreted mucopolysaccharides, and

ruminally undigested dietary starch was small when cattle were limit-fed our soybean hull-based diet. Further, these data suggest that when starch was duodenally infused in subsequent studies (i.e., *Exp. 2* and *3*), amounts of starch from sources other than the infusions accounted for only a minor portion of starch reaching the ileum.

Experiment 2

Nutrient flows to the ileum and feces in cattle limit-fed a soybean hull-based diet and infused with 1,522 g/d raw cornstarch are shown in Table 2.4. As planned, duodenal infusion of raw cornstarch led to considerable amounts of starch flowing to the ileum (1,004 g/d) and feces (747 g/d). Small and large intestinal starch digestion was 34.0% and 16.9% of the infused starch, respectively. Kreikemeier et al. (1991) and Kreikemeier and Harmon (1995) abomasally infused unadapted steers with raw cornstarch (60 and 66 g/h, respectively) for 10 h and reported that small intestinal starch digestion was 57.9% and 63.0%, respectively. Branco et al. (1999) abomasally infused cattle with partially hydrolyzed starch (40 g/h) and reported a small intestinal starch digestibility of 76% which was similar to values of 71% obtained when abomasal infusions of raw cornstarch were provided at an identical rate (71%; Kreikemeier et al., 1991). These data (Kreikemeier et al., 1991; Kreikemeier and Harmon, 1995; Branco et al., 1999) demonstrated little to no differences in response of small intestinal starch disappearance to greater duodenal flows of cornstarch in adapted cattle. In our study, small intestinal starch digestibility was less than these reports (Kreikemeier et al., 1991; Kreikemeier and Harmon, 1995; Branco et al., 1999).

In comparison to *Exp. 1* (Table 2.2), flows of starch, glucose, and lactate to the ileum were much larger with duodenal infusion of cornstarch (Table 2.4). Similarly, flows of starch,

glucose, lactate, acetate, propionate, and butyrate to the feces were larger when starch was infused. These data suggest that appreciable amounts of starch reached the terminal ileum and feces under our experimental conditions, and agree with previous reports (Kreikemeier et al., 1991; Kreikemeier and Harmon, 1995; Branco et al., 1999).

A precise explanation for the lower apparent small intestinal starch disappearance in our model compared to the work of Kreikemeier et al. (1991), Kreikemeier and Harmon (1995), and Branco et al. (1999) remains unclear, but our data demonstrated low small intestinal starch digestion and therefore was considered to be useful for assessing treatments that might improve starch digestion.

Concentrations of CCK were increased 82% when steers were infused with cornstarch (Table 2.3; *Exp. 1* and *Exp. 2*). Swanson et al. (2004) reported that plasma CCK concentration was increased by infusion of hydrolyzed starch, which is in agreement with our observations in cattle receiving duodenal infusion of raw cornstarch.

Experiment 3

In order to determine temporal effects of duodenal casein infusion on small intestinal starch digestion, we analyzed linear and quadratic effects of increasing amounts of casein within each sampling day (Figure 2.1). Effects of casein on small intestinal starch digestion were linear and apparent ($P \leq 0.05$) within 6 d after infusions began and continued through d 10, suggesting that small intestinal starch digestion adapted within 6 d to postruminal infusions of casein (Figure 2.1). Additionally, small intestinal starch digestion increased linearly ($P \leq 0.05$) with greater amounts of casein infusion after adaptation (Table 2.5). We are not aware of any data that has assessed the length of adaptation of small intestinal starch digestion in cattle to postruminal

infusion of casein. Taniguchi et al. (1995) reported that abomasal infusions of casein with cornstarch for 9 d increased the flux of glucose to the portal drained viscera more than 2-fold in cattle. Additionally, Richards et al. (2002) observed linear increases in amount of starch disappearing from the small intestine of cattle provided abomasal infusions of casein for 12 d. Typically, greater postruminal flows of starch and starch hydrolysates are associated with greater amounts of starch digested in the small intestine, but with a decreasing digestibility (Kreikemeier et al., 1991; Nocek and Tamminga, 1991; Branco et al., 1999). Reductions in small intestinal starch digestibility have been associated with decreases in pancreatic α-amylase secretion (Walker and Harmon, 1995; Richards et al., 2003; Swanson et al., 2002); however, reports on pancreatic secretions of α-amylase in cattle in response to postruminal infusions of casein in combination with cornstarch or starch hydrolysates have differed (Richards et al., 2003; Swanson et al., 2004). Richards et al. (2003) observed linear increases in pancreatic α-amylase secretion when they abomasally infused cattle with casein and cornstarch for 7 d. Yet, Swanson et al. (2004) did not detect increases in secretion of pancreatic α-amylase when casein was abomasally infused with cornstarch for an identical length of time. Further, Swanson et al. (2002) reported that expression of α-amylase in pancreatic tissue was not increased when they abomasally infused casein (0.6 g/kg BW daily) and partially hydrolyzed starch (4 g/kg BW daily) for 7 d. Obviously, many factors may have contributed to the apparently disparate results of Swanson et al. (2002, 2004) when compared to those of Richards et al. (2003); differences included initial BW of cattle, type of α-linked glucose infused, relative amount of casein and α-linked glucose, responses in pancreatic secretion and composition versus small intestinal starch disappearance. Our data, however, seem to suggest that adaptation to treatments was not a likely cause for differences because adaptations for those studies were 7 d.

The basal diet in our experiment provided 326 g of metabolizable protein (MP; Table 2.2; calculated as 6.25 × N disappearance between the duodenum and ileum). Apparent small intestinal N digestibility was 57% (diet). If casein is assumed to be entirely digested in the small intestine and contains 98.7% CP (Titgemeyer and Merchen, 1990) and dietary N flows to the duodenum were not altered by infusion of cornstarch and casein, then MP supplies for 200 g/d casein and 400 g/d casein were 531 and 726 g/d, respectively. When Richards et al. (2002) regressed amount of starch digested in the small intestine as a function of the amount of small intestinal CP digested, they reported a 1.2 g increase in small intestinal starch digestion for each 1 g increase in CP digested. Although casein supplementation linearly increased small intestinal starch digestion, our responses were less than those observed by Richards et al. (2002). We observed a 9.2 percentage unit increase (170 g/d) in small intestinal starch digestion when 400 g/d of casein was supplemented, whereas Richards et al. (2002) observed a 226 g/d increase with only 200 g/d of casein supplementation.

Amounts of ethanol-soluble carbohydrates flowing to the ileum increased linearly ($P \leq 0.05$) with increasing amounts of casein infusion. These data provide indirect evidence that pancreatic α-amylase was increased in response to casein infusions, which is similar to the direct observations of pancreatic α-amylase secretions measured by Richards et al. (2003), but different from the observations of Swanson et al. (2004).

