BIOLOGICAL ABNORMALITIES IN THE RUMINANT SMALL INTESTINE AND ITS RELATIONSHIP TO CARBOHYDRATE ASSIMILATION

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MASTER OF SCIENCE

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ABSTRACT

Several biological abnormalities exist between the ruminant and nonruminant small intestine and influences carbohydrate assimilation. Two experiments were conducted to identify potential mechanisms to improve carbohydrate utilization in cattle. Experiment 1 evaluated the effects of duodenal starch infusions with casein or glutamic acid on post-ruminal carbohydrase activities. Experiment 2 evaluated the effects of dietary fructose on visceral organ development and expression of nutrient transporters and digestive enzymes involved in carbohydrate assimilation. In experiment 1, the results suggest that small intestinal starch digestion may be improved in cattle with increased small intestinal flow of casein through increases in post-ruminal carbohydrase activities. In experiment 2, dietary fructose supply influenced nutrient utilization, visceral organ growth, and digestive enzyme mRNA expression and activity in neonatal calves.

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LIST OF ABBREVIATIONS

ACTB.....Beta-Actin

ADF.....Acid Detergent Fiber

ADG.....Average Daily Gain

ANOVA.....Analysis of Variance

AOAC.....Association of Official Analytical Chemists

ATP.....Adenosine Triphosphate

BCA.....Bichinchonic Acid

BW.....Body Weight

C.....Celsius

Ca.....Calcium

cAMP.....Cyclic Adenosine Monophosphate

cm.....Centimeter

CO_2.....Carbon Dioxide

CCK.....Cholecystokinin

cDNA.....Complementary DNA

CF.....Crude Fat

CP.....Crude Protein

CrEDTA.....Chromium Ethylenediaminetetraacetic Acid

CV.....Coefficient of Variation

d.....Day

dL.....Deciliter

Dist. Jej. .....Distal Jejunum

DM.....Dry Matter

DNA.....Deoxyribonucleic Acid

Duod.....Duodenum

FAM.....6-Carboxyfluorescein

g.....Gram

g.....Gravity

GAPDH.....Glyceraldehyde-3-Phosphate Dehydrogenase

G:F .....Gain to Feed

GLM.....General Linear Model

GLUT2.....Glucose transporter 2

GLUT5.....Glucose transporter 5

h.....Hour

H_2O .....Water

IU .....International Unit

kg.....Kilogram

KHK .....Ketohexokinase

KPO_4 .....Potassium Phosphate

kU.....Kilounit

L .....Liter

L-.....L-isomer

LCT .....Lactase

m .....Meter

mg .....Milligram

min .....Minute

mL .....Milliliter

mM.....Millimolar

MGAM.....Maltase-glucoamylase

MGB .....Minor Groove Binder

MP.....Metabolizable Protein

mRNA.....Messenger Ribonucleic Acid

M-value.....Average Expression Stability

n.....Number

N.....Nitrogen

NaCl.....Sodium Chloride

NaOH.....Sodium Hydroxide

NASEM.....National Academy of Science, Engineering, & Math

NCBI.....National Center for Biotechnology Information

NDF.....Neutral Detergent Fiber

NEFA.....Non-Esterified Fatty Acid

NEm.....Net Energy for Maintenance

ng.....Nanogram

nm.....Nanometer

NRC.....National Research Council

OM.....Organic Matter

P.....Phosphorus

PACAP.....Pituitary Adenylate Cyclase-Activating Peptide

PCR.....Polymerase Chain Reaction

PDV.....Portal-Drained Viscera

POLR2A.....RNA Polymerase IIA

P-value.....Probability Value

Prox. Jej.....Proximal Jejunum

RDP.....Ruminally Degradable Protein

RNA .....Ribonucleic Acid

s .....Second

SAS .....Statistical Analysis System

SDHA.....Succinate Dehydrogenase Complex Flavoprotein Subunit A

SEM .....Standard Error of the Mean

SGLT1.....Sodium-Dependent Glucose Cotransporter-1

SI .....Sucrase-Isomaltase

SLC2A2 .....GLUT2

SLC2A5 .....GLUT5

SLC5A1 .....SGLT1

T1R2 .....Taste 1 Receptor 2

T1R3 .....Taste 1 Receptor 3

U.....Unit

µL.....Microliter

µmol .....Micromole

Urea-N.....Urea Nitrogen

UV-VIS .....Ultraviolet-Visible

vs .....Versus

VIP .....Vasoactive Intestinal Peptide

wt/wt .....Weight by Weight

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The ruminant digestive system is anatomically more complex than nonruminants because of their foregut structure containing the rumen, reticulum, and omasum. The reticulorumen allows for digestion of structural carbohydrates that are typically unsuitable for most nonruminant diets. Digestion products of ruminal fermentation are typically volatile fatty acids including acetate, propionate, and butyrate which are used as the primary energy source by the host. Also, ruminal microbial crude protein synthesis from non-protein nitrogen sources offers a clear advantage when formulating diets. After the omasum, the ruminant and nonruminant digestive tracts are similar in structure with the abomasum (gastric compartment), small and large intestines. Shifting the site of carbohydrate digestion and absorption from the rumen to the small intestine can provide energetic advantages because glucose can be used more efficiently and provide more net ATP production than volatile fatty acids. However, several studies have demonstrated that post-ruminal carbohydrate assimilation is functionally different in ruminant than in nonruminant animals. This includes extent of carbohydrate digestion, regulation of digestive enzymes, digesta passage rate and composition, and glucose absorption and transport. This review will highlight the biological abnormalities in the ruminant small intestine and its relationship to carbohydrate assimilation.

1.2. Literature review

1.2.1. Small intestinal carbohydrate digestion

In early nutrition studies in ruminants, various dietary carbohydrates were evaluated in young calves and lambs to determine if there could be potential benefits from their dietary inclusion. Typically, ruminants can be exposed to various carbohydrates at different stages of their production life cycle. This includes glucose (gluconeogenic or dietary origin; Brockman and

Laarveld, 1986), lactose (from milk), galactose (from digestion of lactose), sucrose, starch and starch digestion-products (maltose, isomaltose, limit dextrins), and fructose (during fetal development; Crouse et al., 2019). A common finding between most studies is that ruminants readily utilize glucose, galactose, and lactose but not sucrose, maltose, or starch (Dollar and Porter, 1957; Siddons et al., 1969). Understanding the limitations in carbohydrate assimilation can potentially provide biological targets to improve nutrient utilization and the energetic efficiency of animal production.

In North American finishing cattle and dairy cattle production systems, grain-based diets containing moderate to large proportions of starch are typically fed to increase the net energy concentrations of the diet allowing for more efficient growth and improved product quality. When grain-based diets are fed, up to 40% of dietary starch intake can escape ruminal fermentation and flow to the small intestine for potential enzymatic digestion (Ørskov et al., 1986). Small intestinal starch assimilation occurs in three distinct steps (Huntington et al., 1997): 1) hydrolysis of starch by pancreatic α-amylase into smaller oligosaccharides and limit-dextrins, 2) hydrolysis of small chain oligosaccharides into free glucose by brush border carbohydrases, and 3) glucose transport from the intestinal lumen into an absorptive enterocyte. Initiation of starch digestion in the small intestine begins with pancreatic α-amylase that is secreted into the intestinal lumen via the pancreatic duct. Pancreatic α-amylase is produced in pancreatic acinar cells and is secreted in its active form. In the lumen, pancreatic α-amylase hydrolyzes α-1,4-linked resident glucose molecules in starch and releases smaller oligosaccharides (maltose, maltotriose, and limit-dextrins) (Brake and Swanson, 2018). Membrane-bound brush border carbohydrases (maltase, isomaltase, glucoamylase) hydrolyze small-chain oligosaccharides to release free glucose that is ultimately transported across the apical membrane of the small intestine.

Early work using dietary (Karr et al., 1966; Tucker et al., 1968) or abomasal infusion models (Little et al., 1968) demonstrated that the extent of post-ruminal starch digestion was much lower than in nonruminants. These authors concluded that the extent of starch digestion in the small intestine was inadequate for optimum utilization. Indeed, when Harmon et al. (2004) reviewed the literature, they found that the apparent digestibility of starch in the small intestine averaged 62%. Potential limitations of small intestinal starch assimilation include deficient production of carbohydrases, physiochemical characteristics of starch, reduced glucose transport, or inadequate retention time (Owens et al., 1986; Brake and Swanson, 2018). Interestingly, the limitation is proportional to small intestinal starch flow instead of an absolute maximal value (i.e., plateau) (Ørskov, 1976; Theurer, 1986). Linear relationships between intestinal starch appearance and small intestinal starch digestion were first suggested by Ørskov et al. (1969) and a linear regression model was developed to predict small intestinal starch digestion in lambs (Ørskov, 1976). When Owens et al. (1986) reviewed the literature, they found that there was a positive linear relationship between the amount of starch flowing to the small intestine (g/d) and small intestinal starch disappearance (g/d). Furthermore, there is a negative linear relationship between the amount of starch flowing to the small intestine (g/d) and small intestinal starch digestibility (%) (Harmon et al., 2004). Linear relationships are not normally expected in biology (Harmon and McLeod, 2001), including digestion, which typically conforms to a non-linear relationship because of Michaelis-Menten kinetics. It was previously calculated that at least a 70% digestibility of starch in the small intestine was necessary to avoid the inefficiencies associated with large intestinal starch digestion (Huntington et al., 2006). However, a plateau in efficiency may not be achievable under practical feeding conditions (Harmon and McLeod, 2001).

Moreover, several studies have demonstrated that post-ruminal protein or amino acid flow can increase small intestinal starch disappearance in ruminants. Indirect evidence of increased pancreatic α-amylase (Wang and Taniguchi, 1998) and portal glucose appearance (Taniguchi et al., 1995) led to the speculation that post-ruminal protein flow could increase small intestinal starch disappearance. Increasing levels of post-ruminal casein (0 to 200 g/d) infusion resulted in linear improvements in small intestinal starch disappearance (g/d) and digestibility (%) in cattle abomasally infused with raw cornstarch (Richards et al., 2002). Similar results were obtained in sheep receiving abomasal casein infusions consuming a dry-rolled sorghum grain diet (Mendoza and Britton, 2003) or a cracked corn-based diet (Mabjeesh et al., 2003). Later, Brake et al. (2014a) demonstrated that increasing levels of post-ruminal casein (0, 200, or 400 g/d) infusion could increase small intestinal starch digestion in steers duodenally infused with raw cornstarch within 6 d. In a follow-up study, amino acid treatments were used to represent similar proportions of amino acids to those found in casein. Non-essential amino acid infusions (similar to the profile of casein) increased small intestinal starch digestion (Brake et al., 2014b) but essential amino acid infusions did not. This observation was further supported when glutamic acid or glutamic acid + phenylalanine + tryptophan + methionine increased small intestinal starch digestion and the phenylalanine + tryptophan + methionine treatment had no effect. Furthermore, increasing supply (0, 60, 120 g/d) of duodenal glutamic acid increases small intestinal starch digestion to a similar magnitude achieved with 400 g/d of casein (Blom et al., 2016). Other trials with essential amino acids (leucine and phenylalanine) have not found any effects on small intestinal starch digestion in goats (Yu et al., 2014a). These data suggest that the limitations in small intestinal starch digestion may be improved with post-ruminal non-essential amino acid or protein flow.

1.2.2. Regulation of pancreatic and small intestinal carbohydrases

Ruminant animals have essentially the same complement of digestive enzymes in the pancreas and small intestine as nonruminant animals. However, functionality of post-ruminal digestive enzymes have major biological and regulatory differences from nonruminant digestive enzymes. For the purpose of this review, only post-ruminal carbohydrases will be reviewed. Detailed reviews on post-ruminal protease regulation have been previously published (Harmon, 1993). The pancreas synthesizes and secretes α-amylase in the small intestinal lumen to initiate starch hydrolysis. Four proteins in the small intestine possess carbohydrase activity: sucrase-isomaltase, maltase-glucoamylase, lactase, and trehalase.

1.2.2.1. Amount and distribution of carbohydrases in the gastrointestinal tract

Almost all of the carbohydrases involved in carbohydrate digestion are present in the intestinal mucosa and pancreas of ruminants but in less quantities than nonruminant animals (Walker et al., 1959; Hembry et al., 1967; Siddons, 1968; Coombe and Siddons, 1973; Sir Elkatim and Osman, 1982; Harmon, 1993). Ruminants lack salivary α-amylase and intestinal sucrase but it is unclear how the absence of these enzymes influence carbohydrate digestion. It should be noted that cattle and buffalo possess nasolabial amylase (Majeed et al., 1970) and may indirectly provide the functional role of salivary α-amylase in ruminants. Relative concentrations of pancreatic α-amylase and small intestinal maltase and isomaltase in cattle and sheep are much lower compared to nonruminants (Walker, 1959; Siddons, 1968). Glucoamylase activity in the small intestine has been shown to be much lower in nonruminating and ruminating calves and cows compared to pigs, horses, and dogs (Toofanian et al., 1974).

1.2.2.2. Pancreatic α-amylase

In nonruminants, carbohydrase activities typically increase proportional to luminal substrate flow (Brannon, 1990). However, in ruminants, post-ruminal digestive enzymes respond differently to diet and luminal nutrient flows (Harmon, 1993). Russell et al. (1981) were the first to evaluate the effects of diet and energy intake on the regulation of post-ruminal digestive enzyme activity in cattle. They fed either an alfalfa hay diet or a corn and corn-silage based diet at maintenance or a corn corn-silage based diet at 2× or 3× maintenance. At maintenance intake, they found that steers consuming the corn and corn-silage based diet had lower pancreatic α-amylase specific activity than steers consuming the alfalfa hay diet. Furthermore, increasing the energy intake of the corn and corn-silage based diet from one to two times maintenance increased pancreatic α-amylase specific activity by two-fold, without any additional increases at 3 × maintenance. To further evaluate the effects of diet and energy intake on carbohydrase activities, Kreikemeier et al. (1990) fed either a 90% forage (alfalfa hay) or 90% grain (sorghum:wheat) diet at 1 or 2 times the net energy for maintenance (NE_m) requirement. In steers consuming the grain diet, pancreatic α-amylase concentration and total content was lower than steers consuming forage. Additionally, when energy intake increased from 1 to 2 times NE_m, pancreatic α-amylase activity and total content increased with an increase in pancreatic mass. In contrast, previous studies demonstrated that increasing starch intake could increase pancreatic α-amylase activity (Clary et al., 1969; Janes et al., 1985). However, these studies were confounded with energy intake. Results from Russell et al. (1981) and Kreikemeier et al. (1990) demonstrated that increasing energy intake up to 2 × maintenance can increase pancreatic α-amylase activity. In addition, steers consuming starch-based diets had lower activity of pancreatic α-amylase. However, the diet effects on pancreatic α-amylase were less clear, as the alfalfa hay based diets had greater crude protein levels.

This led to the hypothesis that changes in luminal carbohydrate and protein flow could influence pancreatic α-amylase activity.

While nonruminants increase pancreatic α-amylase activity in response to luminal starch flows (Brannon, 1990), the response is opposite in ruminants. Abomasal infusions of partially hydrolyzed starch decreased pancreatic α-amylase concentration, specific activity, and secretion in steers compared to steers ruminally infused with partially hydrolyzed starch or steers infused with water (Walker and Harmon, 1995). The same decrease in pancreatic α-amylase activity in response to abomasal partially hydrolyzed starch was observed with pancreatic tissue samples (Swanson et al., 2002b). Similarly in wethers, abomasal infusions of raw cornstarch decreased pancreatic α-amylase concentration and secretion compared to control wethers receiving abomasal water infusions (Wang & Taniguchi, 1998). These studies demonstrated that luminal complex carbohydrate flow decreases pancreatic α-amylase activity in cattle. In a study by Swanson et al. (2002a), abomasal infusions of glucose or partially hydrolyzed starch both decreased pancreatic α-amylase concentration, specific activity, and secretion in steers. This study demonstrated that downregulation of pancreatic α-amylase is not due solely to luminal complex carbohydrate flow. It remains unclear whether luminal or absorbed glucose regulates pancreatic α-amylase activity in ruminants. Increasing levels of ruminal glucose infusions had no effect on plasma amylase concentrations in lambs fed a 50% concentrate diet (Krehbiel et al., 1995). In conclusion, high levels of post-ruminal carbohydrate supply as starch, partially hydrolyzed starch, or glucose decreases pancreatic α-amylase activity when energy intake is controlled. Further research is needed to evaluate the effects of other carbohydrates (lactose, fructose, galactose) on pancreatic exocrine function in ruminants.

