INCREASING EFFICIENCY OF FINISHING BEEF PRODUCTION: STRATEGIES TO OPTIMIZE STARCH DIGESTION, IMPROVE FLAKE QUALITY, AND REDUCE METHANE EMISSIONS

DISSERTATION

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requirements for the degree of Doctor of Philosophy in the
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By

Ronald J. Trotta

Lexington, Kentucky

Director: Dr. David L. Harmon, Professor of Animal and Food Sciences

Lexington, Kentucky

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ABSTRACT OF DISSERTATION

INCREASING EFFICIENCY OF FINISHING BEEF PRODUCTION: STRATEGIES TO OPTIMIZE STARCH DIGESTION, IMPROVE FLAKE QUALITY, AND REDUCE METHANE EMISSIONS

To continue to meet the demands of increasing food production and environmental sustainability, new strategies are needed to enhance the efficiency of finishing cattle production. A review of the literature identified several nutritional and physiological constraints that limit the ability for cattle to digest starch and absorb glucose from the small intestine. Comparative sequencing analyses revealed a missing region of the sucrase-isomaltase protein which could affect the capacity for starch digestion in cattle. Post-flaking sampling and handling recommendations were generated based on a series of experiments to improve estimates of starch availability and flaking consistency for commercial feedlots and laboratories. Increasing flake density and increasing starch retrogradation could potentially be implemented as processing strategies to shift the site of starch digestion to the small intestine and reduce substrate available for ruminal methanogenesis. For smaller cattle feeders where flaking corn is not an option, low to moderate inclusion of fine-ground corn in finishing rations might be a suitable processing alternative. Feeding anti-coccidial compounds with saponins did not decrease methane production; yet, future attempts to replace antibiotics in finishing cattle diets should attempt to replace the anti-coccidial and anti-methanogenic activity of ionophores. A novel, *in vivo* animal model was developed to selectively target increased endogenous pancreatic and/or small intestinal carbohydrase activities to evaluate physiological mechanisms to increase small intestinal starch digestion. Next-generation RNA sequencing revealed that exogenous glucagon-like peptide 2 administration affected several metabolic pathways and molecular functions of the jejunal mucosa, which could potentially improve growth, health, and feed efficiency of feedlot cattle. These findings could be used to further develop strategies to optimize starch digestion, improve flake quality, and reduce methane emissions of finishing beef cattle.

KEYWORDS: beef cattle, digestive enzyme activity, glucagon-like peptide 2, starch digestion, starch retrogradation, steam-flaked corn

Ronald J. Trotta

(Name of Student)

04/11/2023

Date

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By
Ronald J. Trotta

Dr. David L. Harmon
Director of Dissertation

Dr. David L. Harmon
Director of Graduate Studies

04/11/2023
Date

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CHAPTER 1. INTRODUCTION

To meet the demands of increasing food production and environmental sustainability, new strategies are needed to enhance the efficiency of finishing beef production. Nutritional and genetic/breeding approaches are typically the most feasible approaches to improving feed efficiency; however, approaches that improve the efficiency of waste management, meat processing, and supply-chain logistics also contribute to increasing the efficiency of finishing beef production. From a nutritional point of view, improvements in diet formulation could help to optimally supply nutrients that meet/exceed nutritional requirements for growth.

1.1 Improving flake quality

Steam-flaking is used as the primary form of grain processing in over 70% of feedlots in the United States (Samuelson et al., 2016). Dry matter content and enzymatic starch availability are used routinely by commercial feedlots to evaluate manufacturing consistency; however, there is variation in enzymatic starch availability measurements that cannot be explained, even when flake density and dry matter content is known (Schwandt et al., 2016). Understanding factors that influence starch availability measurements and developing a repeatable methodology to process steam-flaked corn for starch availability analysis could lead to greater consistency in steam-flaked corn manufacturing, which would ensure greater consistency in nutrient delivery to feedlot cattle.

1.2 Optimizing starch digestion

One of the most practical ways to increase average daily gain and feed efficiency is to maximize nutrient utilization and minimize nutrient excretion. Incomplete digestion of

nutrients is frequently the largest loss of nutrients and improvements in grain processing technologies (steam-flaking, high-moisture processing) has led to improved nutrient utilization and reduced nutrient excretion of modern high-concentrate diets. Approximately 75% of commercial feeders use steam-flaking as their primary or secondary form of grain processing (Samuelson et al., 2016) and thus, fecal starch excretion is minimized because total-tract starch digestibility of steam-flaked corn-based diets is greater than 97% (Huntington, 1997; Owens et al., 2016). Yet, there is also potential to improve nutrient utilization efficiency for the other 25% of feedlot producers who do not use steam-flaking as a grain processing method in their operations. Three problems could be addressed to improve efficiency of nutrient utilization of modern high-concentrate diets: 1) finding a suitable alternative processing method for smaller producers who cannot invest in infrastructure necessary to steam-flake corn, 2) overcoming limitations of small intestinal starch digestibility, and 3) developing practical methods to increase digestible starch flow to the small intestine. Shifting the site of starch digestion from the rumen to the small intestine can increase energetic efficiency because ruminal fermentation of starch yields approximately 65-72% of the energy available from small intestinal digestion of starch because of energy losses to methane formation, urine, and heat of fermentation (Harmon and McLeod, 2001). However, limitations in the extent of small intestinal starch digestibility (55% of duodenal flow in beef cattle) have led to commercial feeders to choose grain processing methods that focus on increasing ruminal starch digestion to maximize nutrient utilization of high-concentrate diets. Even if the limitations of small intestinal starch digestibility could be overcome, there are no practical methods to increase flow of starch to the small intestine under current feeding regimens (Harmon and McLeod, 2001).

To overcome the limitations of small intestinal starch digestibility in cattle, one must first understand the nutritional and physiological constraints that limit small intestinal starch digestibility when high-concentrate diets are fed. The primary limit of small intestinal starch assimilation in ruminants has been debated for decades (Mayes and Ørskov, 1974; Owens et al., 1986; Huntington, 1997; Huntington et al., 2006; Mills et al., 2017; Harmon and Swanson, 2020). Various experimental approaches have been utilized to identify the limiting factors of small intestinal starch assimilation including measures of intestinal starch disappearance (Kreikemeier et al., 1991; Richards et al., 2002), intestinal retention time (Brake et al., 2014b), ileal carbohydrate composition (Mayes and Ørskov, 1974; Kreikemeier and Harmon, 1995; Brake et al., 2014a), portal glucose appearance (Huntington and Reynolds, 1986; Bauer et al., 1995), visceral glucose metabolism (Harmon et al., 2001), pancreatic and intestinal carbohydrase activity and secretion (Walker and Harmon, 1995; Swanson et al., 2002a; Trotta et al., 2020c), and Na^+-dependent glucose transport activity (Bauer et al., 2001a; Rodriguez et al., 2004). Conflicting reports and the lack of a single, conclusive experiment have made interpretations of a primary limitation of intestinal starch assimilation difficult.