Flows of glucose to the ileum increased linearly ($P \leq 0.05$) with greater amounts of casein (Table 2.4), although flows of total organic acids to the ileum were not affected by casein. Kreikemeier and Harmon (1995) abomasally infused glucose (66 g/h) and reported that 84% of infused glucose was absorbed in the small intestine of cattle. Shirazi-Beechey et al. (1991) concluded that glucose absorption in sheep could be increased 50- to 80-fold in response to

greater glucose flows to the small intestine. Several researchers (Bauer et al., 2001; Guimaraes et al., 2007) have studied the relationship of glucose uptake capacity with capabilities for hydrolysis of oligosaccharides along the small intestine of cattle. These authors reported that glucose uptake capacities exceed capabilities for hydrolytic cleavage of oligosaccharides along all locations of the small intestine except the ileum. Bauer et al. (2001) speculated that reports of free glucose at the terminal ileum were likely a result of lower levels of the Na^+/glucose transporter in that region relative to α-glycohydrolases associated with intestinal epithelium. Harmon (1992) concluded that that activity of α-glycohydrolases associated with intestinal epithelium was responsive to dietary energy rather than substrate, and that responses of cattle were less than their nonruminant counterparts. When Huntington (1997) modeled small intestinal starch digestion in cattle he concluded that pancreatic α-amylase activity was most limiting to small intestinal starch digestion. Greater flows of glucose to the ileum in response to casein in our study may be a result of increases in α-amylase secretions (Richards et al., 2003) that allowed for greater flows of small chain α-glycosides to the ileum.

Infusion of casein did not affect plasma CCK (Table 2.6; $P = 0.33$). Plasma glucose increased linearly ($P \leq 0.05$) with casein infusions (Table 2.6). Swanson et al. (2004) reported that plasma CCK concentration was not increased by infusion of casein. Swanson et al. (2004) suggested that either circulating metabolites or other biologically active peptides may regulate α-amylase secretions in cattle. Indeed, stimulation of cattle pancreatic tissue by CCK may be dependent on the concentration of circulating amino acids (Swanson et al., 2003). The role of CCK, as a classical hormone, on pancreatic enzyme secretions has been equivocal since reports that atropine (a cholinergic antagonist) decreased responses of pancreatic secretions to low doses of CCK and to nutritional stimulants of CCK release in dogs (Konturek, 1993; Niebergall-Roth

and Singer, 2001). Benson and Reynolds (2001) reported appreciable hepatic clearance of CCK in cattle, and Swanson et al. (2003) observed consistent increases in enzyme secretion of pancreatic tissue by an analog of acetylcholine but not by CCK.

In summary, small intestinal digestion of raw cornstarch when duodenally infused at a rate of 1.5 kg/d was inefficient. Duodenal infusions of casein linearly improved small intestinal starch digestion, and these responses to casein were apparent within 6 d.

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Table 2.1 Composition of soybean hull-based diet fed to steers

Item	% of DM
Ingredient	
Soybean hulls	72.2
Brome hay, late-bloom	20.0
Corn steep liquor	6.0
Limestone	1.0
Salt	0.5
Mineral and vitamin premix[1]	0.3

Chemical composition	Experiment 1	Experiment 2	Experiment 3
DM[2], %	89.2	89.2	90.0
OM[2], % DM	91.6	91.8	92.4
CP[2], % DM	10.9	13.6	11.1
Starch[2], % DM	2.9	1.7	1.6
RDP[3,4], % CP	59.1	59.1	59.1
NE_m[3,5], Mcal/kg	1.7	1.7	1.7

[1]Provided to diets (per kg diet DM): 50 mg Mn, 50 mg Zn, 10 mg Cu, 0.5 mg I, 0.2 mg Se, 2,200 IU of vitamin A, 275 IU of vitamin D, and 25 IU of vitamin E.
[2]Based on laboratory analyses.
[3]Calculated using the tabular values of NRC (1996).
[4]Ruminally degraded protein
[5]Net energy for maintenance

Table 2.2 Nutrient flows to the duodenum, ileum and feces in cattle fed 3.7 kg DM/d of a soybean hull-based diet (Exp. 1)

Item	Duodenum	SEM	Ileum	SEM	Feces	SEM
No. of observations	6		6		6	
DM content, %	6.3	0.20	10.5	0.32	17.4	0.46
Composition, % DM						
OM	71.2	1.46	78.0	0.47	81.5	0.72
Starch	1.5	0.15	0.18	0.042	0.47	0.050
Glucose	0.12	0.009	0.44	0.040	0.15	0.009
N	3.3	0.07	2.3	0.093	2.65	0.088
pH	2.9	0.05	7.8	0.02	6.7	0.06
Organic acid content, mmol/L						
Acetate	20.2	1.24	23.73	2.1	56.4	3.74
Propionate	1.9	0.22	0.74	0.107	11.3	2.17
Butyrate	0.29	0.022	0.39	0.050	4.32	0.56
Lactate	0.19	0.014	0.47	0.042	0.10	0.035
Flow to segment, g/d						
DM	2,729	49.8	1,676	63.5	1,417	53.2
OM	1,943	64.7	1,309	55.1	1,156	52.6
Starch	37.9	3.96	3.0	0.73	6.6	0.75
Glucose	3.3	0.27	7.2	0.46	2.1	0.09
N	91.3	1.77	39.1	0.89	37.4	1.06
Acetate	49.6	1.57	20.7	2.39	23.1	2.30
Propionate	5.7	0.67	0.77	0.081	5.7	1.17
Butyrate	1.0	0.05	0.49	0.051	2.60	0.40
Lactate	0.7	0.05	0.60	0.026	0.06	0.021

Table 2.3 Nutrient flows to the ileum and feces in cattle fed 3.7 kg DM/d of a soybean hull-based diet and duodenally infused with 1.522 kg/d raw cornstarch (Exp. 2)

Item	Ileum	SEM	Feces	SEM
No. of observations	6		6	
DM content, %	16.3	0.68	24.5	0.50
Composition, % DM				
Starch	34.1	1.31	25.2	1.80
Ethanol-soluble starch	9.84	1.04	5.45	1.48
Glucose	2.90	0.35	2.01	0.24
pH	7.0	0.03	6.1	0.12
Organic acid content, mmol/L				
Acetate	26.0	1.34	55.8	3.37
Propionate	1.0	0.14	10.5	2.04
Butyrate	1.2	0.13	13.8	1.41
Lactate	23.1	1.89	23.4	2.86
Nutrient flow, g/d				
DM	2,963	117.8	2,970	76.3
Starch	1,004	30.6	747	48.9
Ethanol-soluble starch	148.4	12.48	82.1	21.59
Glucose	85.3	8.74	59.9	7.02
Acetate	23.8	1.17	50.8	3.77
Propionate	1.1	0.14	11.8	2.29
Butyrate	1.6	0.16	18.2	1.63
Lactate	31.7	2.56	31.3	3.15
Starch digestion, %	34.0	1.70	49.0	2.94