As stated previously, studies by Russell et al. (1981) and Kreikemeier et al. (1990) demonstrated that pancreatic α-amylase activity was greater in steers fed an alfalfa hay diet compared to a grain-based diet. These authors speculated that differences in dietary crude protein (and therefore, rumen undegradable protein and metabolizable protein) could possibly contribute to differences in pancreatic α-amylase activity. In sheep, Wang & Taniguchi (1998) abomasally infused water (control), raw cornstarch, or raw cornstarch + casein and measured pancreatic exocrine secretion. Pancreatic α-amylase activity was depressed with abomasal starch; however, starch and casein supply restored α-amylase levels to the control. Similarly, increasing levels of abomasal casein supply (0, 60, 120, or 180 g/d) linearly increased pancreatic α-amylase concentration, specific activity, and secretion in steers infused with raw cornstarch (Richards et al., 2003). Additional work feeding a 68.7% concentrate diet with supplemental casein to steers produced increases in duodenal α-amylase concentrations and serum cholecystokinin concentrations (Lee et al., 2013). However, complex interactions exist with starch and protein supply in the ruminant small intestine (Harmon, 2009). Interactions between starch and protein on pancreatic α-amylase were evaluated with the following treatments: 1) water, 2) partially hydrolyzed starch, 3) casein, and 4) partially hydrolyzed starch + casein. In beef steers, casein infusion increased pancreatic α-amylase concentration and secretion and partially hydrolyzed starch infused had the opposite response (Swanson et al., 2004). However, the combination of partially hydrolyzed starch and casein produced a response in pancreatic α-amylase that was less than casein and not different from partially hydrolyzed starch alone. In support of these results, Swanson et al. (2002b) and Swanson et al. (2003) found the same response in pancreatic α-amylase activity to infusion treatments in pancreatic tissue and cell culture models, respectively. The combined results suggest that the benefits of post-ruminal protein supply may be overridden by

the presence of starch in the small intestine. However, it should be noted that no definitive studies have tested the influence of cornstarch source (raw cornstarch or partially hydrolyzed starch) with post-ruminal casein on pancreatic α-amylase activity. Also, it is known that dietary corn starch source (Akay et al., 2002) or endosperm type (Taylor and Allen, 2005) can influence postruminal starch digestibility in lambs and lactating dairy cattle.

Responses in pancreatic α-amylase increase with dietary starch intake in nonruminants (Brannon, 1990), which is similar to responses in ruminants to increasing post-ruminal protein supply. Increasing dietary crude protein concentrations with bypass soybean meal resulted in linear increases in pancreatic α-amylase activity (Swanson et al., 2008). However, at the same time, there were decreasing proportions of high-moisture corn in the ration. Complex interactions between luminal carbohydrate and protein flow on small intestinal starch digestion likely differ between ruminants and nonruminants due, at least in part, to absorption of large amounts of products of ruminal fermentation and microbial crude protein synthesis. In turn, changes in duodenal digesta nutrient composition, osmolality, and volume could potentially influence pancreatic exocrine function.

More recently, the effects of individual amino acids on pancreatic exocrine function in ruminants has been studied. However, responses in digestive enzyme activity to individual amino acids have varied with length of infusion and animal species. Arginine administration through jugular blood had no influence on pancreatic α-amylase activity in non-pregnant ewes (Keomanivong et al., 2017). After 14-d of infusing increasing levels of phenylalanine, Yu et al. (2013) observed linear increases in pancreatic α-amylase specific activity, and a cubic response in α-amylase secretion in goats. In the short-term experiment (10-h), they found a quadratic response in pancreatic α-amylase secretion to increasing levels of phenylalanine. Moreover, increasing

levels of leucine linearly increased α-amylase concentration after 14 d of duodenal infusion (Yu et al., 2014b). In dairy heifers, duodenal infusions of 10 g/d leucine increased total pancreatic secretion, α-amylase concentration, and α-amylase secretion (Liu et al., 2015). Increases in pancreatic α-amylase activity were observed with duodenal infusions or leucine (3 or 9 g/d) and phenylalanine (2 g/d) in goats (Cao et al., 2018a). However, when leucine (1.435 g/L milk), phenylalanine (0.725 g/L milk), or a combination of leucine or phenylalanine (1.435 g leucine/L milk and 0.725 g phenylalanine/L milk) were fed to milk-fed calves, there was no influence on pancreatic α-amylase specific activity (Cao et al., 2018b). Similarly, increasing levels of leucine supplementation to neonatal calves fed milk-replacer had no effect on pancreatic α-amylase activity (Reiners et al., 2019). Duodenal infusions of 20 or 30 g/d of isoleucine have been shown to increase pancreatic α-amylase activity in dairy heifers after 12 h or 10 d of infusion (Liu et al., 2018). In cell culture models using pancreatic acinar cells, amino acids such as phenylalanine (Guo et al., 2018a), leucine (Guo et al., 2018b; Guo et al., 2019; Cao et al., 2019a), and isoleucine (Cao et al., 2019b) increased α-amylase release. Despite increases in small intestinal starch disappearance (Brake et al., 2014b; Blom et al., 2016) with glutamic acid, it is unclear if these increases are related to increases in pancreatic α-amylase activity. Indeed, a great amount of work has contributed to a better understanding of dietary effects of amino acids on pancreatic exocrine function. Because approximately 80% of the amino acids appearing in portal blood are associated with peptides (Koeln and Webb, 1982; Webb et al., 1992), it seems that more focus should be directed towards the influence of peptides on the regulation of digestive enzyme activity.

Despite increases in pancreatic α-amylase activity in response to post-ruminal protein or amino acid flow, it is unclear if increases in pancreatic α-amylase are related to increases in small intestinal starch disappearance. In studies where pancreatic α-amylase increased, small intestinal

carbohydrases were not evaluated. Therefore, there may be confounding effects if there are also simultaneous increases in small intestinal carbohydrase activities. Furthermore, enzyme infusion studies with α-amylase have failed to show a response in small intestinal starch disappearance in cattle. Remillard et al. (1990) infused amylase or bicarbonate in a 2 × 2 factorial design into the jejunum and failed to see any effects on small intestinal starch digestion in steers fed an 85% grain diet. Abomasal infusions of amylase with 880 g/d of raw cornstarch (Westreicher-Kristen et al., 2018) or increasing levels of raw cornstarch up to 1993 g/d (Robbers et al., 2019) had no influence on postruminal starch digestion in steers.

1.2.2.3. Small intestinal carbohydrases

Comparatively, there is far less information describing the influence of diet and luminal nutrient supply on the regulation of small intestinal carbohydrases in ruminants. Early studies demonstrated that diet composition (forage vs grain) and energy intake had little influence on small intestinal carbohydrase activities (Russell et al., 1981; Janes et al., 1985; Kreikemeier et al., 1990). Young bulls consuming a ground corn-based diet had greater duodenal maltase specific activity than young bulls consuming a whole shelled corn-based diet; and there was no diet effect in the jejunum (Carvalho et al., 2019). In dairy calves, milk replacer intake and butyrate supplementation had no influence on lactase or maltase activities or lactase, maltase-glucoamylase, or sucrase-isomaltase mRNA expression (Koch et al., 2019). Increasing the amount of days (0, 7, 13, 21) that steers consumed a 42% dry-rolled barley diet linearly increased proximal jejunal lactase specific activity compared to steers consuming a chopped-hay diet (Górka et al., 2017). However, it should be noted that steers consuming the moderate grain diet had increases in energy intake. Overall, changes in diet or energy status seem to have non-specific effects on small intestinal

carbohydrase activities. This can be due to changes in energy status, tissue mass, and maintenance requirements of the small intestine.

Few studies have investigated the effects of specific nutrients on small intestinal carbohydrase activities in ruminants; although most information is in relation to starch or starch-digestion products. Abomasal infusions of partially hydrolyzed starch increased jejunal maltase activity in sheep but decreased jejunal maltase activity in cattle (Bauer et al., 2001a). In another experiment, partially hydrolyzed starch infusions for 7d had no influence on maltase activity in any site of the small intestine in cattle (Bauer et al., 2001b). Later, steers receiving abomasal infusions of glucose or partially hydrolyzed starch had greater maltase specific activity than steers receiving ruminal starch infusions or consuming 2 × maintenance (Rodriguez et al., 2004). This may indicate that luminal substrate flow (maltose, isomaltose, limit dextrins) can increase carbohydrase activities in the small intestine. In neonatal calves, 18% replacement of lactose with maltodextrin, maltodextrin with a high degree of α-1,6 branching, and maltose decreased jejunal maltase specific activity (Gilbert et al., 2015). Furthermore, jejunal isomaltase specific activity decreased in response to greater amounts of maltodextrin or maltodextrin with a high degree of α-1,6 branching. Additionally, steers with partially hydrolyzed starch infusions in the abomasum had greater maltase specific activity than steers infused with casein, with partially hydrolyzed starch + casein being intermediate (Guimaraes et al., 2007). Conflicting reports on the responses of carbohydrase activity in different animal models make interpretations difficult. A greater understanding of how luminal starch, starch-digestion intermediates, and glucose regulate small intestinal carbohydrase activities is needed.

Information about amino acid regulation of small intestinal carbohydrases in ruminants is scarce. Cao et al. (2019c) found that dietary leucine or phenylalanine had no influence on lactase

activity in small intestinal digesta from calves fed milk and starter. However, increasing levels (0, 0.4, 0.6, or 0.8 g/kg of BW) of leucine supplementation to calves fed milk-replacer showed a quadratic effect on intestinal lactase with the 0.4 g leucine/kg of BW treatment being lower than all other treatments (Reiners et al., 2019). Increasing levels of supplemental leucine linearly decreased maltase and isomaltase activities. It is unclear whether glutamic acid influences small intestinal carbohydrase activities to facilitate improvements in small intestinal starch digestion. However, because of the reduction in ileal ethanol soluble starch flow, researchers have speculated that glutamic acid may increase small intestinal carbohydrase activities (Brake et al., 2014b; Blom et al., 2016).

1.2.3. Sucrase

A remaining enigma of ruminant physiology is the absence of sucrase activity in the small intestine. There is lack of evidence of a detectable sucrase activity at reasonable (physiological) levels in ruminants. Several studies have investigated and characterized digestive enzyme activity along the small intestine with various ruminant animal species, ages, and diets. Yet, there still has been a failure to detect active sucrase in the small intestine. This is in contrast to nonruminant species including the pig, chicken, mouse, and rat. Dollar and Porter (1957) were the first to report the absence of sucrase activity in young calves. Furthermore, no measurable sucrase activity was detected in mucosa or small intestinal digesta contents from lambs (Walker, 1959). Later reports by Huber et al. (1961) and Siddons (1968) corroborated the findings that sucrase activity is absent from the digestive tract of the young calf. With cattle ranging from 4 days of age up to 6 years of age, no detectable amounts of sucrase were found in the small intestine (Siddons, 1968; Kreikemeier et al., 1990). Shirazi-Beechey et al. (1989) attempted to measure sucrase activity in isolated brush-border membrane vesicles from lamb intestine and received the same results as

others. More recently, we did not detect any sucrase activity in the maternal or fetal small intestine of sheep (Trotta et al., 2019).

Alternatively, sucrase activity has been detected in other ruminant species; moose was found to have very lowly detectable amounts of sucrase (Schwartz et al., 1996). However, these values are not physiological (Mean = 0.04 ± 0.03 U/g) and it could be possible that there were not corrections for endogenous glucose concentrations in either the mucosa samples or the substrate (sucrose) or both. In contrast, a comparative study demonstrated that sucrase activity was not detected in any ruminant species including the sheep, goat, roe deer, and moose (Rowell-Schäfer et al., 2001). Although not considered a ruminant, the kangaroo is a foregut-fermenter and does not possess intestinal sucrase activity (Kerry, 1969). In the camel intestine, glucoamylase and maltase activities were 2- and 3-fold greater than sucrase activity (Mohamed et al., 2007). They reported that sucrase activity was 8.2 U/g on average.

Few studies have attempted to induce sucrase activity by nutritional methods. Because little sucrose would typically reach the small intestine, it was unclear if luminal sucrose could induce its enzyme in the ruminant small intestine. However, abomasal infusions of sucrose did not induce sucrase activity in lambs (Swanson and Harmon, 1997). In humans, dietary fructose supplementation has been shown to induce sucrase activity in patients with congenital sucrase-isomaltase deficiency (Greene et al., 1972). It remains unclear whether dietary fructose can induce sucrase activity in ruminants.

Recent evidence from nonruminants may suggest that the absence of sucrase can have other physiological consequences on carbohydrate digestion. The ruminant enzyme profile seems to be similar to humans with congenital sucrase-isomaltase deficiency from genetic mutations in one or both of the subunits of the sucrase-isomaltase complex, leading to limitations in carbohydrate

digestion (Harmon and Swanson, 2019). Nichols et al. (2017) demonstrated that the absence of sucrase activity leads to a reduction in starch digestion and postprandial glucose response with a sucrase-deficient shrew model. It should be noted that maltase and isomaltase activities were not influenced in their sucrase-deficient model. The limit of small intestinal starch digestion in ruminants has been debated for decades (Mayes and Ørskov, 1974; Owens et al., 1986; Kreikemeier and Harmon, 1995; Huntington et al., 1997; Harmon et al., 2004; Huntington et al., 2006; Brake and Swanson, 2018). Despite improvements in small intestinal starch digestion with post-ruminal protein flow (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016), the average digestibility of starch in the small intestine has been inadequate (<70%) to achieve improvements in energetic efficiency (Huntington et al., 2006). Research is needed to investigate if the absence of sucrase activity in the small intestine contributes to the limitations in starch digestion in ruminants.

1.2.4. Carbohydrate absorption

Early studies in ruminants (Schambye, 1951; White et al., 1971) suggested that glucose appearance in portal blood was limited and that the capacity for glucose absorption was less than the rat. While there are numerous mechanisms of transport that vary in abundance within tissue, the main focus of this section will be on sodium-dependent glucose cotransporter-1 (SGLT1), glucose transporter 5 (GLUT5), and glucose transporter 2 (GLUT2). These are thought to be the main carbohydrate transporters in the small intestine of cattle (Harmon, 2009) and this section will primarily focus on nutritional regulation differences of SGLT1, GLUT5, and GLUT2 between ruminants and nonruminants.

1.2.4.1. Sodium-dependent glucose cotransporter-1

Many studies in ruminants have concluded that glucose transport activity and SGLT1 abundance were greatest in milk-fed lambs and declines with age (Scharrer et al., 1979a; Scharrer et al., 1979; Shirazi-Beechey et al., 1989; Shirazi-Beechey et al., 1991). Shirazi-Beechey et al. (1991) demonstrated that duodenal infusions of a 30 mM glucose solution for 4 days in adult sheep increased the rate of glucose transport by 40- to 80-fold which was also accompanied by an increase in SGLT1 abundance. Furthermore, Dyer et al. (1994) demonstrated that duodenal fructose infusions can increase jejunal SGLT1 induction in lambs. These authors concluded that luminal sugar is sensed in the intestine, independent of glucose metabolism, and that the inducing sugar does not need to be a substrate of SGLT1. Moreover, regulation of carbohydrate transport in ruminants has been suggested to be influenced by the presence of sweet taste receptors in the bovine and ovine small intestine (T1R2-T1R3; Moran et al., 2014). The sweet taste receptor signaling mechanism was proposed by Moran et al. (2018), based on research with mice. Luminal sugar is sensed in the small intestine by T1R2-T1R3 and its associated G-protein, gustducin, which induces a signaling cascade, leading to a subsequent increase in glucagon-like peptide-2 secretion. Glucagon-like peptide-2 binds to its receptor on the submucosal plexus, eliciting a neuronal response to evoke the release of vasoactive intestinal peptide (VIP) or pituitary adenylate cyclase-activating peptide (PACAP) in absorptive enterocytes. Binding of either VIP or PACAP to its receptor on the basolateral membrane of absorptive enterocytes results in an increase in intracellular cAMP levels, leading to an upregulation of SGLT1 (Moran et al., 2018).

However, there is an apparent difference between cattle and sheep in carbohydrate transport and their ability to respond to diet or luminal nutrient supply. In companion studies, jejunal sodium-dependent glucose cotransport activity was determined in cattle and sheep

ruminally or abomasally infused with partially hydrolyzed starch for 7 d (Bauer et al. 2001a; Bauer et al., 2001b). Animals receiving post-ruminal infusions of partially hydrolyzed starch had increased glucose uptake by 2-fold, however, the magnitude of the increase was greater in sheep than cattle (Bauer et al., 2001a). In the next experiment, sodium-dependent glucose uptake was evaluated in steers ruminally or abomasally infused with partially hydrolyzed starch for 7 d across multiple sites of the small intestine (Bauer et al., 2001b). There were no increases in SGLT1 activity in response to abomasal partially hydrolyzed starch infusions in any sites of the intestine (Bauer et al., 2001b). Later, after increasing the adaptation length to 35 d, Rodriguez et al. (2004) found that abomasal partially hydrolyzed starch or glucose infusions did not influence SGLT1 abundance or sodium-dependent glucose uptake. It should be noted that increasing dietary energy intake to 2 × NE_m also did not influence SGLT1 abundance or sodium-dependent glucose uptake. Similarly, Liao et al. (2010) infused partially hydrolyzed starch ruminally or abomasally and found only tendencies to influence SGLT1 or GLUT2 mRNA expression. They reported that ruminal partially hydrolyzed starch infusions tended to increase duodenal SGLT1 and that abomasal infusions of partially hydrolyzed starch tended to increase ileal SGLT1 and GLUT2 mRNA expression (Liao et al., 2010). Lohrenz et al. (2011) reported that lactating dairy cows fed a high starch (24% of DM) or low starch diet (12% of DM) had no difference in duodenal or jejunal SGLT1 mRNA or protein expression. Moreover, duodenal or jejunal GLUT2 mRNA expression, protein amount on the apical membrane, or total protein amount was not influenced by diet (Lohrenz et al., 2011). Because of the interactions with luminal protein and carbohydrate in the ruminant small intestine on starch disappearance and enzyme activity, SGLT1 abundance and glucose uptake were evaluated (Guimaraes et al., 2007). Using the same treatments as Swanson et al. (2002b), there was no influence of abomasal partially hydrolyzed starch, casein, or their

combination on SGLT1 abundance or activity in steers (Guimaraes et al., 2007). In contrast, abomasal infusions of casein increased SGLT1 activity in the proximal jejunum and whole small intestine in lambs (Mabjeesh et al., 2003). In goats, SGLT1 activity was greatest when corn- or wheat-based diets were fed but without any changes in transporter affinity or protein expression (Klinger et al., 2013). Collectively, these data suggest that bovine nutrient transporters involved in small intestinal carbohydrate absorption are less sensitive to diet or luminal nutrient supply than sheep.