When the focus is narrowed to only small intestinal starch digestion, it seems probable that insufficient activity of pancreatic and/or small intestinal carbohydrases could limit small intestinal starch digestion. One remaining enigma of ruminant physiology is the lack of sucrase activity in the small intestine of ruminants, which was first reported over 65 years ago (Dollar and Porter, 1957). To assess the limitations of insufficient carbohydrase activity, recent approaches have infused exogenous carbohydrase activity postruminally and measured small intestinal starch disappearance (Remillard et al., 1990; Westreicher-

Kristen et al., 2018; Robbers et al., 2019; Trotta et al., 2020b). However, these experiments did not determine if exogenous infusions of carbohydrase activity increased carbohydrase activity in the lumen of the small intestine. The lack of effects in those experiments could be due to negative effects on endogenous carbohydrase activity and secretion, suboptimal pH, or enzyme degradation/inactivation. To date, there are no physiological explanations for the absence of sucrase activity in ruminants and if it affects the limitations of small intestinal starch digestibility. Also, there are no animal models that describe how to increase endogenous pancreatic and small intestinal carbohydrase activity to assess limitations of small intestinal starch digestibility in cattle.

1.3 Reducing methane emissions

There is rising global interest to decrease methane emissions from ruminants to mitigate some of the negative environmental impacts of animal agriculture. Strategies to reduce enteric methane emissions can largely be grouped into three categories: animal and feed management, diet formulation, and rumen manipulation (Arndt et al., 2022). The feedlot industry can reduce methane emissions compared to pastoral settings by implementing well-established enteric methane mitigation strategies such as increasing feed processing, increasing feeding level, decreasing forage:concentrate, and feeding anti-methanogenic feed additives. After energy losses due to incomplete digestion, methane energy losses represent a considerably large energy loss that could potentially be decreased to provide more energy to the animal. Experimental evidence has demonstrated that shifting the site of starch digestion from the rumen to the abomasum will conserve approximately 6.8 to 9.6% of starch energy intake by reducing methane emissions (Harmon and McLeod, 2001). Therefore, developing practical methods to increase digestible starch

flow to the small intestine would also decrease methane emissions. Starch availability is thought to reflect the rate of ruminal fermentation (Schwandt et al., 2016), and thus, grain processing methods that decrease starch availability might decrease the rate of ruminal fermentation, which could lead to decreased ruminal methane production.

Monensin has been included in finishing cattle diets for decades to control coccidiosis but also to improve average daily gain, feed efficiency, and decrease methane emissions of feedlot cattle. There is also rising interest in developing non-antibiotic feed additives to replace the use of antibiotic compounds in finishing cattle diets. Notably, *Yucca schidigera* extract supplementation has shown effects on ruminal fermentation, growth performance, and feed efficiency that are similar to diets containing monensin (Zúñiga-Serrano et al., 2022). Although it may contain some anti-coccidial activity, a non-antibiotic anti-coccidial compound would need to be fed simultaneously to prevent coccidiosis outbreaks in feedlot cattle. The combination of feeding an anti-coccidial compound with saponins could potentially replace monensin as a non-antibiotic feed additive, but only if it could improve ruminal fermentation, reduce methane emissions, and control coccidiosis similarly to monensin.

1.4 Objectives

The goal of this dissertation was to investigate strategies that could potentially improve the efficiency of finishing beef production. In particular, strategies were targeted to develop new methods to improve flake quality, optimize starch digestion, and reduce methane emissions. Thus, the objectives of this dissertation were to:

1. Understand post-flaking sampling and handling techniques that could influence estimates of enzymatic starch availability of steam-flaked corn to develop a repeatable methodology for commercial feedlot producers and laboratories to assay enzymatic starch availability
2. Understand how changing starch availability by increasing flake density or increasing starch retrogradation influences ruminal digestion and predicted energetic efficiency
3. Evaluate alternative grain processing methods that could be used by feedlot producers who cannot produce steam-flaked corn
4. Determine if combinations of non-antibiotic anti-coccidial compounds and saponins could decrease methane production and affect ruminal fermentation characteristics similarly to monensin
5. Re-assess the nutritional and physiological constraints contributing to limited small intestinal starch digestion and glucose absorption in ruminants
6. Investigate potential physiological mechanisms that could explain the absence of sucrase activity in ruminants
7. Develop an *in vivo* animal model to selectively target increases in endogenous pancreatic and small intestinal carbohydrase activity
8. Identify potential metabolic pathways and molecular functions of the small intestinal mucosa that could potentially be associated with increased small intestinal starch utilization and efficiency of feedlot cattle

CHAPTER 2. NUTRITIONAL AND PHYSIOLOGICAL CONSTRAINTS CONTRIBUTING TO LIMITATIONS IN SMALL INTESTINAL STARCH DIGESTION AND GLUCOSE ABSORPTION IN RUMINANTS

2.1 Introduction

Ruminants consume various types of carbohydrates at different stages of their production life cycle. This includes fructose (during fetal development) (Crouse et al., 2019), lactose (from milk), galactose (from digestion of lactose), glucose (dietary origin) (Brockman and Laarveld, 1986), sucrose (from high sugar feedstuffs), starch and starch-digestion products (maltose, isomaltose, and limit dextrins), and cellulose and hemicellulose (fiber). A common finding 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 will identify biological processes that can be manipulated to improve nutrient utilization and the efficiency of meat and milk production.

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, 1986). Shifting the site of carbohydrate digestion and absorption from the rumen to the small intestine can provide energetic advantages because postruminal glucose can be used more efficiently and provide more net ATP production than ruminal glucose fermentation to short-chain fatty acids (Black, 1971; Owens et al., 1986; Harmon and McLeod, 2001; Harmon et al., 2001).

Several studies have demonstrated that small intestinal carbohydrate assimilation is functionally different in ruminants compared with nonruminant animals. The objective of this review is to explore the nutritional and physiological constraints that contribute to limitations of carbohydrate assimilation in the ruminant small intestine. This includes digesta composition and passage rate, the extent of small intestinal disappearance, regulation of digestive enzymes and absence of sucrase activity, and possible limitations in glucose absorption. Nutrient composition from dietary origin can be substantially altered through the process of ruminal fermentation. Small intestinal starch disappearance as a percentage of duodenal flow decreases with increasing intestinal starch flow but can be improved with increased postruminal protein or non-essential amino acid supply (Brake and Swanson, 2018). Nearly all of the carbohydrases present in the intestinal mucosa or pancreas of ruminants contain less activity compared with nonruminants (Walker, 1959; Hembry et al., 1967; Siddons, 1968; Coombe and Siddons, 1973; Sir Elkhatim and Osman, 1982; Harmon, 1993). It remains unknown why sucrase and palatinase activities are not present in the intestinal mucosa of ruminants (Coombe and Siddons, 1973). The lack of an adaptive response of glucose transporters to dietary substrates (Bauer et al., 2001a; Rodriguez et al., 2004) and the disproportional relationship between intestinal carbohydrate disappearance and portal glucose appearance (Kreikemeier et al., 1991) raise concerns about the capacity for glucose absorption in ruminants. To achieve energetic advantages with increased small intestinal starch digestion and glucose absorption (Huntington et al., 2006), factors limiting these processes must be understood to identify potential strategies or solutions to increase the efficiency of starch utilization.