Table 2.4 Main effect of casein after adaptation on composition of digesta and nutrients flowing to the ileum (Exp. 3)

	Duodenal casein infusion, g/d				*P*-value	
Item	0	200	400	SEM	Linear	Quadratic
No. of observations[1]	6	6	6			
Duodenal starch infused, g/d	1,508	1,509	1,453	18.6		
Ileal DM content[2], %	17.4	16.7	17.0	0.66	0.54	0.43
Composition of ileal digesta, % DM						
Starch	35.8	34.8	33.8	1.3	0.23	0.98
Ethanol-soluble starch, mg/mL	10.52	13.75	15.23	1.59	<0.01	0.48
Glucose	2.57	3.78	4.22	0.10	<0.01	0.53
pH	6.8	6.7	6.5	0.07	<0.01	0.37
Organic acid content, mmol/L						
Acetate	29.7	28.5	31.0	2.32	0.64	0.43
Propionate	1.5	1.1	1.4	0.21	0.54	0.04
Butyrate	1.4	1.4	2.0	0.34	0.02	0.17
Lactate	26.8	24.9	33.2	2.66	0.10	0.12
Nutrient flow to ileum, g/d						
DM	2,726	2,624	2,394	90.2	<0.01	0.49
Starch	972	906	802	37.6	<0.01	0.46
Ethanol-soluble starch	131.9	177.6	175.7	17.01	0.01	0.11
Glucose	68.4	99.1	100.4	8.63	<0.01	0.14
Acetate	23.0	22.4	21.8	1.65	0.62	0.98
Propionate	1.4	1.0	1.2	0.20	0.22	0.07
Butyrate	1.5	1.5	2.1	0.31	0.05	0.26
Lactate	31.3	29.5	35.9	3.81	0.37	0.35
Small intestinal starch digestion, %	35.5	40.0	44.7	2.51	<0.01	0.93

[1]Data collected on d 6, 8 and 10 for each observation.

[2]Reported DM content of feces is representative of 50 g wet feces with 25 g added H_2O.

Table 2.5 Plasma concentrations of circulating metabolites and cholecystokinin in cattle fed 3.7 kg/d of a soybean hull-based diet without (*Exp. 1*) or with (*Exp. 2*) 1,522 g/d raw cornstarch infused duodenally

Plasma	*Experiment 1*	SEM	*Experiment 2*	SEM
No. of observations	6		6	
Cholecystokinin, pmol/L	1.91	0.510	3.48	0.676
Glucose, mmol/L	4.66	0.087	4.72	0.100

Table 2.6 Effect of duodenal casein infusion on plasma concentrations of circulating metabolites and cholecystokinin in cattle (Exp. 3)

	Duodenal casein infusion, g/d				*P*-value	
Item	0	200	400	SEM	Linear	Quadratic
No. of observations	6	6	5			
Cholecystokinin, pmol/L	5.31	4.65	6.17	1.28	0.39	0.20
Glucose, mmol/L	4.55	4.94	4.82	0.16	0.04	0.03

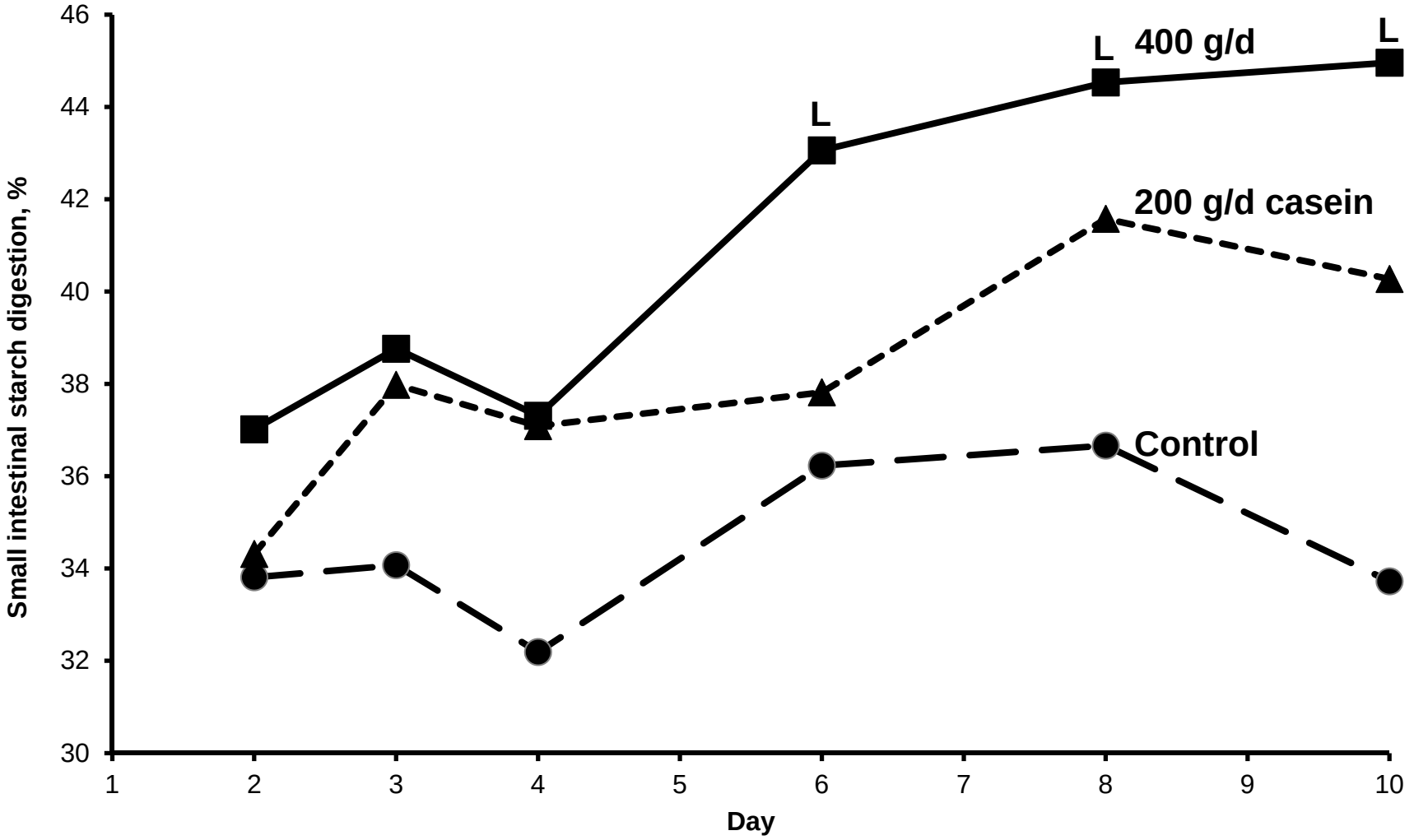

Figure 2.1 Effect of 200 or 400 g/d casein on small intestinal starch digestion in cattle receiving continuous duodenal infusion of 1.5 kg/d cornstarch (Exp. 3). Treatment, P < 0.01. L = linear effect of casein within day, P < 0.05.