1.2.4.2. Glucose transporter 5

There are apparently are no definitive studies that specifically evaluate GLUT2 and its role in apical glucose transport in ruminants. Additionally, there are few studies that have evaluated how diet influences GLUT2 or GLUT5 in ruminants. In nonruminants, nutrient transporters typically respond proportionally to substrate (Ferraris and Diamond, 1989; Shu et al., 1997). There have been no studies evaluating the effects of fructose on GLUT5 regulation in ruminants. Dietary fructose supplementation has been shown to increase GLUT5 expression and enhance intestinal fructose transport in neonatal rats (Shu et al., 1997). Zhao et al. (1993) found that GLUT5 expression in the intestine is significantly lower than in the liver or kidney in cattle. In contrast, many authors have reported that the greatest amount of GLUT5 expression occurs in the small intestine in humans, rats, mice, rabbits, chickens, and horses (Douard and Ferraris, 2008). Nutritional regulation of GLUT5 by fructose requires luminal presence of fructose in the intestine (Shu et al., 1998) and GLUT5 expression is directly proportional to intestinal luminal fructose concentration (Shu et al., 1997). In cattle, ruminal or abomasal infusions of partially hydrolyzed starch did not affect GLUT5 expression in the duodenum, jejunum, or ileum (Liao et al., 2010).

Douard and Ferraris (2008) discussed the complex relationships between age, luminal fructose supply, and induction of GLUT5 in neonatal (milk only), weaning (milk + solid feed), and post-weaning (feed only) rats. In general, GLUT5 expression is nutritionally regulated by luminal fructose during weaning (14-28 days of age) and post-weaning (>28 days of age) in rats (David et al., 1995; Shu et al., 1997; Shu et al., 1998; Jiang and Ferraris, 2001; Jiang et al., 2001; Cui et al., 2004). However, in neonatal rats (<14 days of age), GLUT5 expression can increase with luminal fructose and glucocorticoid supply but not luminal fructose alone (Douard et al., 2008a; Douard et al., 2008b; Suzuki et al., 2011). Therefore, nutritional regulation of GLUT5 by fructose is age-dependent in rats. No studies have defined the GLUT5 regulation by diet in ruminants.

1.2.5. Passage rate

A notable difference in ruminant digestive physiology compared to nonruminants is the rate of digesta passage. Flow of digesta through the duodenum is essentially continuous (Merchen, 1988) while ileal flow is intermittent (Goodall and Kay, 1969). In mature cattle, continuous in- and outflow of digesta leads to a relatively constant and small abomasal capacity (Burgstaller et al., 2017). However, it should be recognized that abomasal emptying occurs in the milk-fed calf and has similarities to non-ruminants (Burgstaller et al., 2017). Another difference is that ileal retrograde flow rarely occurs in ruminants; although sometimes the digesta contents can remain stationary (Ash, 1969). Continuous fermentation and ruminal passage rate has a large influence on digesta flow to the abomasum and subsequently influences abomasal passage rate. Because of the nearly continuous duodenal flow, several other physiological processes seem relatively continuous in the ruminant, as well. For example, the near continuous flow of digesta in the intestine has been thought to minimize diurnal variations in pancreatic exocrine secretion (Merchen, 1988; Walker

and Harmon, 1995). Furthermore, this may affect buffering capacity and in turn, digestive enzyme activity because digestive enzymes are pH-dependent. Although the optimal pH ranges for digestive enzymes are similar between ruminants and nonruminants, it should be noted that the intestinal contents of the ruminant small intestine remain acidic for an appreciable length (7m in sheep; Ben-Ghedalia et al., 1974). Subsequently, the lack of a postprandial glucose increase in response to feeding could also potentially be attributed to relatively constant metabolic processes in nutrient assimilation in ruminants. Because abomasal and intestinal flow of digesta is essentially constant, it is unclear if neural or hormonal signaling to the pancreas or small intestine is impaired. More definitive studies are needed in ruminants to evaluate how continuous abomasal flow affects autonomic control of digestion in the small intestine and if the lack of abomasal emptying could possibly contribute to limitations in digestive enzyme production or secretion.

Owens et al. (1986) suggested that intestinal retention time could potentially limit the extent of small intestinal starch disappearance. Digesta spends less than 3 hours in the small intestine of steers (Zinn and Owens, 1980) and digesta consistency can vary drastically with diet composition. Luminal nutrient composition in the distal intestine can influence hormonal secretion which may act to slow digesta passage to increase digestion in more proximal locations (Croom et al., 1992). However, post-ruminal casein supply had no effect on small intestinal transit time in steers duodenally infused with raw cornstarch (Brake et al., 2014a). In milk-fed calves, casein had no influence on rate of abomasal emptying or intestinal transit time (Smith, 1964). Even if passage rate limited digestion, it is thought that this factor is not independent in the activity or amount of carbohydrases (Brake and Swanson, 2018).

1.2.6. Portal appearance of glucose

After glucose transport into an enterocyte, glucose can be transported across the basolateral membrane into portal blood circulation primarily via GLUT2. Interestingly, many studies have found a disproportional relationship between intestinal carbohydrate disappearance and portal glucose appearance. In mature ruminants, limited amounts of glucose appear in portal blood (Schambye, 1951). To compare the influence of carbohydrate source and breed, Huntington and Reynolds (1986) abomasally infused glucose or raw cornstarch in lactating dairy cows and beef heifers and measured net nutrient absorption across the portal-drained viscera. They reported that approximately 65% of the infused glucose appeared in portal blood and this was similar between lactating dairy cows and beef heifers. However, only 35% and 8% (26% average) of the infused cornstarch appear in portal blood as glucose for the beef heifer and lactating cow, respectively. It should be noted that these calculations were based on the amount of carbohydrate infused, not disappearance of the carbohydrate.

Kreikemeier et al. (1991) were the first to quantify small intestinal carbohydrate disappearance and net portal glucose absorption in cattle simultaneously. Holstein steers infused with glucose, corn dextrin, or corn starch at 60 g/h had 94% of glucose, 38% of corn starch, and 29% of corn dextrin disappearance in the small intestine that could be accounted for in portal blood (Kreikemeier et al., 1991). These authors suggested that glucose could potentially be used as a substrate within the small intestine or that small intestine carbohydrate disappearance could be partially due to microbial fermentation. They concluded that approximately 35% of raw cornstarch that disappears in the small intestine resulted in net portal glucose absorption. In a similar study, Holstein steers were infused with water, glucose, corn dextrin, or corn starch at 66 g/h (Kreikemeier and Harmon, 1995). Seventy-three percent of glucose, 60% of corn dextrin, and 57%

of corn starch that disappeared in the small intestine could be accounted for in net portal glucose flux. In beef steers, abomasal raw cornstarch infusions with casein increased portal glucose appearance by 0.38 g per gram of casein infused (Taniguchi et al., 1995). Shifting the site of starch digestion from the rumen to the small intestine increased glucose utilization by PDV tissues (132%), PDV glucose flux (310%), and irreversible loss of glucose (59%) in growing beef steers infused with partially hydrolyzed starch (Harmon et al., 2001). In general, these studies collectively demonstrate that intestinal carbohydrate digestion is more limiting than glucose absorption. However, compared to nonruminants, the amount of glucose appearing in portal blood is low and raises many questions about the fate of glucose that disappears in the intestine.

In fact, most authors agree that the disproportional relationship between intestinal carbohydrate disappearance and net portal glucose absorption is partially due to both microbial fermentation and visceral metabolism (Kreikemeier et al., 1991; Kristensen et al., 2005; Gilbert et al., 2015). Gilbert et al. (2015) concluded that the largest part of small intestinal starch disappearance is due to fermentation rather than enzymatic hydrolysis to glucose in milk-fed calves. However, this remains to be studied in functional ruminants. Direct quantification of enzymatic hydrolysis of starch in the ruminant small intestine has yet to be quantified. The underdevelopment of anatomical features of the portal-drained viscera such as, vascularity and capillary density, may partially explain why there is a lack of digested starch that appears in portal blood compared to nonruminants. Well-designed, *in vivo* trials are needed to quantify the fate of digested starch in the small intestine.

1.3. Literature summary

Several biological factors differentiate the ruminant and nonruminant small intestine and may contribute to apparent differences in carbohydrate assimilation. The extent of carbohydrate

digestion in ruminants varies with carbohydrate source; however, starch, starch digestion products, and sucrose are limited in their extent of luminal disappearance. Linear negative relationships between intestinal starch flow and intestinal starch disappearance in ruminants are biologically abnormal when compared to non-ruminants. Nutritional regulation of post-ruminal carbohydrases is functionally different and the quantity of carbohydrases are far less than nonruminants. Carbohydrases in the pancreas and small intestine have the opposite response to luminal substrate compared to nonruminants. In general, post-ruminal carbohydrases may increase with protein or amino acid flow and decrease with starch or glucose flow. Sucrase activity cannot be detected in the ruminant small intestine and it remains unclear how its absence influences carbohydrate assimilation. Nutritional regulation of SGLT1 is apparently different between cattle and sheep. Moreover, it remains unclear if GLUT5 or GLUT2 are influenced by luminal fructose supply. Ruminants have a nearly continuous flow of digesta to the duodenum which results in minimal variations in pancreatic exocrine secretion or postprandial glucose concentrations. There is a disproportional relationship between intestinal carbohydrate disappearance and portal glucose appearance. Several biological abnormalities exist between the ruminant and nonruminant small intestine and influences carbohydrate assimilation.

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2. DUODENAL INFUSIONS OF STARCH WITH CASEIN OR GLUTAMIC ACID INFLUENCE PANCREATIC AND SMALL INTESTINAL CARBOHYDRASE ACTIVITIES IN CATTLE[1]

2.1. Abstract

Small intestinal starch digestion in ruminants is potentially limited by inadequate production of carbohydrases. Previous research has demonstrated that small intestinal starch digestion can be improved by post-ruminal supply of casein or glutamic acid. However, the mechanisms by which casein and glutamic acid increase starch digestion are not well understood. The objective of this experiment was to evaluate the effects of duodenal infusions of starch with casein or glutamic acid on post-ruminal carbohydrase activities in cattle. Twenty-two steers (179 ± 4.23 kg BW) were surgically fitted with duodenal and ileal cannulas and fed a soybean hull-based diet containing small amounts of starch at 1.3 × net energy for maintenance requirements. Raw cornstarch (1.61 ± 0.0869 kg/d) was infused into the duodenum alone (control), or with 118 ± 7.21 g/d glutamic acid, or 428 ± 19.4 g/d casein. Treatments were infused continuously for 58 d and then steers were slaughtered for tissue collection. Activities of pancreatic (α-amylase) and intestinal (maltase, isomaltase, glucoamylase, sucrase) carbohydrases were determined. Data were analyzed as a randomized complete block (tissue collection group) design using the GLM procedure of SAS to determine effects of infusion treatment. Pancreatic and small intestinal mass

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and protein concentrations were not influenced by treatment. Duodenal casein infusion increased ($P < 0.05$) pancreatic α-amylase activity by 290%. Duodenal casein infusion increased jejunal maltase ($P = 0.02$) and glucoamylase ($P = 0.03$) specific activity by 62.9% and 97.4%, respectively. Duodenal casein infusion tended to increase ($P = 0.10$) isomaltase concentration by 38.5% in the jejunum. Sucrase activity was not detected in any segment of the small intestine. These results suggest that small intestinal starch digestion may be improved in cattle with increased small intestinal flow of casein through increases in post-ruminal carbohydrase activities.

2.2. Introduction

The ruminant digestive system allows for the consumption of complex feed sources and digestion of structural carbohydrates that are typically unsuitable for non-ruminant diets. However, in North American finishing cattle and dairy cattle production systems, grain-based diets containing moderate to large proportions of starch are typically fed to increase the net energy concentrations of the diet allowing for more efficient growth and improved product quality. When grain-based diets are fed, up to 40% of dietary starch intake can escape ruminal fermentation and flow to the small intestine for potential enzymatic digestion (Ørskov et al., 1986). Host digestion in the small intestine allows for absorption of glucose and provides more energy to the host than short-chain fatty acids produced from fermentation of carbohydrates by the ruminal microbes. Thus, small intestinal starch digestion in cattle is energetically more efficient than ruminal fermentation of starch (Owens et al., 1986; Huntington, 1997; Harmon, 2009; Owens et al., 2016). However, several authors have suggested that the extent of small intestinal starch digestion is potentially limited by inadequate production of post-ruminal digestive enzymes (Little et al., 1968; Owens et al., 1986; Kreikemeier and Harmon, 1995; Brake and Swanson, 2018).

The pancreas and small intestine have important roles in post-ruminal nutrient digestion and there is a limited amount of information on their function in response to nutritional adaptation in ruminants (Harmon, 1993). Specifically, both tissues produce carbohydrases, or glycohydrolases, that are digestive enzymes that hydrolyze glycosidic linkages of saccharides. While non-ruminant digestive enzymes typically increase proportionally to substrate (Brannon, 1990), digestive enzymes in ruminants respond differently to diet and luminal nutrient supply (Harmon, 1992). Previous research has demonstrated improvements in small intestinal starch digestion in response to post-ruminal protein (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b) or glutamic acid (Brake et al., 2014b; Blom et al., 2016) supply. Additionally, increases in post-ruminal protein supply have shown remarkable increases in pancreatic α-amylase activity (Swanson et al., 2002b; Richards et al., 2003; Swanson et al., 2004).

Comparatively, there is significantly less information on the regulation of small intestinal carbohydrases involved in starch digestion (maltase, isomaltase, glucoamylase) in ruminants. Moreover, information on how glutamic acid facilitates improvements in small intestinal starch digestion in cattle remains equivocal. Other studies have shown that individual amino acids such as leucine (Yu et al., 2014; Liu et al., 2015; Cao et al., 2018), isoleucine (Liu et al., 2018; Cao et al., 2019) and phenylalanine (Guo et al., 2018) can increase pancreatic α-amylase activity in ruminants. Understanding the mechanisms by which post-ruminal protein or amino acid flow modulate increases in small intestinal starch digestion potentially could result in the development of nutritional approaches to improve nutrient utilization and the energetic efficiency of animal production. Therefore, the objective of this experiment was to determine the effects of duodenal starch infusions with casein or glutamic acid on pancreatic and small intestinal digestive enzyme activities in cattle.

2.3. Methods

All experimental protocols were approved by the South Dakota State University Institutional Animal Care and Use Committee.

2.3.1. Animals and diets

A duodenal infusion model was used for this experiment as previously described (Brake et al., 2014a). Twenty-two steers (179 ± 4.23 kg BW), predominantly of British and Continental influenced breeds, were surgically fitted with duodenal and ileal cannulas. Double-L intestinal cannulas (Streeter et al., 1991) were placed approximately 10 cm posterior to the pyloric sphincter in the duodenum and approximately 30 cm anterior to the ileocecal junction in the ileum. For 35 d, steers recovered from surgical procedures and were adapted to the basal experimental diet. Steers were limit-fed 3.47 ± 0.326 kg of a soybean hull-based diet (Table 1) that supplied 1.3 × net energy for maintenance requirements for a steer gaining 0.46 kg/d, met the needs for ruminally degradable protein and exceeded requirements for vitamins and minerals (NASEM, 2016). Ruminally degradable protein values for corn steep liquor were calculated using values from DeFrain et al. (2002). The diet contained small amounts of starch (0.6 ± 0.064% DM-basis) by design and was predicted to provide 140 g/d MP above estimated amounts of MP needed for maintenance (NASEM, 2016). The diet was analyzed for dry matter and ash using standard procedures (AOAC, 1990) and neutral and acid detergent fiber (Van Soest et al., 1991). Nitrogen content was determined using the Dumas procedure (AOAC, 1990) with a Rapid N III Elementar Analyzer (Elementar Americas Inc.). Crude protein was calculated by multiplying N concentration × 6.25. Starch content was analyzed using the methods of Herrera-Saldana and Huber (1989). Animals were housed and tethered individually in tie-stalls (1.7 × 2.4 m) in a controlled temperature (21°C) and light (16 h light: 8 h dark) environment.

Table 1. Composition of soybean hull-based diet fed to steers.[1]

Item	Unit
Ingredient	g/kg DM
Soybean hulls	724
Brome grass hay	200
Corn steep liquor	60
Limestone	10
Salt	5
Mineral and vitamin premix[2]	1
Chemical composition	
DM, g/kg	820 ± 10
OM, g/kg of DM	925 ± 3
Starch, g/kg of DM	6 ± 1.6
CP, g/kg of DM	105 ± 11
NDF, g/kg of DM	597 ± 15
ADF, g/kg of DM	438 ± 13
RDP, g/kg of DM	62
NE_m, Mcal/kg	1.37

[1]Diet formulated to supply 1.3 × NE_m requirement, supply adequate ruminally available N, and exceed metabolizable protein requirements (NASEM, 2016).

[2]Provided to diet (per kg diet DM): 50 mg of Mn, 50 mg of Zn, 10 mg of Cu, 0.5 mg of I, 0.2 mg of Se, 2,200 IU of vitamin A, 275 IU of vitamin D, and 25 IU of vitamin E.

[3]Calculated from tabular values (NASEM, 2016) for soybean hulls and brome grass hay, rumen degradable protein values reported by DeFrain et al. (2002) for corn steep liquor.

[4]Calculated from tabular values (NASEM, 2016).