2.2 Small intestinal digesta composition and passage rate

2.2.1 *Small intestinal digesta composition*

Duodenal nutrient flows in ruminants can drastically differ in response to the composition of the diet because of pregastric fermentation of dietary components (Merchen, 1988). Therefore, duodenal digesta composition of ruminants can be substantially different from the composition of duodenal digesta in nonruminants (Swanson, 2019). Obvious factors such as dietary intake, diet composition, foregut retention time, and foregut anatomy and digestive function differentiate the ruminant from nonruminants. These factors contribute to the differences in digesta flow to the small intestine, which in turn, could affect how small intestinal function is coordinated.

The microbes of the reticulorumen contribute substantially to the animal's metabolizable protein supply because large amounts of microbial protein flow to the small intestine for hydrolysis and absorption. With intestinal microbial protein flow, there are simultaneous flows of nucleic acids, as well as α-linked glucose from microbial polysaccharides (McAllan and Smith, 1974).

Early comparative studies demonstrated that short-chain fatty acid concentrations in the small intestine were small across both ruminant and nonruminant species (Elsden et al., 1946). Dietary lipid composition is altered by ruminal microbial populations via lipolysis and biohydrogenation (Jenkins, 1993), which alters duodenal digesta by increasing the concentration of non-esterified saturated fatty acids. Indeed, approximately 90% of dietary lipids reach the duodenum as saturated fatty acids in ruminants (Doreau and Ferlay, 1994). Therefore, concentrations of unsaturated fatty acids in duodenal digesta

are typically much greater in nonruminant species because they do not undergo ruminal biohydrogenation as in ruminants.

Ruminal fermentation of starch is largely affected by level of intake, grain processing, and rate of passage (Theurer, 1986) and this can lead to large proportions of starch flowing to the small intestine for potential enzymatic digestion. Ruminal starch fermentation likely increases the amount of partially hydrolyzed starch and starch-digestion products that are present in duodenal digesta compared with nonruminants. Differences in duodenal carbohydrate, nitrogen, and lipid composition between ruminants and nonruminants could potentially contribute to changes in digestive and absorptive functions in the small intestine because regulation of digestion and absorption can be coordinated through luminal nutrient flows (Ferraris and Diamond, 1989).

2.2.2 *Small intestinal passage rate*

In general, the rate of ruminal passage typically increases with increasing dry matter or energy intake (Staples et al., 1984; Kreikemeier et al., 1990a). Other factors influencing ruminal passage rates include particle size (within a given diet) (Pearce and Moir, 1964), rumination and ingestive behavior, specific gravity (desBordes and Welch, 1984), forage quality (Cherney et al., 1991) or treatment (Berger et al., 1980; Zorrilla-Rios et al., 1985), forage:concentrate (Colucci et al., 1990), protein supplementation (McCollum and Galyean, 1985), animal species and breed (Prigge et al., 1984; Mann et al., 1987; Reid et al., 1990), physiological state (Coffey et al., 1989; Gunter et al., 1990), ambient temperature (Westra and Christopherson, 1976), and reticular motility (Sissons et al., 1984; Okine et al., 1989). Increased rates of ruminal passage can be associated with increased ruminal microbial protein synthesis and efficiency and flow of microbial protein to the

small intestine (Isaacson et al., 1975; Prigge et al., 1978). Therefore, some factors influencing ruminal passage can also potentially influence passage to the small intestine.

However, the flow of digesta through the duodenum is essentially continuous in ruminants (Merchen, 1988) while ileal flow is intermittent (Goodall and Kay, 1965). 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 nonruminants (Burgstaller et al., 2017). Continuous fermentation and ruminal passage rate have 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 (approximately 7 m 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 the abomasal and intestinal flow of digesta are essentially constant, it is unclear if regulatory mechanisms between splanchnic tissues, such as neural or hormonal signaling, are altered. More definitive studies are needed in ruminants to evaluate how near continuous abomasal flow

affects autonomic control of digestion in the small intestine and if the lack of abomasal emptying could 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 typically spends less than 3 h in the small intestine of steers (Zinn and Owens, 1980) which is comparable with intestinal retention time in pigs (Wilfart et al., 2007). 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, postruminal casein supply did not influence small intestinal transit time in steers duodenally infused with raw corn starch (Brake et al., 2014b). In milk-fed calves, casein did not influence the rate of abomasal emptying or intestinal transit time (Smith, 1964). Even if passage rate limited the extent of intestinal carbohydrate disappearance, it is thought that this factor is not independent of the activity or amount of carbohydrases (Brake and Swanson, 2018).

2.3 Small intestinal starch disappearance

2.3.1 *Small intestinal starch disappearance: Linear relationships*

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 postruminal starch disappearance was much lower than in nonruminants. These authors concluded that the extent of starch disappearance in the small intestine was inadequate for optimum utilization. Indeed, summaries have indicated that the extents of small intestinal starch disappearance in beef cattle (55%) (Owens et al., 1986) and dairy cows (60%) (Moharrery et al., 2014) are inadequate to achieve potential energetic advantages over ruminal

fermentation of starch (Huntington et al., 2006). Interestingly, the limitation of small intestinal starch disappearance 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 disappearance were first suggested by Ørskov et al. (1969), and a linear regression model was developed to predict small intestinal starch digestibility 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 disappearance (%) (Harmon et al., 2004). These reviews were compiled data from site of digestion experiments with duodenal starch flows resulting from dietary starch intake. Using data from postruminal starch infusion models in cattle shows that as the amount of raw corn starch infused per hour relative to body weight (BW) increased, small intestinal starch disappearance decreased (Fig. 2.1). 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). Collectively, these data demonstrate a linear relationship between intestinal starch supply and starch disappearance across dietary and infusion models in cattle and sheep.