Chapter 3 - Effects of Postruminal Protein and Amino Acids on Small Intestinal Starch Digestion in Beef Cattle: II. Infusions of essential or non-essential crystalline amino acids in amounts similar to that of casein have disparate effects on small intestinal starch digestion in cattle receiving continuous duodenal infusions of raw cornstarch

Abstract

Previous data suggest that greater amounts of postruminal protein increase small intestinal starch digestion in cattle. Our objective was to determine if increases in small intestinal starch digestion by casein could be replicated by amino acid supplementation. Thus, we fed 5 duodenally and ileally cannulated steers a low-starch soybean hull-based diet and provided continuous duodenal infusion of raw cornstarch in combination with AA or casein in order to measure response of small intestinal starch digestion. Duodenal infusion of casein (400 g/d) increased ($P \leq 0.05$) small intestinal starch digestion. When a mixture of amino acids with a profile similar to casein (CASAA) was infused, small intestinal starch digestion was similar ($P = 0.30$) to casein infusion. Infusion of only non-essential amino acids tended to increase ($P = 0.14$) small intestinal starch digestion relative to control; however, infusion of essential amino acids alone did not affect ($P = 0.84$) small intestinal starch digestion. Additionally, infusion of 400 g/d casein or CASAA increased ileal flows of ethanol-soluble starch (small chain α-glycosides), but non-essential amino acids alone were not different than the negative control. Duodenal infusion of 133 g/d Glu increased ($P \leq 0.05$) small intestinal starch digestion, whereas a mixture of Phe, Trp, and Met (30.4, 6.5, and 17.5 g/d, respectively; PTM) did not. Neither Glu nor PTM increased ileal flow of ethanol-soluble starch, but Glu and PTM provided together tended ($P = 0.07$) to increase ileal flows of small chain α-glycosides. Our data suggest that Glu alone can increase small intestinal starch digestion in cattle similar to casein, but increases in small intestinal starch digestion in response to Glu are not associated with an increase in ileal flows of small chain α-glycosides.

Introduction

Limitations to efficient production of animal products (i.e., meat and milk) by cattle are often associated with dietary restrictions in net energy to support physiologically productive purposes (Lofgreen and Garrett, 1968; Fox et al., 1992; NRC, 1996). Utilization of dietary energy from α-glucosides (starch) is potentially limited by starch assimilation from the alimentary tract in cattle, and a precise understanding of digestion of carbohydrates in the small intestine of cattle remains equivocal (Huntington, 1997; Huntington et al., 2006; Harmon, 2009). Thus, most ruminant nutritionists currently adopt feeding strategies designed to increase ruminal starch fermentation to improve production efficiencies (Vasconcelos and Gaylean, 2007). However, increases in fermentable energy supply can lead to metabolic disorders and reductions in dry matter intake (Krehbiel et al., 1995; Owens et al., 1998; Nagaraja and Titgemeyer, 2007), and energy derived by cattle subsequent to fermentation is less than that derived when glucose is absorbed intestinally (Harmon and McLeod, 2001). Thus, improving small intestinal digestion of starch in cattle may provide great benefit to cattle production systems.

Unfortunately, dietary starch per se apparently reduces secretion of pancreatic enzymes associated with its hydrolysis and subsequent digestion in cattle (Kreikemeier et al., 1990; Swanson et al., 2002; Swanson et al., 2004). However, several reports have demonstrated that greater flow of protein to the small intestine of cattle can increase small intestinal starch digestion (Streeter and Mathis, 1995; Richards et al., 2002; chapter 2 of this dissertation), portal glucose appearance (Taniguichi et al., 1995), and pancreatic α-amylase secretion (Richards et al., 2003). Many studies that evaluated effects of small intestinal protein flows on small intestinal starch digestion and pancreatic α-amylase secretions have used casein, and as a result, little is known of the effects of protein quality or amino acid flow on small intestinal starch digestion.

Indeed, recent reports (Swanson et al., 2004; Liao et al., 2009) suggest that interactions between supplemental proteins and pancreatic α-amylase might be dynamic and that requirements for nutritionally functional amino acids to optimize pancreatic secretions may be proportional to starch appearing in the lumen of the small intestine in cattle.

Our studies were designed to evaluate effects of amino acids on small intestinal starch digestion in cattle. We hypothesized that amino acids were largely responsible for the apparent effects of postruminal protein flows on small intestinal starch digestion in cattle and that responses may be elicited by either groups or individual amino acids found in casein.

Materials and Methods

Experiment 1

Five duodenally and ileally cannulated steers (average initial BW = 259 ± 8.9 kg) were placed in a 5 × 5 Latin square with 6-d periods. Animals were tethered to tie-stalls (1.7 × 1.2 m) in a temperature controlled room (20°C) under 16 h of light (0500 to 2100) and 8 h dark. Cattle were provided ad libitum access to water and limit-fed 4.8 kg/d (DM basis) of a soybean hull-based diet (Table 3.1; about 1.5 × maintenance energy requirement; NRC, 1996).

Treatments were continuous duodenal infusion of raw cornstarch alone (1,529 ± 11 g/d) or cornstarch plus either casein (424 ± 3.0 g/d), crystalline AA similar in amount and AA composition to the casein (CASAA; Table 3.2), non-essential crystalline AA similar to that provided by casein (NEAA; Table 3.2), or essential crystalline AA similar to that provided by casein (EAA; Table 3.2). Glutamine and Asn were excluded from AA infusions. Amounts of Glu were similar to the casein content of both Glu plus Gln, whereas Asp was similar to Asp plus Asn in casein. Because of the limited solubility of Tyr, it was excluded from suspensions.