2.3.2. Experimental design

Steers were blocked into four groups and randomly assigned to continuous duodenal infusion treatments of 1.61 ± 0.0869 kg/d of raw cornstarch with either 0 (control; n = 8), 118 ± 7.21 g/d L-glutamic acid (n = 8), or 428 ± 19.4 g/d of casein (n = 6) on a DM basis. Raw cornstarch was used to facilitate the greatest limitations in small intestinal starch digestion, as the action of both pancreatic and small intestinal carbohydrases are required for complete hydrolysis to glucose.

The amounts of glutamic acid and casein were chosen based on previous reports that have demonstrated that these amounts of glutamic acid and casein resulted in a similar magnitude of increases in small intestinal starch digestion (Brake et al., 2014b; Blom et al., 2016). Treatments were continuously infused through Tygon tubing (2.38 mm i.d.; Saint-Gobain North America) with 14 L of aqueous solution using a peristaltic pump (model CP-78002-10; Cole-Parmer) for 58 d. This infusion period length was chosen to allow adequate time for body composition of steers to respond to treatments (Freetly et al., 1995; McLeod et al., 2001; McLeod et al., 2007). Two containers of treatment suspensions in aqueous solution were prepared daily immediately before infusion for use over 12 h intervals. Suspensions were maintained with continuous stirring by an electric mixer (Arrow 1750; Arrow Engineering Company) and delivered at an infusion rate of 568 ± 31 mL/h. Composition of cornstarch suspensions included 884 g of raw cornstarch (Clinton 184 Food Corn Starch; ADM Corn Processing), CrEDTA (0.075 g Cr/L) as an indigestible flow marker (Binnerts et al., 1968), and deionized H_2O. The pH of the glutamic acid suspension was adjusted to near 7 with addition of 42.8 g of 40% (wt/wt) NaOH. The infusates were prepared daily by weight and the amount infused was determined by the weight of residual infusate after each 12-h period. To prevent accumulation of residual infusate, 100 mL of water was flushed through the tubing every 12 h.

2.3.3. Sample collection and analysis

At the conclusion of the 58 d infusion period, steers were weighed just before slaughter. Then steers were slaughtered via captive bolt stunning and exsanguination and gastrointestinal tracts were removed, weighed, and digestive organs were separated for individual weights and subsample collection. The pyloric and ileocecal junctions were cut to separate the small intestine from the abomasum and cecum, respectively, and the mesentery was cut to separate the entire

small intestine from the viscera. The small intestine was measured in length by looping the intestine between pegs at either end of a 1.5-m board (Kreikemeier et al., 1990). The small intestine was separated into the duodenum (0.1 to 1.1 m caudal to the pyloric sphincter), jejunum (first half of non-duodenal small intestine), and ileum (second half of non-duodenal small intestine) (Liao et al., 2008). The pancreas was trimmed of excess adipose tissue. Mass of the pancreas was recorded and a pancreatic subsample was collected. Twenty-five cm transverse sections of intestinal tissue were weighed and collected from the mid-point of duodenum, jejunum, and ileum, respectively. Tissue masses are reported as fresh weight as previous data has suggested little difference in fresh and dry tissue weights (Swanson et al., 1999; Swanson et al., 2000a; Scheaffer et al., 2004). Pancreatic and intestinal subsamples were flash-frozen in liquid nitrogen and then stored at −80°C until further analyses (Swanson et al., 2008a).

The intestinal samples were thawed and scraped with a microscope glass slide. Pancreatic (253 ± 3.88 mg) and intestinal (527 ± 4.57 mg) tissue were weighed and diluted with 2.25 mL and 2.0 mL of 9 g/L NaCl solution, respectively, in a 10-mL storage tube. Pancreatic and intestinal samples were homogenized (Kinematica Polytron PT 10/35; Brinkmann Instruments Inc.) and protein concentration was measured using the bicinchoninic acid (BCA) procedure (Pierce BCA Protein Assay Kit, Cat no. 23225; Thermo Fisher Scientific Inc.) using bovine serum albumin as the standard (Smith et al., 1985). Activity of α-amylase was determined using the procedure from Wallenfels et al. (1978) that was adapted for analysis of pancreatic tissue. Alpha-amylase activity was assayed kinetically with a commercially available reagent (Amylase Reagent Set, Cat no. A533; Teco Diagnostics) containing p-nitrophenyl-D-maltoheptaoside as the substrate. The reagent was reconstituted [0.225 mM p-nitrophenyl-D-maltoheptaoside; 6,250 U/L α-glucosidase (*Saccharomyces cerevisiae*); 2,500 U/L glucoamylase (*Rhizopus Sp.*); 12.5 mM NaCl; 1.25 mM

CaCl2; 12.5 mM buffer] with 24 mL of distilled water and pre-warmed to 39°C in an incubator. Pancreatic trypsin activity was assayed kinetically using the methods described by Geiger and Fritz (1986) using N-alpha-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (CAS: 911-77-3; Thermo Fisher Scientific Inc.) as the substrate. Five hundred microliters of the pancreas homogenate was combined with 500 µL of 200 U/L enterokinase (CAS: 9014-74-8; Sigma-Aldrich Co.) to activate trypsinogen (Glazer and Steer, 1977) in a 1.5-mL centrifuge tube. Tubes were incubated at 30°C for 60 min in a water bath, followed by drenching in an ice bath.

Each intestinal segment was assayed for brush border carbohydrases: maltase, isomaltase, sucrase, and glucoamylase. Intestinal disaccharidases (maltase, isomaltase, sucrase) were assayed using modified methods of Dahlqvist (1964). Maltose, isomaltose, and sucrose were used as the substrates, respectively. Soluble starch was the substrate used to analyze for glucoamylase activity (Kidder et al., 1972). Five-hundred microliters of the intestinal homogenate were combined with 500 µL of 25 mM KPO_4 buffer (Turner and Moran, 1982) and 100 µL of 60 mM substrate solution in a 1.5-mL centrifuge tube. Tubes were incubated for 30 min at 39°C in a water bath. The reaction was terminated by heating tubes for 2 min in a 90°C water bath, followed by drenching in an ice bath. Tubes were then centrifuged at 4000 × g for 20 min at 4°C. Liberated glucose was measured using the hexokinase/glucose-6-phosphate dehydrogenase procedure (Farrance, 1987).

All assays were optimized to achieve maximal velocity through the linear concentration range. Analyses were adapted for use on a microplate spectrophotometer (Synergy H1; BioTek Instruments, Inc.) at 39°C. One unit (U) of enzyme activity equals 1 µmol of substrate hydrolyzed per min. Blanks of each intestinal homogenate and substrate solution were quantified for endogenous glucose concentrations and subtracted from the total amount of product produced per min. Pancreatic digestive enzyme activity data are expressed as U/g pancreas (concentration), U/g

protein (specific activity), kU/pancreas (content), and U/kg BW (content relative to BW). Small intestinal digestive enzyme activity data are expressed as U/g mucosa (concentration) and U/g protein (specific activity).

2.3.4. Statistical analysis

All variables were tested for normality and homogeneity of variances. The data were analyzed as a randomized complete block design using the GLM procedure of Statistical Analysis System (SAS 9.4) with animal as the experimental unit and slaughter group as the blocking factor. Small intestinal digestive enzyme activity data were analyzed for effects of treatment and slaughter group within each site of the small intestine (duodenum, jejunum, ileum). Mean separation was conducted for data if the probability of a greater F-statistic in the ANOVA was significant for the effect of treatment. Individual mean differences were evaluated using the least significant difference approach, protected by a significant F-test. Results were considered significant if $P \leq 0.05$. Tendencies were declared when $0.05 < P \leq 0.10$.

2.4. Results

The BW of steers at slaughter ($P = 0.003$) was greater in steers infused with casein compared to control or glutamic acid infused steers (Table 2). Small intestinal length and mass:length were not influenced by treatment. Small intestinal and pancreatic mass (g or % of BW) were not influenced by duodenal infusion treatments.

Table 2. Least square means for the effects of duodenal infusions of starch with casein or glutamic acid on body weights, pancreatic and small intestinal mass, and small intestinal length.[1]

Item	Treatment			SEM[2]	*P*-value[3]
	Control	Glutamic Acid	Casein		
Initial BW, kg	179	181	175	5.14	0.60
BW at slaughter, kg	200[a]	206[a]	239[b]	7.42	0.003
Small intestinal mass:length, g/cm	1.18	1.21	1.09	0.105	0.64
Small intestinal length, m	30.5	29.0	31.0	1.62	0.52
Small intestinal mass					
kg	3.58	3.44	3.34	0.195	0.64
% of BW	1.78	1.71	1.43	0.129	0.14
Pancreatic mass					
g	199	205	208	16.7	0.88
% of BW	0.0992	0.100	0.0902	0.00856	0.63

[1]Abbreviations: BW, body weight.

[2]Standard error of the mean (Casein, n = 6).

[3]Means with different superscripts within a row differ ($P \leq 0.05$).

Pancreatic protein concentration, content, and content relative to BW did not differ among treatments (Table 3). Duodenal casein infusion increased α-amylase concentration ($P = 0.05$), specific activity ($P = 0.02$), content ($P = 0.03$), and content relative to BW ($P = 0.01$) compared to control and glutamic acid steers. Pancreatic trypsin activity was not affected by treatment. The α-amylase:trypsin ratio was greatest ($P < 0.001$) in steers infused with casein.

Table 3. Least square means for the effects of duodenal infusions of starch with casein or glutamic acid on pancreatic mass, protein concentration, and digestive enzyme activity in steers.[1]

Item	Treatment			SEM[2]	*P*-value[3]
	Control	Glutamic Acid	Casein		
Protein					
mg/g pancreas	102	99.0	79.8	9.80	0.23
g/pancreas	20.4	21.0	16.3	2.36	0.29
g/kg BW	93.4	94.1	63.0	12.2	0.12
α-Amylase					
U/g pancreas	82.0[a]	71.3[a]	217[b]	44.4	0.05
U/g protein	758[a]	764[a]	2500[b]	456	0.02
kU/pancreas	16.5[a]	14.5[a]	48.1[b]	9.77	0.03
U/kg BW	81.3[a]	69.6[a]	218[b]	36.1	0.01
Trypsin					
U/g pancreas	5.80	5.44	6.72	12.9	0.67
U/g protein	56.4	56.9	85.1	11.8	0.13
kU/pancreas	1.16	1.12	1.43	0.277	0.63
U/kg BW	5.72	6.71	5.37	1.11	0.64
α-Amylase:trypsin	12.8[a]	13.6[a]	41.8[b]	4.75	<0.001

[1]Abbreviations: BW, body weight; U, unit.

[2]Most conservative standard error of the mean (Casein, n = 6).

[3]Means with different superscripts within a row differ ($P \leq 0.05$).

Duodenal mass (g or % of BW) and protein concentration were not affected by different infusion treatments (Table 4). Duodenal isomaltase and glucoamylase activities were not influenced by duodenal infusion treatment. Duodenal maltase concentration ($P = 0.03$) and specific activity ($P = 0.02$) were greater with glutamic acid compared to control and casein.

Table 4. Least square means for the effects of duodenal infusions of starch with casein or glutamic acid on duodenal mass, protein concentration, and carbohydrase activities in steers.[1]

Item	Treatment			SEM[2]	*P*-value[3]
	Control	Glutamic Acid	Casein		
Duodenal mass					
g	177	189	205	11.3	0.21
% of BW	0.0874	0.0942	0.0870	0.00483	0.33
Protein					
mg/g duodenum	64.3	68.5	72.0	3.51	0.25
Isomaltase					
U/g duodenum	0.193	0.219	0.177	0.0466	0.74
U/g protein	3.00	3.10	2.51	0.712	0.80
Maltase					
U/g duodenum	0.157[a]	0.357[b]	0.125[a]	0.0827	0.03
U/g protein	2.41[a]	5.74[b]	1.47[a]	1.29	0.02
Glucoamylase					
U/g duodenum	0.163	0.220	0.130	0.0707	0.54
U/g protein	2.44	3.18	1.79	1.06	0.55

[1]Abbreviations: BW, body weight; U, unit.

[2]Standard error of the mean (Casein, n = 6).

[3]Means with different superscripts within a row differ ($P \leq 0.05$).

Duodenal infusion treatments did not influence jejunal mass (g or % of BW) or protein concentration (Table 5). Jejunal isomaltase concentration tended to be greater ($P = 0.10$) in steers infused with casein than glutamic acid or control. Isomaltase specific activity in the jejunum was not influenced by treatment. Casein infusion increased jejunal maltase concentration ($P = 0.03$) and specific activity ($P = 0.02$) compared to control and glutamic acid. Glucoamylase concentration in the jejunum tended to be greater ($P = 0.06$) in steers infused with casein compared to control or glutamic acid. Jejunal glucoamylase specific activity increased ($P = 0.03$) with casein infusion compared with control or glutamic acid.

Table 5. Least square means for the effects of duodenal infusions of starch with casein or glutamic acid on jejunal mass, protein concentration, and carbohydrase activities in steers.[1]

Item	Treatment			SEM[2]	*P*-value[3]
	Control	Glutamic Acid	Casein		
Jejunal mass					
kg	1.54	1.46	1.51	0.142	0.88
% of BW	0.749	0.730	0.638	0.0682	0.46
Protein					
mg/g jejunum	67.9	65.3	74.7	5.04	0.35
Isomaltase					
U/g jejunum	1.66	1.21	2.30	0.390	0.10
U/g protein	24.1	17.8	31.6	5.15	0.12
Maltase					
U/g jejunum	1.84[a]	1.42[a]	3.14[b]	0.455	0.03
U/g protein	26.7[a]	21.6[a]	43.5[b]	5.71	0.02
Glucoamylase					
U/g jejunum	0.183	0.194	0.390	0.0664	0.06
U/g protein	2.74[a]	2.74[a]	5.41[b]	0.784	0.03

[1]Abbreviations: BW, body weight; U, unit.

[2]Standard error of the mean (Casein, n = 6).

[3]Means with different superscripts within a row differ ($P \leq 0.05$).

Ileal mass (g) was not influenced by duodenal infusions of control, casein, or glutamic acid (Table 6). Ileal mass tended to be less ($P = 0.07$) in steers infused with casein compared to control or glutamic acid. Treatment did not influence ileal protein concentrations or activity of isomaltase and glucoamylase in the ileum; however, maltase concentration and specific activity in the ileum tended to be greater ($P = 0.10$) in steers infused with casein than control or glutamic acid. Sucrase activity was not detected in the duodenum, jejunum, or ileum.

Table 6. Least square means for the effects of duodenal infusions of starch with casein or glutamic acid on ileal mass, protein concentration, and carbohydrase activities in steers.[1]

Item	Treatment			SEM[2]	*P*-value[3]
	Control	Glutamic Acid	Casein		
Ileal mass					
kg	1.70	1.77	1.45	0.129	0.18
% of BW	0.836	0.883	0.621	0.0830	0.07
Protein					
mg/g ileum	65.3	61.2	68.8	3.32	0.22
Isomaltase					
U/g ileum	0.853	1.37	1.99	0.464	0.20
U/g protein	12.6	22.6	29.1	7.08	0.20
Maltase					
U/g ileum	0.934	1.44	2.38	0.476	0.10
U/g protein	13.9	23.5	35.0	7.01	0.10
Glucoamylase					
U/g ileum	0.669	0.851	1.07	0.174	0.23
U/g protein	10.3	13.7	15.8	2.58	0.25

[1]Abbreviations: BW, body weight; U, unit.

[2]Standard error of the mean (Casein, n = 6).

[3]Means with different superscripts within a row differ ($P \leq 0.05$).

2.5. Discussion

Small intestinal starch digestion in ruminants is potentially limited by inadequate production of pancreatic (α-amylase) and small intestinal (maltase, isomaltase, glucoamylase) carbohydrases (2 Little et al., 1968; Owens et al., 1986; Kreikemeier and Harmon, 1995; Brake and Swanson, 2018). Pancreatic and small intestinal carbohydrases contribute to luminal and membrane-bound hydrolysis of starch to oligosaccharides and disaccharides and then to glucose. However, regulation of post-ruminal digestive enzymes in cattle is complex (Swanson et al., 2000b) and there are numerous neurohormonal signaling mechanisms involved in digestive enzyme synthesis and secretion (Swanson et al., 2003). Possible mechanisms for changes in

digestive enzyme activity include changes in tissue mass, mRNA expression, protein concentrations or secretions, or post-translational modifications (Wang et al., 1998). Pancreatic digestive enzymes respond to changes in nutrient intake (Kreikemeier et al., 1990) and changes in tissue mass (Wang et al., 1998; Swanson et al., 2002b). While non-ruminant digestive enzymes typically increase proportionally to substrate (Brannon, 1990), pancreatic digestive enzymes in ruminants respond differently to diet and luminal nutrient supply (Harmon, 1992). Moreover, post-ruminal carbohydrate supply as starch (Walker and Harmon, 1995; Wang and Taniguchi, 1998; Swanson et al., 2002a) or glucose (Swanson et al., 2002a) has been shown to decrease pancreatic α-amylase activity in ruminants.

Previous experiments have demonstrated that post-ruminal protein supply can increase pancreatic α-amylase activity (Swanson et al., 2002b; Richards et al., 2003; Swanson et al., 2004) and small intestinal starch digestion in cattle (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016) and sheep (Mendoza and Britton, 2003). However, differences in ileal ethanol-soluble starch flow has led to the speculation that casein and glutamic acid modulate improvements in intestinal starch digestion by different mechanisms. In the current study, casein dramatically increased α-amylase activity which is in agreement with previous studies using dietary (Kreikemeier et al., 1990; Swanson et al., 2008b; Lee et al., 2013) or abomasal infusion (Swanson et al., 2002b; Richards et al., 2003; Swanson et al., 2004) models. Regulation of pancreatic digestive enzymes in ruminants seems to be more specific to α-amylase, as few studies have demonstrated differences in pancreatic protease activities independent of changes in tissue mass (Swanson et al., 2002b; Swanson et al., 2004; Keomanivong et al., 2017).