2.3.2 *Influence of nitrogenous compounds on small intestinal starch disappearance*

Several studies have demonstrated that postruminal protein or amino acids can increase small intestinal starch disappearance in ruminants. Indirect evidence of increased pancreatic α-amylase (Wang and Taniguchi, 1998) and increased net portal glucose flux (Taniguchi et al., 1995) with abomasal casein infusion led to the speculation that postruminal protein flow could increase small intestinal starch disappearance. Increasing levels of postruminal casein (0 g/d to 200 g/d) infusion resulted in linear improvements in small intestinal starch disappearance (g/d and % of flow) in cattle abomasally infused with raw corn starch (Richards et al., 2002). Similar results were obtained in sheep receiving abomasal casein infusions while 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. (2014b) demonstrated that increasing levels of postruminal casein (0 g/d, 200 g/d, or 400 g/d) infusion could increase small intestinal starch disappearance in steers duodenally infused with raw corn starch 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 digestibility but essential amino acid infusions did not (Brake et al., 2014a). This observation was further supported when Glu or Glu + Phe + Trp + Met increased small intestinal starch digestibility but the Phe + Trp + Met treatment did not (Brake et al., 2014a). Furthermore, increasing supply (0 g/d, 60 g/d, and 120 g/d) of duodenal Glu increased small intestinal starch disappearance to a similar magnitude achieved with 400 g/d of casein (Blom et al., 2016). Other trials with essential amino acids (Leu and Phe)

have not found any effects on small intestinal starch disappearance in goats (Yu et al., 2014b).

2.3.3 *Influence of grain processing on small intestinal starch disappearance*

The effects of grain processing on small intestinal or postruminal starch disappearance have been reviewed extensively (Ørskov, 1986; Owens et al., 1986; Theurer, 1986; Huntington, 1997; Huntington et al., 2006; Owens et al., 2016). The current nutrient requirements of beef cattle (NASEM, 2016) use data from Sniffen et al. (1992) and Owens and Zinn (2005) to show the effects of grain type and degree of processing on postruminal starch disappearance. The postruminal starch disappearance coefficients reported are 30–40%, 65–70%, 80–90%, 85–95%, and 92–97% for whole, dry-rolled or cracked, meal, high-moisture, and steam-flaked methods of corn processing, respectively (NASEM, 2016). Owens et al. (2016) developed linear models to predict postruminal starch disappearance in dairy and finishing diets. In general, starch digestibility of diets containing whole or rolled corn typically decreases linearly with increasing intestinal flow, as described previously. However, extensive methods of corn processing, such as high-moisture and steam-flaking, are typically not affected by increasing intestinal flow because they are highly digestible postruminally. It is not clear whether or not enhanced postruminal starch digestibility with extensive corn processing methods are directly related to increased small intestinal starch digestibility. The issues of variable duodenal flow, maintenance of ileal cannulas, and maintaining production levels of intake in cattle with ileal cannulas are largely why measurements of small intestinal disappearance with different grain processing methods have not been evaluated. A complicating factor is that extensive grain processing methods that increase postruminal digestibility also increase

ruminal digestibility (Zinn et al., 2002). Therefore, steam-flaking and high-moisture processing methods result in greater ruminal starch disappearance and decreased intestinal starch flows relative to whole-shelled or dry-rolled processing methods. Because of this, comparisons of postruminal starch digestibility coefficients that were obtained using dietary models had differing intestinal starch flows. More studies in which duodenal flow is controlled are needed to clarify how grain processing methods affect the extent of small intestinal starch disappearance in ruminants. This will aid in understanding the limits of the extent of small intestinal starch disappearance in ruminants.

2.4 Pancreatic α-amylase

2.4.1 *Influence of dry matter or energy intake on pancreatic α-amylase activity*

The effects of nutrition on pancreatic exocrine function in ruminants have been reviewed previously (Croom et al., 1992; Harmon, 1992b, a, 1993, 2009; Harmon and Swanson, 2020; Guo et al., 2021; Trotta and Swanson, 2021). In nonruminants, carbohydrase activities typically increase proportional to luminal substrate flow (Brannon, 1990). However, in ruminants, postruminal digestive enzymes respond differently to diet and luminal nutrient flows (Harmon, 1993). Russell et al. (1981) evaluated the effects of diet and energy intake on pancreatic α-amylase activity in steers. They fed either an alfalfa hay diet (hay) or a corn and corn-silage-based diet (grain) at 1 × net energy of maintenance (NE_m) or the grain diet at 2 × or 3 × NE_m. At 1 × NE_m intake, they found that steers consuming the grain diet had lower pancreatic α-amylase activity per gram protein than steers consuming the hay diet. Furthermore, increasing the energy intake of the grain diet

from 1 to 2 × NE_m increased pancreatic α-amylase activity per gram protein by two-fold, without any additional increases at 3 × NE_m.

To further evaluate the effects of diet and energy intake on carbohydrase activities, Kreikemeier et al. (1990b) fed either a 90% forage (alfalfa hay) or 90% grain (sorghum and wheat) diet at 1 or 2 × the 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 × 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., 1968; Janes et al., 1985). However, these studies were confounded with energy intake. Results from Russell et al. (1981) and Kreikemeier et al. (1990b) 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.

More recent studies have evaluated the effects of dietary intake restriction on pancreatic α-amylase activity in ruminants. Dietary intake restriction decreased pancreatic α-amylase activity in nonpregnant ewes (Keomanivong et al., 2017b), pregnant ewes (Keomanivong et al., 2016; Trotta et al., 2020d), and pregnant beef cows (Awda et al., 2016; Keomanivong et al., 2017a). Changes in pancreatic α-amylase activity in response to changes in dry matter or energy intake may be related to the abundance and activity of

pancreatic proteins involved in energy metabolism. Increasing dry matter intake increased the abundance of ATP synthase, Na^+/K^+-ATPase, proliferating cell nuclear antigen, and ubiquitin in the pancreas of steers (Wang et al., 2009b). Dietary intake restriction of pregnant beef cows decreased ATP synthase abundance in the pancreas (Wood et al., 2013). Proteomic analyses suggest that intracellular activity and abundance of proteins related to energy metabolism in the pancreas may be associated with pancreatic α-amylase activity (Holligan et al., 2013).

2.4.2 *Influence of dietary or luminal carbohydrate on pancreatic α-amylase activity*

While pancreatic α-amylase activity in nonruminants increases in response to luminal starch flows (Brannon, 1990), the response is opposite in ruminants. High levels of postruminal carbohydrate supply as starch, partially hydrolyzed starch, or glucose decreases pancreatic α-amylase activity when energy intake is controlled. Abomasal infusions of partially hydrolyzed starch decreased pancreatic α-amylase concentration, specific activity, and secretion in steers compared with 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., 2002a). Similarly in wethers, abomasal infusions of raw corn starch decreased pancreatic α-amylase concentration and secretion compared with control wethers receiving abomasal infusion of water (Wang and Taniguchi, 1998). These studies demonstrated that luminal complex carbohydrate flow decreases pancreatic α-amylase activity in cattle. In a study by Swanson et al. (2002b), abomasal infusions of either glucose or partially hydrolyzed starch 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. However, it remains unclear whether luminal glucose concentration, absorbed glucose, or both regulate pancreatic α-amylase activity in ruminants. Increasing levels of ruminal glucose infusions did not affect plasma amylase concentrations in lambs fed a 50% concentrate diet (Krehbiel et al., 1995). In neonatal dairy calves, supplementing fructose at 2.2 g/kg of BW did not statistically increase pancreatic α-amylase activity; however, pancreatic α-amylase activity was 42% greater in fructose-fed calves (Trotta et al., 2020e). This could partially result from an increase in metabolizable energy intake.