Cattle were infused through Tygon tubing (i.d. = 2.38 mm; Saint-Gobain North America, Valley Forge, PA) with a peristaltic pump (Model CP-78002-10; Cole-Parmer Instrument Company, Vernon Hills, IL) with 12.6 L/d of treatment suspensions. Suspensions (2 containers of 7 L) were prepared daily immediately prior to infusion for use over 12-h intervals. Cornstarch was maintained in suspension with continuous stirring (Arrow 6000, Arrow Engineering Company, Hillsdale, NJ) during infusion, and the pumping rate was 525 mL/h. Cornstarch suspensions contained CrEDTA (6.4 g/d; Binnerts et al., 1968) which served as an indigestible marker, 800 g of raw cornstarch (Pure food powder cornstarch; Tate and Lyle Ingredients North Americas, Decatur, IL) and deionized H_2O to prepare to the final solution weight. The pH of

suspensions containing CASAA and NEAA were adjusted to near 7 with addition of 39.5 g NaOH (40% wt/wt). Each infusate was prepared daily by weight and the exact amount infused was determined by recording the weight of residual infusate after each 12-h infusion period. Suspensions were prepared in slight excess so that infusions were continuous; this led to some variation in total infusion amounts. Infusate containers, the peristaltic pump, and infusion lines were above the cattle, and containers were exchanged twice daily (0700 and 1900 h). Additionally, every 48 h tap water was flushed through the lines (approximately 100 mL) to prevent accumulation of residual infusate in the infusion lines.

On d 6 of each period, spot samples of ileal digesta and feces were collected 2, 4, 6, 8, 10, and 12 h after the 0700 h feeding. Samples of ileal digesta (200 g) were collected by attaching a plastic bag to the cannula (140 × 229 mm) and feces (200 g) was collected directly from the rectum via stimulation with a gloved hand. Immediately after sampling, pH of ileal samples were measured with a mobile pH meter (Portable Meter Model 250A, Orion Research, Beverley, MA). The pH of feces was determined after mixing an aliquot (~5 g) of feces with 15 mL of deionized H_2O. To preserve starch content of digesta and feces, α-amylase was deactivated immediately after measuring pH by increasing sample pH to near 11 by mixing samples with 2.1 mL of 40% (wt/wt) NaOH. Samples were composited and frozen (-20°C) between collections.

Composite digesta and feces were thawed at room temperature (22°C) prior to analysis. For analyses of DM, starch, glucose, ethanol-soluble starch, and organic acids, pH of an aliquot of previously alkalized ileal digesta (75 g) or wet feces (50 g with addition of 25 mL H_2O) was neutralized with an 1.0 to 1.5 mL of 6 *M* HCl. A portion wet ileal digesta or wet feces (50 g with addition of 25 mL H_2O) was analyzed for DM. Dry matter was determined by drying at 105°C

for 24 h. Starch concentrations were subsequently measured using the glucogenic assay described by Herrera-Saldana and Huber (1989) with glucose quantified using glucose oxidase linked assay (Gochmann and Schmitz, 1972). Unpolymerized glucose was determined from assay tubes to which no enzyme was added. Ethanol-soluble starch was determined using techniques similar to those described by Kreikemeier and Harmon (1995). Briefly, a portion of wet digesta or feces (with added H_2O) was centrifuged (20,000 × *g*; 15 min; 4°C). Following centrifugation, supernatant was collected and 0.5 mL transferred to a microcentrifuge tube containing 1.25 mL of anhydrous ethanol. Samples were kept overnight (about 16 h) at 4°C and then centrifuged (17,000 × *g*; 10 min; 4°C), and supernatant decanted in another microcentrifuge tube. The precipitate was subsequently resuspended in 1 mL of ethanol and centrifuged. This rinsing procedure was conducted for a total of 3 times and the resulting supernatant from each rinse was pooled with supernatant collected from the overnight incubation. Ethanol was then evaporated from the pooled supernatant and starch content measured using procedures previously described.

Samples were analyzed for Cr concentration with atomic absorption from supernatant collected after centrifugation (20,000 × *g*; 15 min; 4°C). Also, a portion of supernatant was retained, and 1 mL was mixed with 0.25 mL of 25% (wt/vol) meta-phosphoric acid and analyzed for VFA and lactic acid by GLC as described by DeFrain et al. (2002).

Thirty minutes prior to the final digesta sampling (11.5 h after feeding), jugular blood (20 mL) was collected by venipuncture into glass vacuum tubes (16 × 100 mm; Monoject Blood Collection Tubes, Sherwood Medical, St. Louis, MO) containing sodium heparin isolated from porcine mucosa (143 USP) to allow measures of circulating glucose, cholecystokinin (CCK), α-amino-N, and urea-N concentrations. Blood was immediately placed on ice, and plasma was

harvested by centrifugation (2,200 × *g*; 15 min; 4°C). Plasma was transferred to plastic tubes (Fisherbrand Premium 2.0 mL MCT Graduated Natural, Fisher Scientific), and aprotinin (500 KIU per mL plasma) was added to a portion of plasma (3 mL). Plasma samples were immediately frozen (-20°C), and plasma containing aprotinin was vortexed for 10 sec prior to freezing (-80°C). Plasma CCK was analyzed with the double antibody radioimmunoassay described by Benson and Reynolds (2001). Plasma urea (Marsh et al., 1965), plasma glucose (Gochman and Schmitz, 1972), plasma α-amino-N (Palmer and Peters, 1969) were measured with an AutoAnalyzer (Technicon Analyzer II, Technicon Industrial Systems, Buffalo Grove, IL).

Samples of the diet (200 g/d) were collected 1 and 2 d prior to sampling of digesta and feces, composited and stored at room temperature (22°C) until analysis. Feed samples were ground to pass a 1-mm screen (Thomas-Wiley Laboratory Mill Model 4, Thomas Scientific USA, Swedesboro, NJ). Dry matter content was determined by drying samples at 105°C for 24 h in a forced-air oven, and OM was subsequently determined by ashing in a muffle furnace for 8 h at 450°C. The technique of Van Soest et al. (1991) was used to quantify NDF (with α-amylase and sodium sulfite). Nitrogen content of feed was determined through combustion (Nitrogen Analyzer Model FP-2000, Leco Corporation, St. Joseph, MI), and CP was calculated as 6.25 × N.

Experiment 2

This experiment was conducted to measure response of small intestinal starch digestion in cattle either to Glu or to Phe, Trp, and Met (PTM), or to a combination of Glu and PTM (Glu+PTM) in amounts 1.5-times those contained in 400 g casein (Table 3.3). Infusions of raw

cornstarch alone (1,571 ± 5.4 g/d) and raw cornstarch with casein (436 ± 1.5 g/d) served as negative and positive controls. The pH of suspensions containing Glu were adjusted to near 7 with addition of 40.0 g NaOH (40% wt/wt).