Interestingly, duodenal casein supply also increased maltase, glucoamylase, and isomaltase activities in the jejunum in the current experiment. It is unclear if increases in small intestinal

carbohydrases with casein infusion are directly related to increased luminal protein flow, as peptide hydrolysates and free amino acids from casein may influence neuroendocrine signaling to increase carbohydrase activity in the small intestine. Alternatively, increased luminal protein flow may cause increases in carbohydrase activities indirectly. Increased flow of luminal substrates (maltose, isomaltose, limit dextrins) as a result of greater hydrolysis of amylose and amylopectin in response to increases in pancreatic α-amylase activity may modulate increases in small intestinal carbohydrase activities. Indeed, Rodriguez et al. (2004) suggested that increases in luminal substrate flow may increase small intestinal carbohydrase activities indirectly in cattle. Further delineations are needed to understand the hydrolytic limit to small intestinal starch digestion and regulation of post-ruminal digestive enzyme activity in cattle.

Brake et al. (2014b) demonstrated that a combination of non-essential amino acids similar to the non-essential amino acid profile of casein increased small intestinal digestion while essential amino acids did not. Additionally, duodenal glutamic acid infusions have resulted in increases in small intestinal starch digestion similar to casein (Brake et al., 2014b; Blom et al., 2016). With exception of duodenal maltase, it appears that glutamic acid failed to increase most enzymes important to small intestinal starch digestion. One major difference from previous studies where glutamic acid increased starch digestion (Brake et al., 2014b; Blom et al., 2016) was the length of the infusion period. In the current study, steers were infused with starch or a combination of starch and casein or glutamic acid for 58 d while previous experiments only evaluated small intestinal starch digestion up to 6-d (Brake et al., 2014b) or 12-d (Blom et al., 2016). This may suggest that glutamic acid is not effective in improving small intestinal starch digestion long-term. A greater understanding of intestinal sensing and signaling in cattle is needed to identify specific

mechanisms that may limit long-term improvements in small intestinal starch digestion in response to glutamic acid.

There have been multiple reports (Huber et al., 1961; Siddons, 1968; Kreikemeier et al., 1990) that sucrase activity is absent in the ruminant small intestine. Furthermore, abomasal infusions of sucrose does not induce mucosal sucrase activity in lambs (Swanson and Harmon, 1997). Likewise in the current study, sucrase activity was undetectable in the duodenum, jejunum, and ileum. Using the sucrase-deficient shrew as a model for sucrase deficiency, Nichols et al. (2017) determined that the absence of sucrase activity results in decreased starch digestion. It should be noted that maltase and isomaltase activities were not affected in their sucrase-deficient shrew model. This may suggest that the absence of sucrase activity in ruminants plays a role in the reduced capacity for small intestinal starch hydrolysis. More research on the transcriptional regulation and post-translational modification of sucrase-isomaltase is needed to better understand how the absence of sucrase activity may influence carbohydrate digestion in the ruminant small intestine.

2.6. Conclusions

Duodenal casein supply increased both pancreatic and small intestinal carbohydrase activities in steers. Glutamic acid increased duodenal maltase activity but failed to induce changes in pancreatic α-amylase or other small intestinal carbohydrases. Data from the current study suggest that casein may modulate increases in small intestinal starch digestion by increasing post-ruminal carbohydrase activities in cattle. A greater understanding of the complex relationships between luminal nutrient flows, gut signaling, and digestive enzyme regulation is needed to identify mechanisms to potentially increase small intestinal starch digestion in cattle.

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3. INFLUENCE OF DIETARY FRUCTOSE ON VISCERAL ORGAN MASS, CARBOHYDRASE ACTIVITY, AND mRNA EXPRESSION OF GENES INVOLVED IN CARBOHYDRATE ASSIMILATION IN NEONATAL CALVES

3.1. Abstract

Ten Holstein steer calves (BW = 41.2 ± 1.45 kg) were fed equal amounts of milk-replacer twice daily at 2.0% of BW and assigned to one of two dietary treatment groups: 1) milk-replacer (control; n = 6) or 2) milk-replacer + fructose (fructose; n = 4). Dietary fructose was provided at 2.2 g/kg of BW. Calves were fed dietary treatments for 28 days with blood sampled every 7 days. On day 29, calves were slaughtered, visceral weights were recorded, and the pancreas and small intestine were collected. Pancreatic and small intestinal carbohydrase activities were assayed. Quantitative, real-time PCR was conducted for small intestinal mRNA expression of nutrient transporters (GLUT5, SGLT1, GLUT2), digestive enzymes (lactase, maltase-glucoamylase, sucrase-isomaltase), and ketohexokinase. Data were analyzed using MIXED procedures in SAS. Dietary fructose decreased (P=0.004) serum glucose concentration but did not affect serum urea-N concentration. Feeding fructose increased (P=0.01) serum NEFA concentrations on day 0 and 7, but not on other days. Feeding fructose increased (P<0.05) the mass of the small intestine and liver and tended to increase (P=0.06) kidney mass. Pancreatic α-amylase and small intestinal isomaltase, maltase, and lactase activities were not influenced by dietary fructose. Sucrase activity was not detected in the small intestine. Fructose increased (P=0.05) small intestinal glucoamylase concentration by 30% and increased (P<0.001) maltase-glucoamylase mRNA expression by 6.8-fold. Dietary fructose did not influence mRNA expression of GLUT5, SGLT1, GLUT2, or ketohexokinase. In calves fed fructose, small intestinal lactase mRNA expression increased (P=0.001) by 3.1-fold. Sucrase-isomaltase mRNA expression in the small intestine decreased

(P<0.001) by 5.1-fold with dietary fructose. Dietary fructose supply influences nutrient utilization, visceral organ mass, and digestive enzyme mRNA expression and activity in neonatal calves.

3.2. Introduction

The digestive tract of neonatal ruminants allows for flow of digesta to the abomasum, and subsequently, the small intestine without pre-gastric fermentation. Carbohydrate utilization in pre-ruminant calves has been studied extensively and it is well established that young calves readily utilize glucose, galactose, and lactose but not sucrose, maltose, or starch (Dollar and Porter, 1957; Siddons et al., 1969). Dietary sucrose cannot be enzymatically digested in the calf small intestine (Huber et al., 1961; Siddons et al., 1969) because of the absence of sucrase activity in the brush border membrane of the small intestine (Dollar and Porter, 1957; Huber et al., 1961; Siddons, 1968). In humans, dietary fructose supplementation has been shown to induce sucrase activity in patients with congenital sucrase-isomaltase deficiency (Greene et al., 1972).

The pancreas and small intestine have important roles in post-ruminal carbohydrate digestion and absorption and there is limited information on regulation of digestive enzymes and nutrient transporters in response to luminal carbohydrate flow in ruminants. Previous research in cattle has demonstrated that post-ruminal carbohydrate supply as starch or glucose decreases pancreatic α-amylase activity (Swanson et al., 2002b). Four proteins in the small intestine possess carbohydrase activity: sucrase-isomaltase, maltase-glucoamylase, lactase, and trehalase. Conflicting reports demonstrate that differences in the composition of luminal carbohydrate flow can alter the regulation of small intestinal disaccharidases. Specifically, Rodriguez et al. (2004) demonstrated that abomasal infusions of glucose increased maltase activity in steers. In contrast, Gilbert et al. (2015) found that replacing 18% of the lactose in milk-replacer with maltose or maltodextrins decreased maltase activity in the small intestine in calves. Indeed, a greater

understanding of the regulatory mechanisms involved in digestive enzyme activity are needed to potentially improve post-ruminal carbohydrate digestion.

Fructose is a monosaccharide that is passively transported through the apical membrane of the small intestine by the facilitated glucose transporter 5 (GLUT5) (Ferraris et al., 2018). Dietary fructose supplementation has been shown to increase GLUT5 expression and enhance intestinal fructose transport in neonatal rats (Shu et al., 1997). Zhao et al. (1993) found that GLUT5 expression in the intestine is significantly lower than in the liver or kidney in cattle. In contrast, many authors have reported that the greatest amount of GLUT5 expression occurs in the small intestine in humans, rats, mice, rabbits, chickens, and horses (Douard and Ferraris, 2008). Nutritional regulation of GLUT5 by fructose requires luminal presence of fructose in the human and rodent small intestine (Shu et al., 1998; Ferraris et al., 2018) and GLUT5 expression is directly proportional to intestinal luminal fructose concentration (Shu et al., 1997). In cattle, ruminal or abomasal infusions of partially hydrolyzed starch did not affect GLUT5 mRNA expression in the duodenum, jejunum, or ileum (Liao et al., 2010).

Many previous research studies have suggested that the liver is the principal site of fructose metabolism (Caliceti et al., 2017; Jegatheesan and De Bandt, 2017; Lee and Cha, 2018). However, recent research has demonstrated that a significant proportion of dietary fructose is metabolized in the small intestine in rats (Jang et al., 2018). Yet, fructose has a physiological role in many tissues including the kidney (Johnson et al., 2010), testis (Angulo et al., 1998), and placenta (White et al., 1979; Crouse et al., 2019). Fructose is phosphorylated by ketohexokinase to fructose-1-phosphate which bypasses the main regulatory steps in glycolysis (Elliott et al., 2002), leading to a relatively unregulated source of acetyl-CoA, an important substrate for the tricarboxylic acid cycle, fatty acid synthesis, and lipogenesis (Samuel, 2011; Crescenzo et al., 2014). It is unclear how dietary

fructose supplementation affects gastrointestinal development and small intestine digestive and absorptive function in ruminants. Therefore, the objective of this study was to evaluate the effects of dietary fructose on visceral organ development, nutrient utilization, and mRNA expression of digestive enzymes and nutrient transporters in the small intestine.

3.3. Materials and methods

All animal procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee. A17037

3.3.1. Animals and diets

Twelve Holstein steer calves (initial BW = 40.5 ± 1.80 kg) were housed and fed in individual pens (0.91 × 1.2 m) in a temperature-controlled environment (14°C) with Tenderfoot flooring at the North Dakota State University Animal Nutrition and Physiology Center. Calves were fed milk-replacer at 2.0% of BW daily (Nurture Calf Formula Basic Bov Medicated; Provimi, Inc., Brookville, OH) in equal amounts at 0730 and 1630 during a 7-d adaptation period. During the adaptation period, calves were trained to consume feed from a bucket and acclimated to their pens. Following the adaptation period, calves were randomly assigned to dietary treatment groups: milk-replacer (control) or milk-replacer + fructose (fructose). Fructose (Archer Daniels Midland Co., Chicago, IL) was fed at 2.2 g/kg BW. Calves had *ad libitum* access to water. Approximately 1.5 L of warm water was measured into each bucket and the fructose was added and dissolved for the fructose treatment. Milk-replacer was added and diets were hand-mixed for 30 s with a whisk. Bulk samples of the milk-replacer were obtained and analyzed for chemical composition (93.25% DM, 93.44% OM, 21.68% CP, 9.19% CF, 4.63% starch, 0.70% Ca, 0.51% P) including DM, and ash (AOAC, 1990), N using a Kjeltec Auto 1030 Analyzer (Foss Tecator AB, Höganäs, Sweden; AOAC, 1990). Crude protein was calculated by multiplying N concentration x 6.25. Crude fat, Ca,

and P concentrations were analyzed using standard procedures (AOAC, 1990). Starch content was analyzed using the methods of Herrera-Saldana and Huber (1989). Calves were fed dietary treatments over a 28-d period and then slaughtered. Two calves in the fructose treatment were removed from the study due to illness and all data pertaining to those calves were excluded from analyses.

3.3.2. Sample collection

Blood samples were collected by jugular venipuncture on d 0, 7, 21, and 28 before and 2 hr after the morning feeding. Blood samples were allowed to clot for 20 min and centrifuged at 2000 × *g* for 20 min at 4°C and serum was stored at -20°C for later analysis. Glucose concentrations in serum samples were measured using the hexokinase/glucose-6-phosphate dehydrogenase procedure (Farrance, 1987). Serum urea-N concentrations were analyzed using the QuantiChrom Urea Assay Kit (BioAssay Systems, Hayward, CA) containing *o*-phthaldialdehyde and primaquine diphosphate, based on the procedures of Jung et al. (1975). Serum non-esterified fatty acid (NEFA) concentrations were determined using the modified methods of Eisemann et al. (1988) with a commercial enzymatic kit (HR Series NEFA-HR; Fujifilm Waco Diagnostics, Mountain View, CA). Serum glucose, urea-N, and NEFA procedures were adapted for use on a microplate spectrophotometer (Synergy H1; BioTek Instruments, Inc., Winooski, VT). L-lactate concentrations in serum were analyzed using the lactate dehydrogenase procedure (Gutmann and Wahlefeld, 1974) on a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Inc., Brea, CA).

Body weights were measured every 7 days with a two-day weight recorded at the start of the collection period. On day 29, each calf was fed, in 30-min increments, and slaughtered 2 h after feeding via captive bolt and exsanguination. Viscera were removed, weighed, and separated for individual weights and subsample collection. Omental, mesenteric, and perirenal fat was

removed, composited, and weighed. Visceral fat was calculated as the sum of omental, mesenteric, and perirenal fat masses (Favre et al., 2017). The gastrointestinal tract, foregut (reticulorumen, omasum, and abomasum), small intestine, large intestine, pancreas, liver (gallbladder removed), spleen, heart, kidney, and lung masses were recorded on a wet-tissue basis after removing digesta. The small intestine was removed at the pyloric and ileocecal junctions and cut into four segments: duodenum, proximal jejunum, distal jejunum, and ileum. Demarcations of the four sections of the small intestine were made using the modified methods of Bauer et al. (2001b). Briefly, one meter sections were measured from the pylorus and ileocecal junction to represent portions of the duodenum and ileum, respectively. The remainder of the small intestine was measured, and cut in half to represent the proximal and distal jejunum. One meter segments were cut and sampled from the midpoint of the previously cut sections. Each one meter intestinal segment was weighed, and scraped using a glass microscope slide (Siddons, 1968). The tissue remaining after the mucosal scrape was weighed and mucosal density calculated. The longissimus dorsi was removed from the 12^{th} rib, and trimmed of all external fat and epimysial connective tissue. Subsamples of the pancreas, liver, longissimus dorsi (12^{th} to 13^{th} rib), duodenum, proximal jejunum, distal jejunum, and ileum were flash-frozen in isopentane (2-Methylbutane, J. T. Baker, Center Valley, PA) that was super-cooled in liquid nitrogen and then stored at −80 °C until further analyses (Keomanivong et al., 2016). Crude fat concentrations of the liver, longissimus dorsi, and pancreas were measured using standard procedures (AOAC, 1990).

3.3.3. Quantitative, real-time PCR

Samples of duodenal, proximal and distal jejunal, and ileal mucosa from each animal were used for quantitative real-time PCR analysis. The RNA was extracted and purified using the Qiagen RNeasy Mini Kit and genomic DNA (gDNA) was eliminated using a gDNA Eliminator

column (Qiagen Inc., Germantown, MD). Ribonucleic acid concentrations were quantified using a fluorometer (Qubit 3.0; Thermo-Fisher Scientific, Inc., Waltham, MA) and RNA quality was assessed using a microplate spectrophotometer (Synergy H1; BioTek Instruments, Inc., Winooski, VT) at wavelengths 230, 260, and 280 nm. Complementary DNA was synthesized from 1000 ng/µL RNA for each sample utilizing the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) containing reverse transcriptase. Hydrolysis probes were utilized for each gene containing 6-carboxyfluorescein (FAM) dye and minor groove binder (MGB) label. Hydrolysis probes were sourced (TaqMan; Applied Biosystems, Grand Island, NY), except maltase-glucoamylase which was custom designed (PrimeTime Std qPCR Assay; Integrated DNA Technologies, Inc., Coralville, IA) using the NCBI reference sequence from GenBank (XM_024991197.1; Bethesda, MD) to produce amplicons bridging exon-exon junctions (Cui et al., 2007). Amplification efficiency was assessed for each primer and probe set by generating a five point 10-fold serial dilution standard curve (Table 7). Gene expression was quantified using a 7500, Fast, Real-Time PCR System (Applied Biosystems, Grand Island, NY), with TaqMan Fast Advanced Master Mix, using a 20 µL total reaction volume (10 µL Master Mix; 7 µL nuclease-free water; 2 µL cDNA; 1 µL probe) for all genes.

Systematic and common gene names, probe assay numbers, and primer efficiencies are reported in Table 7. Quantitative, real-time PCR analysis (intraplate CV = 0.562, interplate CV = 0.357) was conducted with 40 oscillating cycles of denaturing (95°C for 20s) and annealing/extension (60°C for 30 s) temperatures. Multiple reference genes were evaluated for the stability of expression across treatments and intestinal segments: beta-actin, glyceraldehyde-3-phosphate dehydrogenase, RNA polymerase II subunit A, and succinate dehydrogenase complex flavoprotein subunit A. Stability was by geNorm analysis (Vandesompele et al., 2002) using

qbase+ software (Biogazelle, Zwijnarrde, Belgium). The gene with the lowest M-value (<0.05) was glyceraldehyde-3-phosphate dehydrogenase, which was therefore selected as the reference gene. Experimental genes of interest were ketohexokinase, solute carrier family 2 member 5 (GLUT5), solute carrier family 5 member 2 (SGLT1), solute carrier family 2 member 2 (GLUT2), sucrase-isomaltase, maltase-glucoamylase, and lactase. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with glyceraldehyde-3-phosphate dehydrogenase used as the reference gene.