2.4.3 *Influence of dietary or luminal nitrogenous compounds on pancreatic α-amylase activity*

As stated previously, studies by Russell et al. (1981) and Kreikemeier et al. (1990b) demonstrated that pancreatic α-amylase activity was greater in steers fed an alfalfa hay diet compared with a grain-based diet. These authors speculated that differences in dietary crude protein (and therefore, rumen undegradable protein and metabolizable protein) contribute to differences in pancreatic α-amylase activity. In sheep, Wang and Taniguchi (1998) abomasally infused water (control), raw corn starch, or raw corn starch + casein and measured pancreatic exocrine secretion. Pancreatic α-amylase activity was depressed with abomasal starch infusion; however, abomasal infusion of starch with casein restored α-amylase activity to the same level as the control. Similarly, increasing levels of abomasal casein supply (0 g/d, 60 g/d, 120 g/d, or 180 g/d) linearly increased pancreatic α-amylase concentration, specific activity, and secretion in steers postruminally infused with raw corn starch (Richards et al., 2003). Feeding a 68.7% concentrate diet with supplemental casein

to steers produced increases in duodenal α-amylase concentrations and serum cholecystokinin (CCK) concentrations (Lee et al., 2013).

More information is needed to understand how the association between luminal nutrient supply, hormones and neuropeptides, and enzyme activities are coordinated to influence intestinal starch disappearance in ruminants. The effects of individual amino acids on pancreatic exocrine function have been studied predominantly with preruminant calves and lambs (Trotta and Swanson, 2021). Several amino acids including Arg, Leu, Ile, and Phe have been shown to influence pancreatic α-amylase activity in ruminants. Similarly, rumen-protected Trp supplementation to steers consuming a high-concentrate diet was associated with greater postruminal starch disappearance, increased luminal amylase activity in the duodenum, and increased serum CCK and melatonin (Lee et al., 2020). We have found that melatonin supplementation to gestating ewes increased maternal pancreatic α-amylase activity (Keomanivong et al., 2016) and small intestinal maltase, isomaltase, and glucoamylase activities (Trotta et al., 2021c). Tryptophan and its metabolites are precursors to the synthesis of biogenic amines such as serotonin and melatonin.

Responses in pancreatic α-amylase activity to individual amino acids have varied with the type of amino acid, length of infusion, and animal species. Arginine administration through jugular blood did not influence pancreatic α-amylase activity in non-pregnant ewes (Keomanivong et al., 2017b). Similarly, dietary rumen-protected Arg supplementation to ewes during mid- to late-gestation did not influence pancreatic α-amylase activity of lamb offspring at 54 d of age (Trotta et al., 2020a). After 14 d of duodenally infusing increasing levels of Phe, 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 Phe. Moreover, increasing levels of Leu linearly increased α-amylase concentration in pancreatic juice after 14 d of duodenal infusion (Yu et al., 2014a). In dairy heifers, duodenal infusions of 10 g/d Leu 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 Leu (3 g/d or 9 g/d) and Phe (2 g/d) in goats (Cao et al., 2018). However, when Leu (1.435 g/L milk), Phe (0.725 g/L milk), or a combination of Leu and Phe (1.435 g Leu/L milk and 0.725 g Phe/L milk) were fed to milk-fed calves, pancreatic α-amylase specific activity was not influenced (Cao et al., 2019c). Similarly, increasing levels of Leu supplementation to neonatal calves in milk replacer did not affect pancreatic α-amylase activity (Reiners, 2021). These data suggest that Leu can increase pancreatic α-amylase activity in post-weaning ruminants but not in milk-fed calves. Duodenal infusions of 20 g/d or 30 g/d of Ile 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 Phe (Guo et al., 2018b), Leu (Guo et al., 2018a; Cao et al., 2019b; Guo et al., 2019), and Ile (Cao et al., 2019a) increased α-amylase release. Despite increases in small intestinal starch disappearance with Glu (Brake et al., 2014a; Blom et al., 2016), it is unclear if these increases are related to increases in pancreatic α-amylase activity, as our recent experiment found duodenal glutamic acid infusion did not influence pancreatic α-amylase in steers (Trotta et al., 2020c).

Indeed, a few studies have begun to explore cellular and molecular mechanisms driving associations between increased pancreatic α-amylase activity in ruminants and amino acid supply. Phenylalanine increases α-amylase activity in dairy calves and the initiation of messenger ribonucleic acid (mRNA) translation through phosphorylation of ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) (Guo et al., 2018a). Leucine and Ile have been shown to increase α-amylase synthesis and phosphorylation of the mammalian target of rapamycin (mTOR) signaling pathway (Guo et al., 2018a; Cao et al., 2019a). Proteomic analysis has suggested that Leu modulates increases in pancreatic α-amylase activity in dairy calves by increasing citrate synthase activity in the tricarboxylic acid cycle, ATPase activity and oxidative phosphorylation, and stimulating the general secretory signaling pathway in pancreatic acinar cells (Guo et al., 2020). In these studies, Phe, Leu, and Ile were the only amino acids studied and future research is needed to evaluate how other amino acids could affect pancreatic exocrine function in ruminants.

2.4.4 *Interactions between starch and protein supply on pancreatic α-amylase activity*

Complex interactions can occur with simultaneous 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 Holstein calves, abomasal casein infusion for 7 d increased pancreatic α-amylase activity relative to BW (Fig. 2.2) (Swanson et al., 2002a). 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 or control. In support of these results,

Swanson et al. (2003) and Swanson et al. (2004a) found similar responses in pancreatic α-amylase activity to infusion treatments in pancreatic explant and secretion models, respectively. The combined results suggest that the benefits of postruminal protein supply may be overridden by the presence of starch in the small intestine. In contrast, we recently demonstrated that duodenal infusion of raw corn starch with casein for 58 d increases pancreatic α-amylase activity by 290% compared with raw corn starch infusion alone (Trotta et al., 2020c). Interestingly, the responses in pancreatic α-amylase activity relative to BW were numerically similar across studies (casein: 226 U/kg BW and raw starch + casein: 218 U/kg BW). Furthermore, activity of pancreatic α-amylase in response to starch treatments was similar, as well (partially hydrolyzed starch: 49.5 U/kg BW and raw starch: 81.3 U/kg BW). The results from Trotta et al. (2020c) may suggest that a longer adaptation to postruminal protein supply could overcome the negative effects of starch on pancreatic α-amylase activity.