Five duodenally and ileally cannulated steers (average initial BW = 361 kg) were used in a 5 × 5 Latin square with 6-d periods. Animals were limit-fed 5.5 kg/d (DM basis) of the same diet and housed under identical conditions as Exp. 1.

Cattle infusions, treatment suspensions, sampling and sample analyses were identical to those methods previously described (*Experiment 1*).

Calculations

Digesta flow at the ileum and feces in Exp. 2 and Exp. 3 were calculated as described by Kreikemeier and Harmon (1995). These calculations were as follows:

$$\text{Ileal or fecal fluid flow (g/d)} = \frac{duodenal\ infusion\ rate\ of\ Cr\ (mg/d)}{Cr\ concentration\ in\ ileal\ or\ fecal\ fluid\ (mg/g)}$$

$$\text{Ileal or fecal total digesta flow (g/d)} = \frac{fluid\ flow\ (g/d)}{(1-digesta\ DM\ concentration)}$$

$$\text{Ileal or fecal DM flow} = total\ digesta\ flow - fluid\ flow$$

Flows of ethanol-soluble starch and organic acids were calculated as the product of fluid flow and nutrient concentration in supernatant. Starch and free glucose flows were calculated as the product of DM flow and starch (or free glucose) concentration (DM basis). Small intestinal starch digestibility was calculated as:

$$\text{Small intestinal starch digestibility} = 1 - \left(\frac{ileal\ starch\ flow\ (g/d)}{starch\ infused\ on\ the\ day\ of\ sampling\ (g/d)}\right)$$

Statistical Analyses

Data within each experiment were analyzed using the MIXED procedure of SAS. Fixed effects included treatment and period, and steer was included as a random effect. The LSMEANS option was used to calculate treatment means. For Exp. 1, when the *F*-statistic for treatment was significant ($P \leq 0.05$) all pair-wise comparisons of least squares means were evaluated using the PDIFF option. For Exp. 2, effects of Glu and PTM were analyzed by treatment contrasts that considered Glu and PTM as a 2 × 2 factorial; the positive control (casein) was compared to the negative control by a *t*-test. For pH data of digesta and feces, fixed effects were treatment, period, time and time × treatment, and steer was included as a random effect. The repeated term was time, with steer×period serving as the subject. The covariance structure was autoregressive(1).

Results

Experiment 1

Nutrient flows and small intestinal starch digestibilities are reported in Table 3.3. Small intestinal starch digestion was different among treatments ($P = 0.05$). When CASAA was infused small intestinal starch digestion was greater ($P < 0.01$) than control, and both casein ($P = 0.07$) and NEAA ($P = 0.14$) tended to increase small intestinal starch digestion relative to control; however, when cattle received EAA small intestinal starch digestion was not different ($P = 0.84$) than the control. Additionally, flows of ethanol-soluble starch (small-chain α-glycosides) to the ileum were affected ($P = 0.01$) by treatment. Ethanol-soluble starch flows were not different ($P = 0.83$) than control when NEAA was infused, but infusion of casein ($P = 0.03$) and EAA ($P <$

0.01) increased ileal flow of ethanol-soluble starch in comparison to control. Ethanol-soluble starch flows were intermediate for CASAA and least for control and NEAA. Ileal flows of unpolymerized glucose were not different among treatments ($P = 0.26$). We detected a tendency ($P = 0.07$) for differences among circulating concentrations of glucose (Table 3.4). Infusions of casein ($P = 0.05$), CASAA ($P = 0.05$), and EAA ($P = 0.11$) tended to increase plasma glucose relative to control, but NEAA ($P = 0.87$) did not. Also, the overall treatment effect for plasma CCK concentrations (Table 3.4) tended to be present ($P = 0.08$), but none of the treatments were different than the control ($P \geq 0.17$).

Experiment 2

Flows of starch to the ileum were decreased when Glu ($P < 0.01$) or casein ($P = 0.02$) were infused, but were not affected by PTM (Table 3.5). Similarly, both Glu ($P < 0.01$) and casein ($P = 0.02$) increased apparent small intestinal digestion of starch, but small intestinal starch digestion was not affected by PTM. We observed a tendency ($P = 0.07$) for an interaction between Glu and PTM among ileal flows of ethanol-soluble starch. Infusion of Glu ($P = 0.86$) or PTM ($P = 0.97$) alone did not affect ileal flow of ethanol-soluble starch in comparison to control, but Glu + PTM increased ($P = 0.01$) ileal flow of ethanol-soluble starch in relation to control. Circulating levels of glucose were increased ($P < 0.01$) by Glu, but were not affected ($P \geq 0.11$) by PTM or casein.

Discussion

Starch digestion occurs by similar means in both ruminants and nonruminants, and its digestion can be separated into 3 independent segments (Huntington, 1997): 1) hydrolysis by α-amylase to smaller oligosaccharides, 2) release of glucose from oligosaccharides by α-

glycohydrolases closely associated with intestinal epithelium, and 3) transport of glucose from the lumen by transmembrane glucose transporters of intestinal epithelium. Flows of ethanol-soluble starch to the terminal ileum provide indirect information about the relative activities of luminal pancreatic α-amylase and α-glycohydrolases closely associated with enterocytes (Kreikemeier et al., 1995); α-amylase produces the short-chain α-glycosides that comprise ethanol-soluble starch, whereas luminally anchored α-glycohydrolases hydrolyze short-chain α-glycosides to unpolymerized glucose. Infusion of casein and Glu + PTM increased small intestinal starch digestion and ileal flows of ethanol-soluble starch. This suggests that casein increased hydrolysis of starch by both pancreatic α-amylase and luminally anchored α-glycohydrolases; however, increases in ethanol-soluble carbohydrate flows suggest that hydrolysis of starch via α-amylase is increased by casein to a greater extent than the short-chain α-glycoside hydrolyzing capacity of the luminally anchored α-glycohydrolases.

The apparently disparate response in ethanol-soluble starch flows ($P \leq 0.05$) as well as a numeric difference ($P = 0.19$) in small intestinal starch digestion between NEAA and EAA was unexpected. In nonruminants, optimal pancreatic α-amylase secretion depends on a combination of carbohydrate and AA content flowing to the duodenum (Snook, 1971; Johnson et al., 1977; Riepl et al., 1996). Snook et al. (1971) reported that pancreatic α-amylase secretions were increased when up to 30% of the diet was replaced with casein in rats. Johnson et al. (1977) fed rats diets with containing zein or casein, and they observed greater pancreatic enzyme secretions when casein was fed. Additionally, Johnson et al. (1977) noted that when zein was reconstituted with AA, to resemble casein, responses in enzymatic secretions of pancreata were similar to when casein was provided. Thus, Johnson et al. (1977) concluded that high-quality protein flow to the small intestine was essential for optimal enzymatic secretions of pancreata. Schick et al.