Table 7. Selected genes in the small intestine that were analyzed using quantitative, real-time PCR.

Name	Gene	Assay No.	Primer Efficiency
Solute carrier family 2 member 5 (GLUT5)	*SLC2A5*	Bt03258299_g1	92.432
Solute carrier family 2 member 2 (GLUT2)	*SLC2A2*	Bt03258678_m1	92.511
Solute carrier family 5 member 1 (SGLT1)	*SLC5A1*	Bt03223889_m1	105.067
Sucrase-isomaltase	*SI*	Bt03259068_m1	91.756
Maltase-glucoamylase[1]	*MGAM*	XM_024991197.1	97.247
Lactase	*LCT*	Bt04285580_m1	96.149
Ketohexokinase	*KHK*	Bt03249204_m1	99.362
Beta-actin	*ACTB*	Bt03279174_g1	94.901
RNA polymerase IIA	*POLR2A*	Bt04294167_m1	97.892
Succinate dehydrogenase complex flavoprotein subunit A	*SDHA*	Bt04307498_m1	101.496
Glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	Bt03279174_g1	97.892

[1]Maltase-glucoamylase was assayed using a PrimeTime Assay Standard Probe from Integrated DNA technologies. Probe: 5'-/56FAM/CAGCATTCC/ZEN/ATCTGGCACCTCTGA/3IABkFQ/-3'; Reverse Primer: 5'-CGGCGAATTTCAATCCCAAAT-3'; Forward Primer: 5'-GTTCCAGTCCCTCTCAACATAC-3'

3.3.4. Digestive enzyme activity

Pancreatic (0.50 ± 0.007 g) or intestinal (0.51 ± 0.021 g) tissue were weighed and diluted with 9 g/L NaCl solution in a 10-mL storage tube. Samples were homogenized (Kinematica Polytron PT 10/35; Brinkmann Instruments Inc.) and protein concentrations were measured using the bicinchoninic acid procedure (Pierce BCA Protein Assay Kit, Cat no. 23225; Thermo Fisher Scientific Inc.) using bovine serum albumin as the standard (Smith et al., 1985). Activity of α-amylase was determined using the procedure from Wallenfels et al. (1978) that was adapted for analysis of pancreatic tissue. Alpha-amylase activity was assayed kinetically with a commercially available reagent (Amylase Reagent Set; Cat no. A533; Teco Diagnostics, Anaheim, CA) containing p-nitrophenyl-D-maltoheptaoside as the substrate. The reagent was reconstituted (0.225 mM p-nitrophenyl-D-maltoheptaoside; 6,250 U/L α-glucosidase (*Saccharomyces cerevisiae*); 2,500 U/L glucoamylase (*Rhizopus Sp*.); 12.5 mM NaCl; 1.25 mM CaCl2; 12.5 mM buffer) with 24 mL of distilled water and pre-warmed to 39°C in an incubator.

Each intestinal segment was assayed for brush border carbohydrases: lactase, maltase, isomaltase, sucrase, and glucoamylase. Intestinal disaccharidases (lactase, maltase, isomaltase, and sucrase) were assayed using modified methods of Dahlqvist (1964). Lactose, maltose, isomaltose, and sucrose were used as the substrates, respectively. Soluble starch was the substrate used to analyze for glucoamylase activity (Kidder et al., 1972). Five-hundred microliters of the intestinal homogenate was combined with 500 µL of 25 mM KPO_4 buffer (Turner and Moran, 1982) and 100 µL of 60 mM substrate solution in a 1.5-mL centrifuge tube. Tubes were incubated for 30 min at 39°C in a water bath. In a preliminary assay, the sucrase incubation time was increased to 24 h because of failure to detect activity after 30 min or 1 h. The reaction was terminated by heating tubes for 2 min in a 90°C water bath, followed by drenching in an ice bath.

Tubes were then centrifuged at 4000 × g for 20 min at 4°C. Liberated glucose was measured using the hexokinase/glucose-6-phosphate dehydrogenase procedure (Farrance, 1987).

All assays were optimized to achieve maximal velocity through the linear concentration and adapted for use on a microplate spectrophotometer (Synergy H1; BioTek Instruments, Inc., Winooski, VT) at 39°C. One unit (U) of enzyme activity equals 1 μmol of substrate hydrolyzed per min. Blanks of each intestinal homogenate and substrate solution were quantified for endogenous glucose concentrations and subtracted from the total amount of product produced per min. Enzyme activity data are expressed as U/g tissue (concentration) and U/g protein (specific activity).

3.3.5. Statistical analysis

Data were analyzed as a completely randomized design using SAS (SAS 9.4, SAS Institute, Cary, NC). Normality was assessed using the Shapiro-Wilk test of the UNIVARIATE procedure. The TTEST procedure of SAS was used to analyze visceral weights and fat concentrations for homogeneity of variances (Folded F-test) and effects of treatment. Body weight, and serum metabolite data were analyzed using the repeated measures statement of the MIXED procedure of SAS for effects of day, time, treatment, and their interactions. Small intestinal mass, protein concentration, mucosal density, carbohydrase activity, and mRNA expression data were analyzed using the repeated measures statement of the MIXED procedure for effects of site, treatment, and the site × treatment interaction. Least square means were separated using the least significant difference approach, protected by a significant F-test. Appropriate (minimize information criterion) covariance structures were utilized (Wang and Goonewardene, 2004). Results were considered significant if $P \leq 0.05$. Tendencies were declared when $0.05 < P \leq 0.10$. For small

intestinal data, there were no significant site × treatment interactions and therefore, only main effects of treatment are presented.

3.4. Results

3.4.1. Growth performance and nutrient utilization

Initial or final BW, average daily gain, and gain:feed were not affected by fructose supplementation (Table 8).

Table 8. Least square means for the effect of dietary fructose supplementation on initial and final body weight (BW), average daily gain (ADG), and gain:feed (G:F).

	Treatment			
Item	Control	Fructose[1]	SEM[2]	*P*-value
Initial BW, kg	39.9	43.2	2.26	0.29
Final BW, kg	54.0	57.1	3.47	0.51
ADG, kg/d	0.51	0.50	0.054	0.91
G:F, kg/d	0.64	0.54	0.05	0.18

[1]Fructose was provided in the diet at 2.2 g/kg of BW.
[2]Standard error of the mean (Fructose, n =4).

There were no interactions among day, time, or treatment for serum glucose or urea-N concentrations. Dietary fructose decreased (P = 0.004) serum glucose concentrations but did not affect serum urea-N concentrations (Fig. 1).

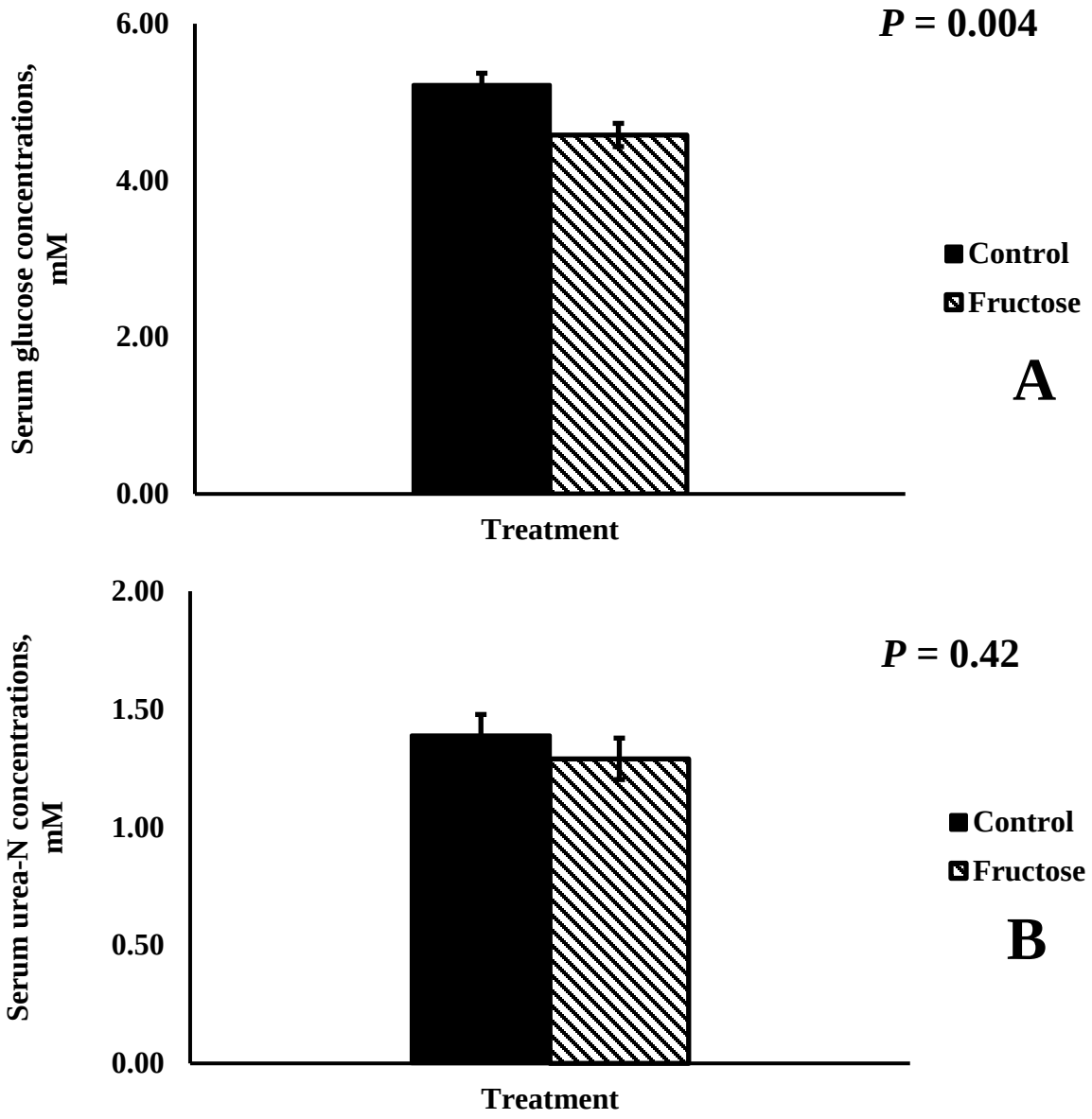

Figure 1. Least square means for the effect of dietary fructose on serum A) glucose and B) urea-N concentrations in neonatal calves.

Serum glucose concentrations were greater ($P < 0.001$) 2-h after feeding but serum urea-N concentrations were not influenced by feeding time (Table 9).

Table 9. Least square means for the effect of sampling time (pre-feeding or 2 h post-feeding) on serum glucose and urea-N concentrations.

	Time			
Item	Fasting	Fed[1]	SEM[2]	*P*-value
Serum glucose, mM	4.50	5.31	0.135	<0.001
Serum urea-N, mM	2.78	2.98	0.172	0.43

[1]Blood samples were collected 2 h post-feeding.

[2]Standard error of the mean (FRUC, n =4).

Serum glucose concentrations linearly increased ($P < 0.001$) from day 0 to 28 of feeding (Table 10). In contrast, serum urea-N concentrations linearly decreased ($P = 0.01$) with day of feeding.

Table 10. Least square means for the effect of day on body weight and serum glucose and urea-N and concentrations.

	Day						*P*-value	
Item	0	7	14	21	28	SEM[2]	Linear	Quad.
Body weight, kg	41.5	44.8	48.5	51.6	54.3	2.30	<0.001	0.81
Serum glucose, mM	4.26	4.50	5.42	4.91	5.41	0.206	<0.001	0.16
Serum urea-N, mM	3.41	3.17	2.54	2.75	7.51	0.90	0.01	0.33

[1]Fructose was provided in the diet at 2.2 g/kg of BW.

[2]Standard error of the mean (Fructose, n =4).

There were no time × treatment or day × time × treatment interactions for serum NEFA concentrations. There was a day × treatment interaction ($P = 0.01$) for serum NEFA as on day 0 and 7, feeding fructose increased ($P = 0.01$) serum NEFA concentrations but not on other days (Fig. 2).

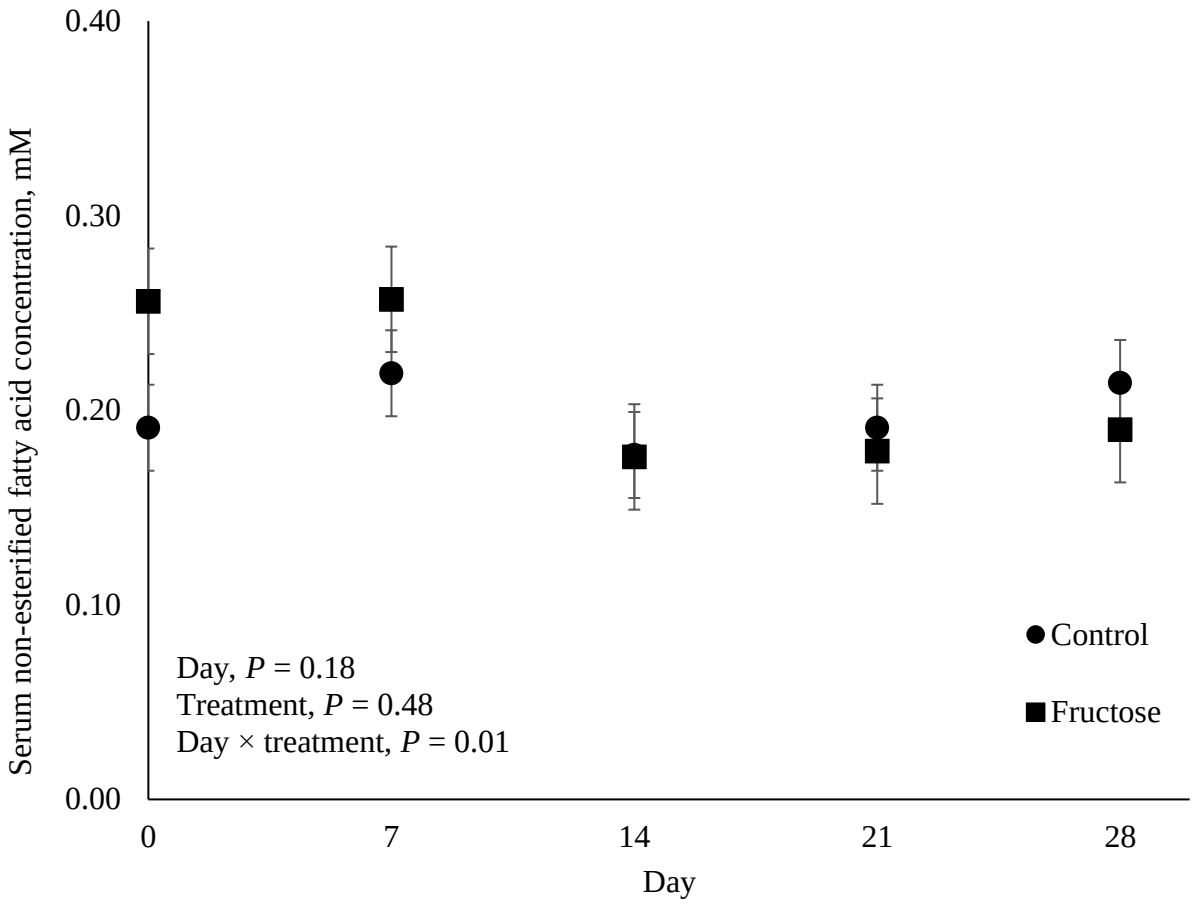

Figure 2. Least square means for the day × treatment interaction for non-esterified fatty acid concentrations in serum from calves fed milk-replacer (control) or milk replacer + 2.2 g/kg of BW fructose (fructose).

There also was a day × time interaction ($P < 0.001$) for serum NEFA concentrations as there were no differences between fasting- or fed-state NEFA concentrations in serum on day 0 or 14 but on days 7, 21, and 28, serum NEFA concentrations were greater ($P < 0.001$) 2-h post-feeding than during the fasted state (Fig. 3).

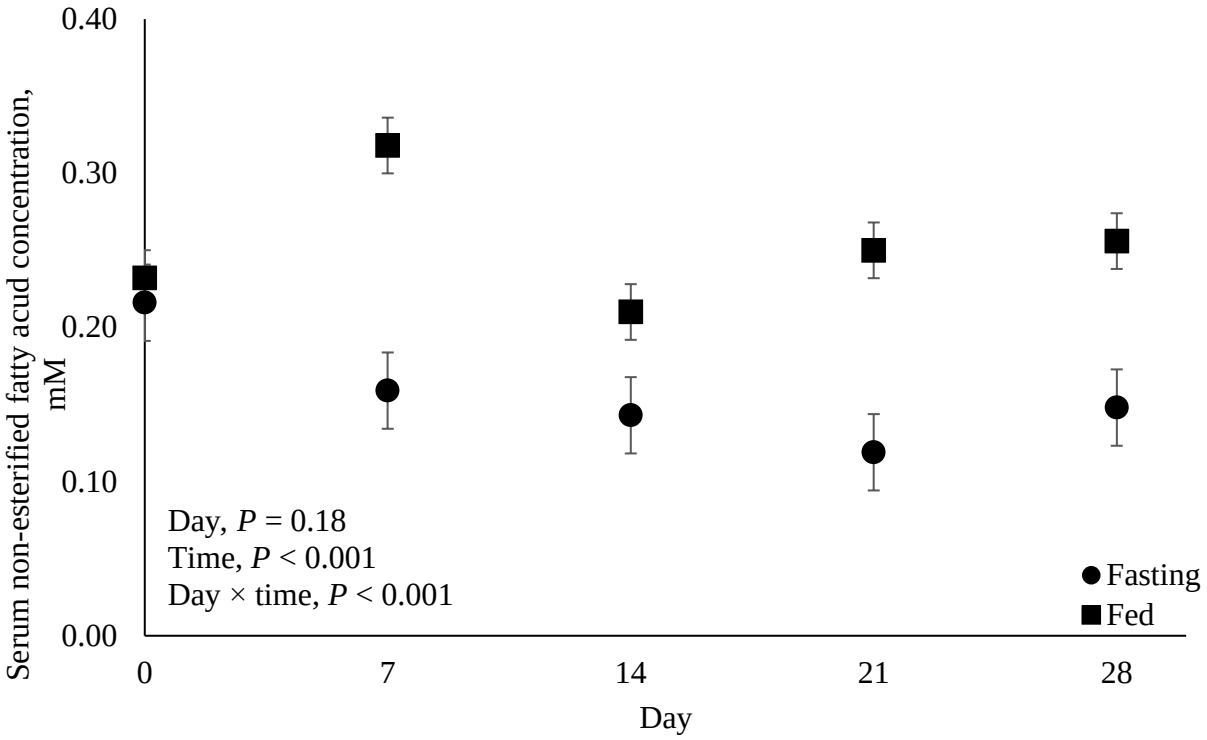

Figure 3. Least square means for the day × time interaction for non-esterified fatty acid concentrations in serum from calves that were fasting or fed (2 h after feeding).