Alternatively, partially hydrolyzed starch and raw corn starch may elicit different responses in pancreatic α-amylase activity. Branco et al. (1999) reported that the coefficient of true small intestinal digestibility for partially hydrolyzed starch infused at 40 g/h was 88%. Therefore, the high digestibility of partially hydrolyzed starch may not require the amount of pancreatic α-amylase that raw corn starch does and thus, decreased activity of α-amylase with partially hydrolyzed starch flow might be a function of the starch source. The high small intestinal digestibility of partially hydrolyzed starch relative to raw corn starch likely allows for more glucose to be absorbed and it is still unclear if luminal or absorbed glucose regulates pancreatic α-amylase activity. No definitive studies have tested

the influence of corn starch source (raw corn starch or partially hydrolyzed starch) with postruminal casein on pancreatic α-amylase activity.

Secretion of pancreatic α-amylase increases with increasing dietary starch intake in nonruminants (Brannon, 1990), which is similar to responses in ruminants to increasing postruminal protein supply. Increasing dietary crude protein concentrations with ruminal escape 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. Increasing dry matter or energy intake results in increased microbial-N flow to the small intestine (Clark et al., 1992). Moreover, ruminal starch supply increased duodenal bacterial-N and total-N flow compared with postruminal starch supply (Alman, 2009). Synchrony of ruminal starch and protein fermentation increases ruminal bacterial-N outflow (Huntington, 1997) and responses of the pancreas to luminal nutrients may be related to changes in the evolutionary control of pancreatic exocrine function.

2.4.5 *Pancreatic α-amylase activity and small intestinal starch disappearance*

Despite increases in pancreatic α-amylase activity in response to postruminal protein or amino acid flow, it is unclear if increases in pancreatic α-amylase are related to increases in small intestinal starch disappearance. Associations between increased small intestinal starch disappearance (Richards et al., 2002) and increased pancreatic α-amylase activity (Richards et al., 2003) with postruminal casein supply have led to speculation that pancreatic α-amylase could be the primary factor limiting the extent of small intestinal starch disappearance. However, in most studies where pancreatic α-amylase increased,

small intestinal carbohydrases were not evaluated. Therefore, there may be confounding effects in determining the limiting factor if there are also simultaneous increases in small intestinal carbohydrase activities.

Furthermore, several enzyme infusion studies with exogenous α-amylase have failed to show a response in small intestinal starch disappearance in cattle. Remillard et al. (1990) infused porcine pancreatic α-amylase or bicarbonate in a 2 × 2 factorial design into the jejunum and failed to see any effects on small intestinal starch disappearance in steers fed an 85% grain diet. Abomasal infusions of exogenous amylase (*Bacillus subtilis* origin) with 880 g/d of raw corn starch (Westreicher-Kristen et al., 2018) or increasing levels of raw corn starch up to 1993 g/d (Robbers et al., 2019) did not influence postruminal starch disappearance in heifers. Likewise, duodenal infusion of exogenous porcine pancreatic α-amylase in amounts equivalent to two, four, or five times the endogenous amount of bovine pancreatic α-amylase secretion (22 kU/h) did not result in increased small intestinal starch disappearance in steers (Trotta et al., 2020b).

2.5 Small intestinal carbohydrases

2.5.1 *Influence of energy intake on small intestinal maltase, isomaltase, and glucoamylase activity*

Compared with pancreatic α-amylase, 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 excess energy intake had little influence on small intestinal carbohydrase activities (Russell et al., 1981; Janes et al., 1985; Kreikemeier et al., 1990b). Increasing dietary intake from 1 to 2 × NE_m did not influence intestinal maltase activity in steers

(Rodriguez et al., 2004). 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 did not influence maltase activity or mRNA expression of maltase-glucoamylase or sucrase-isomaltase in the small intestine (Koch et al., 2019). Sixty-percent nutrient restriction of ewes during mid- to late-gestation increased small intestinal maltase, isomaltase, and glucoamylase activities (Trotta et al., 2020d). Overall, changes in energy intake status do not seem to have specific effects on small intestinal carbohydrase activities (Harmon, 1993; Rodriguez et al., 2004).

2.5.2 *Influence of macronutrients on small intestinal maltase, isomaltase, glucoamylase activity*

Few studies have investigated the effects of specific nutrients on small intestinal carbohydrase activities in ruminants; most information is about 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., 2001b). In another experiment, partially hydrolyzed starch infusions for 7 d did not influence maltase activity in any site of the small intestine in cattle (Bauer et al., 2001a). Later, steers receiving abomasal infusions of glucose or partially hydrolyzed starch for 35 d had greater maltase specific activity than steers receiving ruminal starch infusions (Rodriguez et al., 2004). This may indicate that luminal substrate flow (maltose, isomaltose, and 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 infused with partially hydrolyzed starch into the abomasum had greater maltase specific activity than steers infused with casein, with partially hydrolyzed starch + casein being intermediate (Guimaraes et al., 2007). Dietary fructose differentially regulated small intestinal maltase-glucoamylase (increase) and sucrase-isomaltase (decrease) mRNA expression in neonatal calves (Trotta et al., 2020e). In the same study, glucoamylase activity was found to increase with dietary fructose while maltase activity was unchanged (Trotta et al., 2020e).

In rats, 80% of apparent maltase activity is derived from sucrase-isomaltase and the remaining 20% is derived from maltase-glucoamylase (Galand, 1989; Lin et al., 2012). The concept that maltase activity is derived from multiple enzyme subunits is supported by heat-inactivation studies by Coombe and Siddons (1973). Therefore, changes in maltase-glucoamylase and sucrase-isomaltase mRNA expression with dietary fructose may explain why glucoamylase and maltase activities were differentially regulated. 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.

There is limited information about how dietary or luminal amino acid supply influences small intestinal carbohydrase activity in ruminants. Increasing supplemental Leu (0 g/kg, 0.4 g/kg, 0.6 g/kg or 0.8 g/kg of BW) in milk replacer linearly decreased maltase and isomaltase activities of calves (Reiners, 2021). Similarly, feeding Leu at 2.9% of DM to neonatal lambs in milk replacer for 42 d decreased small intestinal maltase and

isomaltase activity at slaughter after an 82 d finishing period (Reiners, 2021). Rumen-protected Arg supplementation to gestating ewes fed at 60% of nutrient requirements did not influence small intestinal digestive enzyme activities of lamb offspring at day 54 of age (Trotta et al., 2020a). Although Glu has been shown to increase small intestinal starch disappearance in steers after 12 d (Brake et al., 2014a; Blom et al., 2016), our recent study found only a small increase in duodenal maltase activity in steers duodenally infused with raw corn starch and Glu for 58 d (Trotta et al., 2020c).