(1984) replaced dietary carbohydrate with casein in rat diets and observed that responses in α-amylase secretion were quadratic and greatest when casein composed 22% of the diet. Therefore, Schick et al. (1984) concluded that a proportional relationship was required between small intestinal carbohydrate and AA flow for optimal starch digestion, and they speculated that this response was likely conserved across many species. Indeed, similar responses have been reported in man (Riepl et al., 1996). Studies in cattle have reported a positive association between increased duodenal protein as casein (Richards et al., 2002; chapter 2 of this dissertation) or fishmeal (Streeter and Mathis, 1995) and small intestinal starch digestion and pancreatic α-amylase secretion (Richards et al., 2003) in cattle. Swanson et al. (2008) fed increasing levels of ruminal-escape soybean meal and reported increased pancreatic α-amylase activity in removed pancreata, and they speculated that greater amounts of postruminal protein led to greater rates and amounts of pancreatic enzyme secretions.

Interestingly, small intestinal starch digestion was numerically greater ($P = 0.19$) when cattle were duodenally infused with NEAA rather than EAA, but ethanol-soluble starch flows were not different than the control when NEAA were infused. We interpret the increase in ethanol-soluble starch flow and lack of response in small intestinal starch digestion in response to EAA to suggest that luminal hydrolysis of starch via pancreatic α-amylase was increased by EAA, but small intestinal starch digestion was limited by hydrolysis of small-chain α-glycohydrolases. Swanson et al. (2003) noted that pancreatic tissue from cattle with greater postruminal flows of casein had increased responsiveness to CCK- and acetylcholine-agonists. Hara et al. (2001) reported in rats that non-essential AA play an integral role in secretion of α-amylase, and specific non-essential AA can be essential for optimal activity of α-glycohydrolases anchored to intestinal epithelium (Schroder et al., 1995; Horvath et al., 1996;

Weiss et al., 1999). Quezada-Calvillo et al. (2007) reported that luminal hydrolysis of starch via pancreatic α-amylase is inhibited by increasing concentration of small chain α-glycosides. The increase in ileal flows of ethanol-soluble carbohydrate but lack of response in small intestinal starch digestibility in cattle infused with EAA may be explained by increased secretions of pancreatic α-amylase. Yet, a lack of response in small intestinal starch digestion may have resulted from an increasing concentration of ethanol-soluble carbohydrate in the small intestinal lumen if activity of intestinally anchored α-glycohydrolases was not increased by EAA. Clearly, pancreatic α-amylase and small intestinal α-glycohydrolases are responsive to luminal nutrient flows, and these responses are apparently complex.

Infusion of Glu alone, Glu + PTM, and casein increased small intestinal starch digestion. Additionally, flows of ethanol-soluble starch were not different for control, PTM, and Glu, but Glu + PTM had the greatest flows of small chain α-glycosides to the terminal ileum. Harmon (1992) reviewed the available literature and concluded that α-glycohydrolases activity associated with intestinal epithelium was responsive to dietary energy rather than substrate, and that responses of cattle were less than their nonruminant counterparts. It is well known that Gln, Glu, and Asp are key metabolic fuels for small intestinal mucosa (Windmueller and Spaeth, 1975, 1976; Wu, 1998). Indeed, Harmon (2009) indicated that greater intestinal flows of protein apparently increase small intestinal starch digestion in cattle. Our data seem to agree with this conclusion, and indicate that 132 g/d of Glu affected small intestinal starch digestion similar to 400 g/d of casein. Yet, hydrolysis of small chain α-glycosides was apparently increased (measured as decrease in flow of ethanol-soluble starch to the ileum) 32% by NEAA compared to EAA, which seems to suggest an appreciable response to non-essential AA in hydrolytic capacity of cattle small intestine for small chain α-glycosides.

In summary, casein and EAA appeared to increase pancreatic α-amylase secretion, but EAA did not increase small intestinal starch digestion. In contrast, NEAA and Glu increased small intestinal starch digestion, but not flows of small-chain α-glycosides. A possible explanation for these observations is that EAA may have affected pancreatic α-amylase but not small intestinal α-glycohydrolases anchored to enterocytes. Indeed, when Harmon (1992) reviewed the available literature on energy supplementation to the small intestine of cattle, he concluded that responses among intestinally anchored α-glycohydrolases in cattle were limited and that small intestinal hydrolytic capacity of small-chain α-glycosides in cattle may be more closely associated with increases in small intestinal length. We did not measure small intestinal length, but we are skeptical that appreciable differences in small intestinal length occurred within these studies. Thus, a possible explanation for effects of NEAA and Glu may be that NEAA and Glu may have increased hydrolysis of small-chain α-glycosides by affecting anchored α-glycohydrolases. An increase in the small intestinal hydrolytic capacity of small-chain α-glycosides may have indirectly facilitated greater hydrolytic activity of α-amylase or NEAA and Glu may have increased pancreatic α-amylase secretions in cattle.

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Table 3.1 Composition of soybean hull-based diet fed to steers

Item	% of DM
Ingredient	
Soybean hulls	72.2
Brome hay, late-bloom	20.0
Corn steep liquor	6.0
Limestone	1.0
Salt	0.5
Mineral and vitamin premix[1]	0.3

Chemical composition	*Experiment 1*	SEM	*Experiment 2*	SEM
DM[2], %	88.6	0.38	88.9	0.27
OM[2], % DM	92.2	0.17	92.1	0.09
CP[2], % DM	14.5	0.06	13.8	0.05
Starch[2], % DM	1.8	0.06	2.1	0.05
RDP[3,4], % CP	59.1	—	59.1	—
NE_m[3,5], Mcal/kg	1.7	—	1.7	—

[1]Provided to diets (per kg diet DM): 50 mg Mn, 50 mg Zn, 10 mg Cu, 0.5 mg I, 0.2 mg Se, 2,200 IU of vitamin A, 275 IU of vitamin D, and 25 IU of vitamin E.
[2]Based on laboratory analyses.
[3]Calculated using the tabular values of NRC (1996).
[4]Ruminally degradable protein.
[5]Net energy available for maintenance.