There was a day × treatment interaction (Fig. 4) for serum L-lactate concentrations. On day 0, serum L-lactate concentrations were greatest ($P = 0.02$) in both treatment groups compared to all other days. Furthermore, on days 7, 14, 21, and 28, dietary fructose decreased ($P = 0.02$) serum L-lactate concentrations.

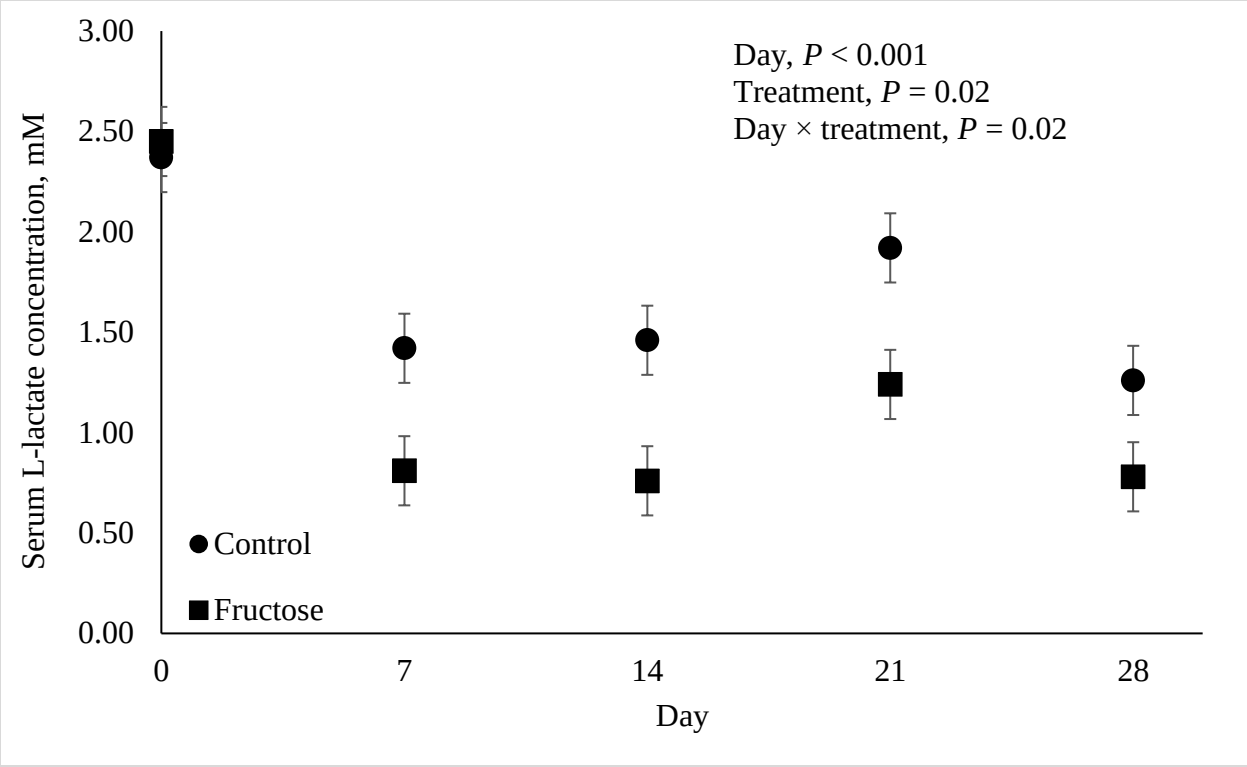

Figure 4. Least square means for the day × treatment interaction for L-lactate concentrations in serum from calves fed milk-replacer (control) or milk replacer + 2.2 g/kg of BW fructose (fructose).

3.4.2. Visceral organ mass and fat content

Mass of the gastrointestinal tract, foregut complex, reticulorumen, omasum, abomasum, large intestine, colon, spleen, pancreas, heart, and longissimus dorsi were not affected by dietary fructose (Table 11). Dietary fructose increased ($P < 0.05$) the mass of the small intestine and liver and tended to increase ($P = 0.06$) kidney mass. When mass was expressed per kg of BW, small intestine and kidney mass were not affected by dietary fructose. Mass of the duodenum, proximal jejunum, and ileum were not affected by dietary fructose. However, feeding fructose increased distal jejunum mass ($P = 0.01$) but, did not influence distal jejunum mass per kg of BW. Feeding fructose tended to increase ($P < 0.10$) liver and lung mass per kg of BW and tended to decrease ($P = 0.08$) cecum mass per kg of BW.

Table 11. Least square means for the effects of dietary fructose on visceral organ mass.

Item	Treatment		SEM[2]	*P*-value
	Control	Fructose[1]		
Gastrointestinal tract				
g	6280	6910	431.7	0.29
g/kg of BW	118	122	1.0	0.78
Foregut complex[3]				
g	628	611	45.7	0.78
g/kg of BW	11.7	10.7	0.73	0.32
Reticulorumen				
g	340	271	18.1	0.18
g/kg of BW	5.72	4.76	0.438	0.13
Omasum				
g	71.9	71.3	11.1	0.97
g/kg of BW	1.35	1.22	0.168	0.57
Abomasum				
g	251	269	24.1	0.59
g/kg of BW	4.74	4.65	0.310	0.84
Small intestine				
g	1133	1313	56.1	0.04
g/kg of BW	21.4	23.1	1.75	0.49
Duodenum				
g	74	83	6.66	0.35
g/kg of BW	1.39	1.43	0.187	0.86
Proximal jejunum				
g	57	61	2.98	0.26
g/kg of BW	1.05	1.07	0.104	0.92
Distal jejunum				
g	46	58	2.65	0.01
g/kg of BW	0.857	1.00	0.0651	0.13
Ileum				
g	90	89	6.95	0.85
g/kg of BW	1.66	1.54	0.162	0.58
Large intestine[4]				
g	398	422	23.0	0.44
g/kg of BW	7.52	7.42	0.646	0.91
Cecum				
g	64	54	4.46	0.12
g/kg of BW	1.20	0.937	0.102	0.08
Colon				
g	335	369	24.3	0.31
g/kg of BW	6.31	6.49	0.620	0.84
Spleen				
g	154	179	16.5	0.28
g/kg of BW	2.84	3.13	0.177	0.24
Pancreas				
g	55	56	5.13	0.83
g/kg of BW	1.01	1.00	0.084	0.85
Liver				
g	1328	1594	86.7	0.05
g/kg of BW	24.8	27.9	1.24	0.09
Kidney				
g	239	295	19.7	0.06
g/kg of BW	4.46	5.17	0.303	0.11

Table 11. Least square means for the effects of dietary fructose on visceral organ mass (continued).

	Treatment			
Item	Control	Fructose[1]	SEM[2]	*P*-value
Heart				
g	436	446	21.3	0.72
g/kg of BW	8.17	7.84	0.446	0.58
Lungs				
g	731	930	106.1	0.18
g/kg of BW	13.45	16.12	1.090	0.10
Longissimus dorsi				
g	48	56	3.77	0.14
g/kg of BW	0.889	0.979	0.0051	0.20

[1]Fructose was provided in the diet at 2.2 g/kg of BW.

[2]Standard error of the mean (Fructose, n =4).

[3]Calculated as sum of reticulorumen + omasum + abomasum.

[4]Calculated as sum of cecum + colon.

Omental and mesenteric fat mass were not affected by dietary fructose (Table 12). Perirenal fat mass tended to increase ($P = 0.06$) in calves fed fructose but was not affected when expressed per kg of BW. Fat concentration of the longissimus dorsi, pancreas, or liver were not influenced by fructose feeding.

Table 12. Least square means for the effects of dietary fructose on gross fat content of the viscera, kidney, longissimus dorsi, pancreas, and liver.

	Treatment			
Item	Control	Fructose[1]	SEM[2]	*P*-value
Visceral fat[3]				
g	585	629	43.8	0.46
g/kg of BW	10.7	10.9	0.88	0.86
Omental fat				
g	177	170	14.4	0.72
g/kg of BW	3.27	3.00	0.188	0.28
Mesenteric fat				
g	219	215	23.0	0.88
g/kg of BW	4.09	3.79	0.412	0.58
Perirenal fat				
g	189	245	19.6	0.06
g/kg of BW	3.57	4.34	0.459	0.23
Longissimus dorsi fat[4,5]				
mg/g	3.98	3.41	0.81	0.61
g/longissimus dorsi	0.40	0.38	0.079	0.83
mg/kg of BW	7.57	6.92	1.74	0.78
Pancreatic fat[5]				
mg/g	37.9	28.2	5.33	0.20
g/pancreas	5.38	4.16	0.85	0.30
mg/kg of BW	99.7	72.5	13.7	0.16
Hepatic fat[5]				
mg/g	4.73	4.44	0.37	0.57
g/pancreas	15.45	18.03	1.92	0.33
mg/kg of BW	285	315	23.1	0.35

[1]Fructose was provided in the diet at 2.2 g/kg of BW.

[2]Standard error of the mean (Fructose, n =4).

[3]Calculated as sum of omental + mesenteric + perirenal fat.

[4]Fat in longissimus dorsi sample taken between 12th and 13th rib.

[5]Fat concentrations are expressed on a dry-tissue basis.

3.4.3. Digestive enzyme activity

Dietary fructose increased (P = 0.04) small intestinal mucosal density (Table 13). Pancreatic and small intestinal protein concentrations (mg/g) were not influenced by feeding

fructose. Feeding fructose did not influence pancreatic α-amylase concentration or specific activity. Glucoamylase concentration increased (P = 0.05) with dietary fructose and glucoamylase specific activity tended to increase (P = 0.09) with dietary fructose. Isomaltase, maltase, and lactase concentrations and specific activities were not influenced by dietary fructose. Sucrase activity was not detected in any segment of the small intestine in calves from either treatment.

Table 13. Least square means for the effect of dietary fructose on pancreatic and small intestinal protein concentration and carbohydrase activities.

	Treatment			
Item	Control	Fructose[1]	SEM[2]	*P*-value
Mucosal density, %	63.4	66.8	1.21	0.04
Small intestinal protein, mg/g	70.6	72.7	1.94	0.40
Pancreatic protein, mg/g	88.3	80.5	3.70	0.14
α-Amylase				
U/g pancreas	70.8	102.1	18.0	0.21
U/g protein	805	1330	261.6	0.16
Glucoamylase				
U/g intestine	0.480	0.624	0.0544	0.05
U/g protein	6.86	8.56	0.767	0.09
Isomaltase				
U/g intestine	0.486	0.497	0.0320	0.79
U/g protein	7.09	6.90	1.20	0.81
Lactase				
U/g intestine	5.85	4.62	1.473	0.21
U/g protein	79.5	62.5	20.07	0.20
Maltase				
U/g intestine	0.967	1.01	0.049	0.50
U/g protein	14.1	14.0	1.02	0.97

[1]Fructose was provided in the diet at 2.2 g/kg of BW.

[2]Standard error of the mean (Fructose, n =4).

[3]Least square mean for the effect of treatment across all sampling sites (duodenum, proximal jejunum, distal jejunum, ileum).

Small intestinal sampling site mass was greatest ($P < 0.001$) in the ileum and least in the proximal and distal jejunum (Table 14). When expressed per kg of BW, duodenal and ileal mass were greater ($P < 0.001$) than proximal and distal jejunal mass. Protein concentration did not differ among sites of the small intestine. Mucosal density was greatest ($P < 0.001$) in the proximal jejunum. Glucoamylase activity was not influenced by site of the small intestine. Isomaltase and maltase specific activity were not influenced by site of the small intestine. Isomaltase and maltase concentrations were greatest ($P < 0.03$) in the distal jejunum. Lactase concentration and specific activity were greatest in the proximal jejunum and least in the ileum ($P < 0.001$).

Table 14. Least square means for the effect of sampling site on small intestinal mass, protein concentration, mucosal density, and carbohydrase activities.

	Site					
Item	Duod.	Prox. Jej.	Dist. Jej.	Ileum	SEM[2]	*P*-value
Mass						
g	78.4[b]	59.1[a]	52.1[a]	89.4[c]	3.36	<0.001
g/kg BW	1.41[b]	1.06[a]	0.927[a]	1.60[b]	0.08900	<0.001
Protein						
mg/g	74.0	73.5	71.2	67.9	2.50	0.31
Mucosal density, %	61.3[a]	73.6[b]	61.2[a]	64.4[a]	1.56	<0.001
Glucoamylase						
U/g intestine	0.640	0.482	0.610	0.475	0.0703	0.24
U/g protein	8.68	6.58	8.53	7.05	0.990	0.35
Isomaltase						
U/g intestine	0.471[a]	0.440[a]	0.603[b]	0.451[a]	0.0414	0.03
U/g protein	6.40	6.05	8.56	6.96	0.772	0.12
Lactase						
U/g intestine	5.80[b]	11.68[c]	3.37[b]	0.109[a]	0.9881	<0.001
U/g protein	77.6[b]	157[c]	47.1[b]	1.95[a]	12.957	<0.001
Maltase						
U/g intestine	0.933[a]	0.936[a]	1.18[b]	0.906[a]	0.0631	0.01
U/g protein	12.7	12.8	16.7	14.0	1.31	0.13

[1]Fructose was provided in the diet at 2.2 g/kg of BW.

[2]Standard error of the mean (Fructose, n =4).

[a-c]Means within a row with the same letter do not differ.

3.4.4. mRNA expression

Dietary fructose did not influence mRNA expression of GLUT5, SGLT1, GLUT2, or ketohexokinase (Table 15). In calves fed fructose, small intestinal lactase mRNA expression increased (P = 0.001) by 3.1-fold. Sucrase-isomaltase mRNA expression in the small intestine decreased (P < 0.001) by 5.1-fold with dietary fructose. Fructose increased (P < 0.001) maltase-glucoamylase mRNA expression in the small intestine by 6.80-fold.

Table 15. Least square means for the effect of dietary fructose on mRNA expression of genes in the small intestine.

		Treatment			
Item	Fold Change	Control	Fructose[1]	SEM[2]	*P*-value
Ketohexokinase	↓1.03	1.15	1.12	0.205	0.91
GLUT5	↑1.14	0.655	0.744	0.1131	0.55
SGLT1	↓1.21	1.00	0.828	0.1242	0.29
GLUT2	↑1.21	0.869	1.05	0.1537	0.37
Lactase	↑3.10	0.701	2.17	0.3218	0.001
Sucrase-isomaltase	↓5.07	1.36	0.268	0.2222	<0.001
Maltase-glucoamylase	↑6.80	1.94	13.2	2.142	<0.001

[1]Fructose was provided in the diet at 2.2 g/kg of BW.

[2]Standard error of the mean (Fructose, n =4).

[3]Least square mean for the effect of treatment across all sampling sites (duodenum, proximal jejunum, distal jejunum, ileum).

Glucose transporter 5 mRNA expression was greatest in the duodenum and proximal jejunum and least in the distal jejunum and ileum (P < 0.001; Table 16). The mRNA expression of SGLT1 and GLUT2 were greatest in the proximal jejunum and least in the distal jejunum and ileum (P < 0.001). Lactase mRNA expression was greatest (P = 0.001) in the proximal jejunum. Furthermore, duodenal and distal jejunum lactase mRNA expression was greater (P = 0.001) than ileal lactase expression. Sucrase-isomaltase and ketohexokinase mRNA expression did not differ across sites of the small intestine.

Table 16. Least square means for the effect of sampling site on mRNA expression in the small intestine.

Item	Site				SEM[2]	*P*-value
	Duod.	Prox. Jej.	Dist. Jej.	Ileum		
Ketohexokinase	1.01	1.39	1.45	0.674	0.2648	0.16
GLUT5	0.980[b]	1.35[b]	0.420[a]	0.053[a]	0.1460	<0.001
SGLT1	0.952[b]	1.50[c]	0.817[ab]	0.388[a]	0.1603	<0.001
GLUT2	1.10[b]	2.44[c]	0.287[a]	0.007[a]	0.2088	<0.001
Lactase	1.52[b]	2.68[c]	1.53[b]	0.015[a]	0.4155	0.001
Sucrase-isomaltase	0.766	0.564	1.26	0.668	0.2869	0.35
Maltase-glucoamylase	6.28	6.18	8.98	8.79	3.044	0.86

[1]Fructose was provided in the diet at 2.2 g/kg of BW.
[2]Standard error of the mean (Fructose, n =4).
[a-c]Means within a row with the same letter do not differ.