Duodenal infusions of raw corn starch with casein increased jejunal maltase, isomaltase, and glucoamylase activities in steers (Trotta et al., 2020c). It is unclear if increases in small intestinal carbohydrases with postruminal casein infusion are directly related to increased luminal protein flow because peptide hydrolysates and free amino acids from casein might influence neuroendocrine signaling to increase carbohydrase activity in the small intestine. Alternatively, increased luminal protein flow might cause increased carbohydrase activity indirectly. Increased flow of luminal substrates (maltose, isomaltose, and limit dextrins) as a result of greater hydrolysis of amylose and amylopectin in response to increases in pancreatic α-amylase activity might modulate increases in small intestinal carbohydrase activities. In Caco-2 cells, supply of maltose induced synthesis of a higher molecular weight sucrase-isomaltase immunoblot band compared with glucose, fructose, isomaltose, and fructose (Cheng et al., 2014). Using Caco-2 cells, Chegeni et al. (2018) suggested there was a luminal maltose-sensing mechanism that increases apparent maltase activity by enhancing intracellular trafficking of sucrase-isomaltase to the apical membrane. These effects were associated with an increased mRNA expression of *TAS1R2* and *TAS1R3*, the genes encoding the dimeric sweet taste receptor subunits T1R2-T1R3.

These authors suggested that T1R2-T1R3 could potentially mediate effects of luminal maltose on sucrase-isomaltase activity (Chegeni et al., 2018).

2.6 Sucrase

2.6.1 *Intestinal sucrase activity is absent in multiple ruminant species*

A remaining enigma of ruminant digestive physiology is the absence of sucrase activity in the small intestine. Several studies have investigated and characterized digestive enzyme activity along the small intestine with various ruminant species, ages, and diets. Yet, there has been a failure to detect active sucrase in the small intestine. This is in contrast to nonruminant species including the pig and human. 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., 1990b). Shirazi-Beechey et al. (1989) attempted to measure sucrase activity in isolated brush-border membrane vesicles (BBMV) from lamb intestine and also did not detect any sucrase activity. More recently, we have been unable to detect sucrase activity in intestinal mucosa samples from neonatal calves (Trotta et al., 2020e), growing steers (Trotta et al., 2020c), or fetal, neonatal, or gestating sheep (Trotta et al., 2020a; Trotta et al., 2020d).

The lack of sucrase activity seems to expand to a wider range of ruminants other than sheep and cattle and even some nonruminant foregut fermenters. Marine mammals

such as whales and dolphins have similarities in the sucrase-isomaltase protein sequence to even-toed ungulates. Although it is unknown if dolphins or whales possess intestinal sucrase activity (Ridgway, 2013), the sea lion does not (Kretchmer and Sunshine, 1967). A comparative study demonstrated that sucrase activity was not detected in any ruminant species including 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 pseudoruminant camel intestine, glucoamylase and maltase activities were two- and three-fold greater than sucrase activity (Mohamed et al., 2007).

2.6.2 *Congenital sucrase-isomaltase deficiency and multiple sequence alignment*

Interestingly, the absence of sucrase activity in the small intestine of ruminants appears to be similar to congenital sucrase-isomaltase deficiency (CSID) in infants (Coombe and Siddons, 1973). There are seven distinct phenotypes of CSID in humans with phenotypes I, II, and III resulting in completely inactive sucrase and isomaltase activities (Naim et al., 2012). It would seem unlikely that mechanisms driving phenotypes I, II, or III of CSID would explain the inactivity of sucrase in ruminants because ruminants do have active isomaltase activity (Coombe and Siddons, 1973). However, in phenotype V of CSID, it is characterized by the presence of isomaltase activity and absence of sucrase activity.

The primary structure of the sucrase-isomaltase complex was deduced in the rabbit intestine and a "stalk" region (amino acids 33–70) was identified as the connection between the transmembrane domain and *N*-terminal sucrase-isomaltase (isomaltase subunit) (Hunziker et al., 1986). In phenotype V of CSID, pro-sucrase-isomaltase is cleaved

intracellularly in the *trans*-Golgi network, and the sucrase subunit is subsequently degraded while the isomaltase subunit is transported to the apical membrane (Fransen et al., 1991; Naim et al., 2012). Later experiments determined that the signals for apical sorting were located in the *O*-glycosylated "stalk" region and membrane anchoring domain of the isomaltase subunit (Alfalah et al., 1999; Jacob et al., 2000).

The stalk region of sucrase-isomaltase may be important to understanding the sucrase phenotype of ruminants. Using multiple sequence alignment, the sucrase-isomaltase amino acid sequence was compared between several ruminant species and the camel, pig, horse, and human (Fig. 2.3). At amino acid 46 of the sequence, the selected ruminant species (cattle, bison, water buffalo, goat, and Reeve's muntjac) all have an Arg residue, while sheep have a Gly residue. Furthermore, the camel, pig, horse, and human all contain a Ser residue at this position. Immediately following amino acid 46, the sequence from amino acids 47–60 is missing in the selected ruminant species. This region is enriched with Ser and Thr residues and has been suggested to be *O*-glycosylated to be protected from degradation from pancreatic proteases (Hunziker et al., 1986). Indeed, bypassing the pancreatic duct in rats to prevent luminal pancreatic secretions resulted in a decreased rate of sucrase degradation and increased intestinal sucrase activity (Riby and Kretchmer, 1985). *N*- and *O*-glycosylation sites of sucrase-isomaltase are essential components for proper folding and intracellular trafficking to the apical membrane (Alfalah et al., 1999; Naim et al., 1999).

This missing region of the sucrase-isomaltase protein could perhaps explain why ruminants do not have intestinal sucrase activity. Using multiple sequence alignment, mutations in the coding regions of the sucrase or isomaltase subunits that result in other

phenotypes of CSID (Naim et al., 2012) did not occur in the sucrase-isomaltase sequences of the selected ruminant species (data not shown). It should be noted that although the horse and the camel are herbivores that consume mostly forage-based diets, these species have been shown to have active intestinal sucrase activity (Dyer et al., 2002; Mohamed et al., 2007) and the 47–60 amino acid sequence of sucrase-isomaltase. These comparative sequence findings provide evidence that the absence of sucrase activity in ruminants could potentially be similar to phenotype V of CSID due to the absence of the 47–60 amino acid sequence of the stalk region, which is important for intracellular sorting to the apical membrane. Cellular localization and molecular characterization of the sucrase-isomaltase complex in ruminants is warranted, with specific regard to phenotype V of CSID.