Table 3.2 Crystalline AA treatments in *Exp. 1* and *Exp. 2*

	Exp. 1			Exp. 2	
L-AA, g/d	CASAA[1]	NEAA[1]	EAA[1]	Glu[2]	PTM[2]
Asp	28.9	28.9	—	—	—
Thr	17.0	—	17.0	—	—
Ser	22.2	22.2	—	—	—
Glu	86.2	86.2	—	133	—
Pro	41.5	41.5	—	—	—
Lys[3]	31.3	—	31.3	—	—
Gly	7.3	7.3	—	—	—
Ala	12.2	12.2	—	—	—
Val	25.2	—	25.2	—	—
Met	11.3	—	11.3	—	17.5
Ile	19.3	—	19.3	—	—
Leu	38.2	—	38.2	—	—
Phe	19.7	—	19.7	—	30.4
His[4]	11.3	—	11.3	—	—
Arg	14.9	—	14.9	—	—
Cys	1.9	1.9	—	—	—
Trp	4.2	—	4.2	—	6.5

[1]Amounts provided are those infused when 1,529 g/d of cornstarch was infused.
[2]Amounts provided are those infused when 1,571 g/d of cornstarch was infused.
[3]Provided as Lys-HCl.
[4]Provided as His-HCl-H_2O.

Table 3.3 Effect of duodenal infusion of AA or casein on nutrients flowing to the ileum of cattle receiving 1.5 kg/d duodenally infused raw cornstarch (Exp. 1)

	Treatment[1]						
Item	Control	EAA	NEAA	CASAA	Casein	SEM	*P*
No. of observations	5	5	5	5	5		
Duodenal starch infused, g/d	1,499	1,542	1,547	1,535	1,520	19	—
Ileal DM content, %	14.6	15.0	15.4	15.4	16.0	0.59	0.53
Ileal pH	6.92[a]	6.87[a]	6.84[ab]	6.80[ab]	6.72[b]	0.05	0.04
Nutrient flow to ileum, g/d							
DM	3,364	3,213	3,195	2,828	3,020	118	0.06
Starch	882	895	817	742	788	44	0.08
Ethanol-soluble starch	178[a]	240[b]	182[a]	209[ab]	219[b]	13	0.01
Glucose	47	50	79	55	76	16	0.26
Acetate	28.3	28.5	28.5	23.8	25.0	3.7	0.80
Propionate	1.4	1.7	1.4	1.1	1.1	0.36	0.70
Butyrate	2.3	2.0	2.4	1.7	2.0	0.50	0.74
Lactate	41.6	43.6	40.5	33.6	41.0	4.9	0.30
Small intestinal starch digestion, %	41.2[a]	41.9[a]	46.8[ab]	52.0[b]	48.2[ab]	2.8	0.05

[1]EAA = 194 g/d AA in profile similar to essential AA of casein; NEAA = 203 g/d AA in profile similar to non-essential AA in casein; CASAA = 394 g/d AA in profile similar to casein; Casein = 424 g/d casein.

Table 3.4 Effect of duodenal infusion of AA or casein on plasma concentrations of circulating metabolites and cholecystokinin in cattle receiving 1.5 kg/d duodenally infused raw cornstarch (Exp. 1)

	Treatment[1]						
Plasma	Control	EAA	NEAA	CASAA	Casein	SEM	*P*
No. of observations	5	5	5	5	5		
Cholecystokinin, pmol/L	7.6	4.8	8.2	9.9	4.7	1.65	0.08
Glucose, mmol/L	4.7	4.9	4.7	4.9	4.9	0.16	0.07

[1]EAA = 194 g/d AA in profile similar to essential AA of casein; NEAA = 203 g/d AA in profile similar to non-essential AA in casein; CASAA = 394 g/d AA in profile similar to casein; Casein = 424 g/d casein.

Table 3.5 Effect of duodenal infusion of AA or casein on ileal nutrient flows and small intestinal (SI) starch digestion in steers receiving 1.5 kg/d duodenally infused raw cornstarch (Exp. 2)

	Treatment[1]						Contrast[2]			
Item	Control	PTM	Glu	Glu + PTM	Casein	SEM	Glu	PTM	Glu × PTM	Casein vs. Control
No. of observations	5	5	5	5	5					
Duodenal starch infused, g/d	1,569	1,577	1,558	1,573	1,579	12.6	0.52	0.31	0.78	0.53
Ileal DM content, %	14.3	14.2	14.0	14.4	14.5	0.41	0.86	0.81	0.48	0.81
Ileal pH	6.69	6.72	6.72	6.73	6.62	0.03	0.47	0.49	0.73	0.05
Nutrient flow to ileum, g/d										
DM	3,433	3,533	3,680	3,648	3,833	191	0.36	0.86	0.73	0.16
Starch	879	829	727	759	752	41	<0.01	0.79	0.22	0.02
Ethanol-soluble starch	134	134	136	177	164	21	0.05	0.06	0.07	0.06
Glucose	15.0	18.3	17.4	17.8	13.7	2.9	0.71	0.48	0.56	0.72
Acetate	26.7	26.0	25.0	25.6	29.7	1.9	0.48	0.99	0.63	0.17
Propionate	1.3	0.9	1.0	1.0	1.0	0.18	0.47	0.37	0.16	0.23
Butyrate	2.5	2.7	2.4	2.4	2.5	0.50	0.60	0.76	0.76	0.92
Lactate	33.9	34.0	35.5	33.6	35.0	2.3	0.80	0.69	0.64	0.72
Total organic acid	64.8	63.9	64.1	63.0	68.5	3.7	0.80	0.76	0.96	0.43
SI starch digestion[2], %	44.1	47.4	53.4	51.8	52.4	2.6	<0.01	0.70	0.28	0.02

[1]PTM = 55 g/d AA in profile similar to Phe, Trp, and Met of casein; Glu = 132 g/d Glu; Casein = 436 g/d casein.
[2]Glu = (Glu and Glu + PTM) vs. (Control and PTM); PTM = (PTM and Glu + PTM) vs. (Control and Glu).

Table 3.6 Effect of duodenal infusion of AA or casein on plasma of steers receiving 1.5 kg/d duodenally infused raw cornstarch (Exp. 2)

	Treatment[1]						Contrast[2]			
Plasma	Control	PTM	Glu	Glu + PTM	Casein	SEM	Glu	PTM	Glu × PTM	Casein vs. Control
No. of observations	5	5	5	5	5					
Cholecystokinin, pmol/L	6.5	6.3	6.5	7.3	7.2	1.37	0.57	0.76	0.61	0.57
Glucose, mmol/L	4.29	4.37	4.44	4.53	4.40	0.13	<0.01	0.11	0.92	0.14

[1]PTM = 55 g/d AA in profile similar to Phe, Trp, and Met of casein; Glu = 132 g/d Glu; Casein = 436 g/d casein.
[2]Glu = (Glu and Glu + PTM) vs. (Control and PTM); PTM = (PTM and Glu + PTM) vs. (Control and Glu).