3.5. Discussion

Shifting the site of soluble carbohydrate digestion and absorption to the small intestine can provide a greater amount of energy available for maintenance and production compared to ruminal fermentation (Owens et al., 1986). While there is information about glucose absorption and utilization in ruminants, there is much less known about fructose as it is not a typical component of the diet and negligible amounts of fructose would typically flow to the small intestine. However, because fructose is metabolized differently than glucose, it serves as a relatively unregulated source of acetyl-CoA by bypassing the main regulatory steps in glycolysis (Elliott et al., 2002). High dietary intakes of fructose can increase lipogenesis and fatty acid synthesis in humans (Samuel, 2011) and rats (Crescenzo et al., 2014). Dietary carbohydrates, such as glucose, can be utilized in ruminants as a substrate to increase intramuscular fat (Smith and Crouse, 1984) or milk fat synthesis in beef and dairy cattle, respectively. Volpi-Lagreca and Duckett (2016) speculated that intestinal supply of fructose may result in increased intramuscular fat deposition in beef cattle. Moreover, about one-half of milk fatty acids are derived from *de novo* synthesis in dairy cows,

with a majority of the carbon source as acetate (Bauman and Mikko Griinari, 2003). Therefore, ectopic lipid accumulation from excess dietary energy intake (as fat or carbohydrate) may provide value for animal nutrition feeding strategies (Arai, 2014). However, the neonatal calf is an ontogenetic ruminant and may not have the genetic capacity to utilize large quantities of dietary carbohydrates for *de novo* fatty acids synthesis compared to mature ruminants (Roehrig et al., 1988; Pantophlet et al., 2016). Therefore, the lack of change in visceral, hepatic, and longissimus dorsi fat concentrations is likely because of the age of the calves rather than dietary inclusion of fructose. Despite this, the neonatal calf is a suitable model for studying effects of post-ruminal carbohydrate flow on post-ruminal digestion and absorption.

Feeding fructose to neonatal calves apparently altered carbohydrate metabolism and utilization in the current study, based on differences in serum glucose, L-lactate, and NEFA concentrations. Many previous studies in calves have demonstrated suppressed glucose concentrations in response to feeding fructose (Siddons et al., 1969; Daniels et al., 1974; Tyler and Ramsey, 1993; Keller et al., 1998; Becker et al., 2000; Pantophlet et al., 2016) and this effect is thought to be independent of insulin sensitivity (Pantophlet et al., 2016). Rapid catabolism of fructose by ketohexokinase to fructose-1-phosphate increases AMP concentrations, which is a negative allosteric regulator of fructose 1,6-bisphosphatase (Timson, 2019). Therefore, decreased serum glucose concentrations in response to dietary fructose could potentially be due to accumulation of fructose-1-phosphate and AMP, leading to a reduction in gluconeogenesis. In the current study, feeding fructose increased serum NEFA concentrations on days 0 and 7 of the feeding period but did not differ from control calves on days 14, 21, or 28. The convergent response for serum NEFA concentrations may suggest that the liver adapts to fructose supply by increasing fatty acid metabolism in the liver. This could partially be because of the increase in liver mass in

calves supplemented with fructose. More information is needed to determine if serum NEFA differences among days in calves fed fructose are because of increased incorporation of fatty acids into triglycerides and/or reduced fatty acid synthesis. A greater understanding of nutrient flux across the portal-drained viscera and liver are needed to evaluate tissue-level and whole-body changes in glucose and lipid metabolism in response to dietary fructose.

Although BW was not affected by treatment, mass of the small intestine, liver, and kidney increased in response to dietary fructose. The small intestine, liver, and kidney are the primary tissues involved in nutrient absorption, metabolism, and excretion. It is unclear if these observed differences in tissue mass influence energy demands or if changes in tissue mass during neonatal growth could influence post-weaning performance. Increases in small intestinal and hepatic mass could potentially increase energy expenditure for the maintenance of these tissues and potentially decrease energy balance. Alternatively, an increase in small intestinal or hepatic mass may increase the capacity for digestion, absorption, or metabolism resulting in an increase energy supply. Evidence for increases in digestive or absorptive capacities may also be supported by the increase in small intestinal mucosal density in calves fed fructose. Recent research has demonstrated that a significant proportion of dietary fructose is metabolized in the small intestine in rats (Jang et al., 2018). In contrast, many authors have suggested that the liver is the principal site of fructose metabolism (Caliceti et al., 2017; Jegatheesan and De Bandt, 2017). There is valid evidence to support this concept because of the high levels of expression of enzymes involved in fructose catabolism, sensitivity to fructose, and association of fructose with the incidence of non-alcoholic fatty liver disease (Koo et al., 2008; Ishimoto et al., 2013; Lanaspa et al., 2013; Zhang et al., 2017; Jang et al., 2018). Regardless, dietary fructose may have specific effects on both the small intestine and liver in many species including rats, mice, humans, and cattle.

In rats, dietary fructose, independent of energy intake, has been shown to increase kidney weight, renal hypertrophy, and tubular cell proliferation (Nakamaya et al., 2010). Although not measured in the current study, it is thought that hepatic fructose metabolism, which subsequently leads to depletion of ATP (Yair and Allen, 2017) and production of uric acid, may contribute to adverse effects on renal function (Johnson et al., 2010). There was also a tendency for increased perirenal fat mass in the fructose-fed calves. It is unclear if the tendency for an increase in perirenal fat mass is directly due to dietary fructose intake or indirectly through increases in kidney mass. Others have reported that dietary carbohydrates do not significantly contribute to body fat deposition in milk-fed calves (van den Borne et al., 2007). Rats fed fructose have been shown to have increased perirenal fat pad mass per unit of BW (Rönn et al., 2013), independent of changes in visceral adiposity. Indeed, research in rodent and ruminant models, similar to the current study, suggests that fructose can affect kidney mass and function.

Regulation of post-ruminal digestive enzyme activity in ruminants has typically been thought to respond to dietary intake level or energy intake more so than luminal carbohydrate (Kreikemeier et al., 1990; Harmon, 1992; Swanson et al., 2000). In the current study, pancreatic α-amylase concentration was 44.2% greater in calves supplemented with fructose. Previous research has demonstrated that post-ruminal carbohydrate supply as starch or partially hydrolyzed starch (Walker and Harmon, 1995; Wang and Taniguchi, 1998; Swanson et al., 2002b) or glucose (Swanson et al., 2002b) decreases pancreatic α-amylase activity in ruminants. Swanson et al. (2002b) infused 1.37 g/kg of BW or 2.76 g/kg of BW of glucose into the abomasum, which is similar to the 2.2 g/kg of BW of fructose provided in the current study. Fructose increases cholecystokinin (CCK) secretion in humans (Kuhre et al., 2014) and CCK has been thought to be a major regulator of pancreatic exocrine secretion in nonruminants (Miyasaka and Funakoshi,

1998). Much less is known in ruminants; however, *in vitro* incubations of bovine pancreatic acinar cells with caereulein, a CCK mimic, increased α-amylase release when incubated with amino acids or when tissue was collected from ruminating calves infused with casein (Swanson et al., 2003). It should be noted that neonatal calves in our study had a large supply of post-ruminal protein, as the milk-replacer contained 21.68% CP on a DM basis. In contrast to nonruminants, pancreatic α-amylase typically increases with post-ruminal protein or amino acid flow in ruminants (Swanson et al., 2002a; Liu et al., 2015)

Dietary fructose increased small intestinal glucoamylase concentration by 30.0% and maltase-glucoamylase expression by 6.8-fold. Approximately 80% of the apparent maltase activity is derived from sucrase-isomaltase and the remaining 20% from maltase-glucoamylase in humans and mice (Galand, 1989; Lin et al., 2012). Differential regulation of maltase-glucoamylase (increase) and sucrase-isomaltase (decrease) mRNA expression by dietary fructose may explain why there was no change in maltase activity yet an increase in glucoamylase activity. Koch et al. (2019) found that the level milk replacer intake did not influence mRNA expression of sucrase-isomaltase, maltase-glucoamylase, or lactase. This suggests that, in the current study, changes in small intestinal carbohydrase mRNA expression with dietary fructose occurred independent of energy intake. More information is needed to understand how dietary carbohydrates influence digestive enzyme activity in ruminants.

To our knowledge, no studies have evaluated the effects of dietary fructose on the regulation of GLUT5 mRNA expression in the ruminant small intestine. In the current study, dietary fructose supply did not influence GLUT5 mRNA expression in the small intestine. Previous work in cattle has demonstrated that GLUT5 does not respond to ruminal or abomasal infusions of 3.1 g/kg of BW of starch hydrolysate (Liao et al., 2009). Douard and Ferraris (2008)

discussed the complex relationships between age, luminal fructose supply, and induction of GLUT5 in neonatal (milk only), weaning (milk + solid feed), and post-weaning (feed only) rats. In general, GLUT5 expression is nutritionally regulated by luminal fructose during weaning (14-28 days of age) and post-weaning (>28 days of age) in rats (David et al., 1995; Shu et al., 1997; Shu et al., 1998; Jiang and Ferraris, 2001; Jiang et al., 2001; Cui et al., 2004). However, in neonatal rats (<14 days of age), GLUT5 expression can increase with luminal fructose and glucocorticoid supply but not luminal fructose alone (Douard et al., 2008a; Douard et al., 2008b; Suzuki et al., 2011). Calves in the current study were fed milk-replacer with no solid feed. Thus, results from the current study in neonatal calves support the work of others in rats, suggesting that GLUT5 expression is not influenced solely by luminal fructose supply in mammals during the neonatal phase. However, it remains unclear whether GLUT5 can be nutritionally regulated by luminal fructose in weaned or mature cattle.

Sodium-dependent glucose cotransporter-1 is likely the primary apical membrane transporter for glucose absorption in ruminants (Shirazi-Beechey, 1995; Harmon, 2009). Many studies in ruminants have concluded that glucose transport activity and SGLT1 abundance were greatest in milk-fed lambs and declines with age (Scharrer et al., 1979a; Scharrer et al., 1979b; Shirazi-Beechey et al., 1989; Shirazi-Beechey et al., 1991). Shirazi-Beechey et al. (1991) demonstrated that duodenal infusions of a 30 mM glucose solution for 4 days in adult sheep increased the rate of glucose transport by 40- to 80-fold which was also accompanied by an increase in SGLT1 abundance. Furthermore, Dyer et al. (1994) demonstrated that duodenal fructose infusions can increase jejunal SGLT1 induction in lambs. These authors concluded that luminal sugar is sensed in the intestine, independent of glucose metabolism, and that the inducing sugar does not need to be a substrate of SGLT1. However, several studies with post-ruminal

carbohydrate infusions in cattle have found little or no change in SGLT1 activity (Bauer et al., 2001a; Bauer et al., 2001b; Rodriguez et al., 2004; Guimaraes et al., 2007), mRNA expression (Liao et al., 2010), or protein abundance (Guimaraes et al., 2007). Similarly, there were no effects of dietary fructose on SGLT1 or GLUT2 mRNA expression in the small intestine in the current study, further suggesting that carbohydrate transporters in the small intestine of cattle are not sensitive to luminal carbohydrate flow.

Gilbert et al. (2016) reported that replacing 15% of lactose with fructose on a gross energy basis decreased total-tract DM, energy, and N digestibility. It should be noted that lactase activity was numerically lower by 21% in fructose-fed calves, which may have resulted in decreased digestibility. Dietary fructose increased lactase mRNA expression in the small intestine by 3.1-fold. Similarly in rats, fructose supplementation (40% of energy intake) increased lactase mRNA expression and the response was greater than with other carbohydrates such as glucose (Tanaka et al., 1998). Because lactase activity was numerically lower in fructose-fed calves and fructose-feeding may have decreased digestibility, it seems logical that lactase mRNA expression would increase to attempt to increase lactase protein synthesis and activity. However, regulation of digestive enzyme activity is complex and there are numerous regulatory mechanisms involved in controlling lactase activity (Auricchio, 1994).

In humans, dietary sucrose or fructose increases sucrase activity in the small intestine (Rosensweig and Herman, 1968). Additionally, dietary fructose supplementation can induce sucrase activity in patients with congenital sucrase-isomaltase deficiency (Greene et al., 1972). However, several authors have failed to detect sucrase activity in the small intestine of cattle (Huber et al., 1961; Siddons, 1968; Kreikemeier et al., 1990) or sheep (Walker, 1959; Shirazi-Beechey et al., 1989). Moreover, abomasal infusions of sucrose does not induce sucrase activity

in lambs (Swanson and Harmon, 1997). Similar to previous reports, sucrase activity was not detected in the small intestine in the current study, even when the incubation time was increased to 24 h. This demonstrates that dietary fructose does not induce sucrase activity in cattle. In the current study, feeding fructose to calves decreased sucrase-isomaltase mRNA expression in the small intestine. This demonstrates that sucrase-isomaltase can be regulated at the transcriptional level in cattle. In rats, dietary fructose (40% of energy intake) has been shown to increase jejunal sucrase-isomaltase expression within 12 h (Kishi et al., 1999). However, results from the current study suggest that fructose presence in the small intestine selectively decreases sucrase-isomaltase mRNA expression through a negative feedback mechanism via product inhibition. Since fructose is a product of sucrose hydrolysis, it is possible that luminal and/or absorbed fructose decreases sucrase-isomaltase mRNA expression after feeding fructose for 28 d.

3.6. Conclusions

Data from the current study demonstrates that dietary fructose supply influences visceral organ growth, carbohydrate metabolism, and small intestinal digestive enzyme mRNA expression in neonatal calves. Specifically, feeding fructose to neonatal calves increased mass of the small intestine, liver, and kidney. Altered carbohydrate metabolism in response to dietary fructose was evident by decreased serum glucose and L-lactate concentrations and an increase in serum NEFA concentrations. Dietary fructose did not influence nutrient transporter expression but differentially regulated lactase, maltase-glucoamylase, and sucrase-isomaltase expression. Sucrase-isomaltase is transcriptionally regulated by fructose in the ruminant small intestine. However, feeding fructose did not induce sucrase activity. Future research should focus on differential regulation of small intestinal function between glucose and fructose in ruminants. Additional work is needed to

quantify fructose transport and metabolism in ruminants and determine if there are potentially production benefits from increasing post-ruminal supply of fructose.

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4. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

In experiment 1, duodenal casein increased both pancreatic and small intestinal carbohydrases. Specifically, casein increased pancreatic α-amylase and small intestinal maltase, isomaltase, and glucoamylase. Therefore, these data suggest that post-ruminal casein increases small intestinal starch digestion by increasing post-ruminal carbohydrase activities. However, glutamic acid did not affect any of the post-ruminal carbohydrases, with the exception of duodenal maltase. Although previous research has shown increases in small intestinal starch digestion with post-ruminal glutamic acid supply, these studies were limited to up to 14-d of sampling.

In experiment 2, dietary fructose influenced nutrient utilization, visceral organ mass, carbohydrase activity, and mRNA expression of small intestinal carbohydrases. Fructose decreased serum glucose concentrations, and also had interactions with day of sampling for L-lactate and NEFA concentrations in serum. These data suggest that fructose alters carbohydrate metabolism in calves. Furthermore, fructose increased mass of the liver, small intestine, kidney, and perirenal fat. These tissues are heavily involved in metabolism and contribute largely to whole-body energy expenditure. It is unclear how changes in visceral organ mass in response to fructose influences energy balance and maintenance energy requirements. Interestingly, fructose did not influence GLUT5, GLUT2, or SGLT1 mRNA expression. The lack of change in these carbohydrate transporters could potentially be due to the age of the calves. On the other hand, carbohydrate transporters in cattle seem to be relatively insensitive to changes in diet. Maltase-glucoamylase and lactase mRNA expression were greater with dietary fructose. Also, glucoamylase activity in the small intestine was increased with dietary fructose. In contrast, sucrase-isomaltase mRNA expression was decreased by 5.1-fold with dietary fructose. This

suggests that sucrase-isomaltase is transcriptionally regulated by dietary fructose via a negative feedback mechanism.

In both experiments, sucrase activity was not detected in the small intestine. Previous research in humans has shown that dietary fructose can induce sucrase activity in patients with congenital sucrase-isomaltase deficiency. In nonruminants, the absence of sucrase activity decreases starch digestion. It is unclear how the absence of sucrase activity influences carbohydrate digestion in ruminants. Although post-ruminal casein supply increases pancreatic and small intestinal carbohydrases and small intestinal starch digestion, the digestion coefficients are still low (<70%). Therefore, only modest improvements may be achieved practically and other factors may be limiting to small intestinal starch digestion.

Future research is needed to understand how glutamic acid influences small intestinal starch digestion in the short- and long-term. Although post-ruminal protein supply had positive effects on carbohydrase activities, more information is needed on the mechanisms by which these processes occur. This could generate a more-targeted approach for future research to target specific genes, proteins, or metabolic pathways associated with increasing carbohydrase activity.

Future studies are needed to determine if GLUT5 can respond to luminal fructose post-weaning or pre-weaning with glucocorticoid enhancement. Fructose transport and metabolism is still undefined in ruminants and will need to be determined in future experiments. This is an important step because fructose needs to be absorbed through the small intestine, transported to the liver, and metabolized by ketohexokinase if energetic advantages over glucose are to be observed. If this is possible, then future studies should evaluate the effects of fructose and glucose on marbling deposition in beef cattle and milk fat synthesis in dairy cattle.