2.6.3 *Nutritional influences on sucrase-isomaltase mRNA expression and activity*

Few studies have attempted to induce sucrase activity by nutritional methods in ruminants. Milk-feeding or dietary supplementation of sucrose to lambs did not induce sucrase activity and small intestinal disappearance of sucrose was small (Ørskov et al., 1972). Because sucrase activity was not induced by its own substrate and small intestinal disappearance was low, those authors concluded that intestinal disappearance of sucrose was most likely due to microbial fermentation (Ørskov et al., 1972). These conclusions were supported by increased cecal microbial counts and increased fecal N excretion with sucrose inclusion (Ørskov et al., 1972). Likewise, abomasal infusions of sucrose did not induce sucrase activity in lambs (Swanson and Harmon, 1997).

In one case report in humans, dietary fructose supplementation increased sucrase activity by nearly four-fold in a patient with CSID (Greene et al., 1972). Although the phenotype of the patient was not revealed, the patient had deficient, but not absent, activity

of sucrase and isomaltase before beginning fructose treatment (Greene et al., 1972). After treatment, sucrase activity levels were still approximately 18.5% of the amount of sucrase activity from the patient's family members (Greene et al., 1972). Fructose supplementation at 2.2 g/kg of BW did not induce sucrase activity in neonatal calves fed milk replacer (Trotta et al., 2020e). However, dietary fructose decreased sucrase-isomaltase mRNA expression, suggesting that sucrase-isomaltase may be transcriptionally regulated by dietary fructose in the ruminant small intestine. These studies indicate that fructose supplementation is not effective at inducing or increasing sucrase activity in patients with CSID or in ruminants.

2.6.4 *Impacts on carbohydrate digestion*

Recent evidence from nonruminant studies suggests that the absence of sucrase can have other physiological consequences on carbohydrate digestion. 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. Furthermore, when supplemented with an oral glucoamylase enzyme, sucrase-deficient shrews had blood glucose concentrations that were similar to the control shrews (containing normal sucrase activity). These authors concluded that sucrase was the predominant mucosal enzyme involved in starch digestion because of its affinity towards multiple starch substrates (Lee et al., 2016). In steers, duodenal infusions of exogenous glucoamylase increased small intestinal starch disappearance (Trotta et al., 2020b). Collectively, these data suggest that ruminants have quantitative limits in carbohydrate digestion similar to humans with congenital sucrase-isomaltase deficiency and supplemental enzymes may

replace missing intestinal hydrolytic activity to improve small intestinal carbohydrate digestibility.

2.7 Glucose absorption

2.7.1 *Sodium/glucose cotransporter-1 (SGLT1)*

Sodium/glucose cotransporter-1 (SGLT1), glucose transporter 5 (GLUT5), and glucose transporter 2 (GLUT2) are thought to be the predominant carbohydrate transporters in the small intestine of ruminants (Harmon, 2009). Many studies in ruminants have concluded that SGLT1 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 d 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 activity 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, duodenal infusion of raw corn starch did not influence SGLT1 activity in sheep (Shirazi-Beechey et al., 1995).

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 cyclic adenosine monophosphate (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 (Table 2.1). In companion studies, jejunal Na^+-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). Postruminal infusion of partially hydrolyzed starch increased SGLT1 activity by two-fold in both sheep and cattle compared with ruminal infusion of partially hydrolyzed starch (Bauer et al., 2001b). In the next experiment, SGLT1 activity 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., 2001a). Abomasal infusion of partially hydrolyzed starch did not increase SGLT1 activity in any site of the small intestine (Bauer et al., 2001a). 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 activity. It should be noted that increasing dietary energy intake to 2 × NE_m also did not influence SGLT1 abundance or activity (Rodriguez et al., 2004). Similarly, Liao et al. (2010) infused partially hydrolyzed starch ruminally or abomasally and found only tendencies to influence *SGLT1* mRNA

expression. They reported that ruminal partially hydrolyzed starch infusions tended to increase duodenal *SGLT1* mRNA expression and that abomasal infusions of partially hydrolyzed starch tended to increase ileal *SGLT1* mRNA expression (Liao et al., 2010). Lohrenz et al. (2011) reported that in lactating dairy cows fed a high starch (24% of DM) or low starch diet (12% of DM) there was 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 activity were evaluated (Guimaraes et al., 2007). Using the same treatments as Swanson et al. (2002a), abomasal partially hydrolyzed starch, casein, or their combination did not influence 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 abundance (Klinger et al., 2013). Although Dyer et al. (1994) determined that duodenal fructose infusions can increase jejunal SGLT1 activity and abundance in lambs, dietary fructose supplementation to neonatal calves did not influence *SGLT1* mRNA expression (Trotta et al., 2020e). 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.

2.7.2 *Glucose transporter 5 (GLUT5)*

Fructose is passively transported across the intestinal apical membrane by GLUT5 (Ferraris et al., 2018). Dietary fructose supplementation has been shown to increase *GLUT5* mRNA expression and enhance intestinal fructose transport in neonatal rats (Shu et al., 1997). Zhao et al. (1993) found that *GLUT5* mRNA 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* mRNA expression is found 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* mRNA expression is directly proportional to intestinal luminal fructose concentration in weaning rats (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). Dietary fructose supplementation to neonatal calves fed milk replacer did not influence *GLUT5* mRNA expression in the small intestine (Trotta et al., 2020e).

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 (solid 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, 1998; Jiang et al., 2001; Jiang and Ferraris, 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 and this process could be similar in cattle.

2.7.3 *Glucose transporter 2 (GLUT2)*

Glucose transporter 2 is thought to be the primary basolateral transporter of monosaccharides from intestinal enterocytes. The apical GLUT2 hypothesis (Kellett and Helliwell, 2000) in which GLUT2 translocates to the apical membrane and contributes to apical (luminal) sugar transport has been controversial (Ferraris et al., 2018). Whether or not GLUT2 translocation occurs in ruminants or contributes to apical sugar uptake under physiological substrate concentrations have not been adequately evaluated. Abundance of GLUT2 in the small intestine has been specifically evaluated in BBMV from lactating dairy cows (Lohrenz et al., 2011), newborn calves (Steinhoff-Wagner et al., 2014), and lactating ewes (Klinger et al., 2016). Lohrenz et al. (2011) quantified GLUT2 abundance in BBMV, as well as, crude cell membrane extracts (CCM) from duodenal and jejunal mucosal tissue of lactating dairy cows. They found that GLUT2 was present in duodenal and jejunal BBMV. Steinhoff-Wagner et al. (2014) demonstrated that GLUT2 was present in BBMV prepared from mid-duodenal and proximal-, mid-, and distal-jejunal mucosa of newborn calves. Additionally, they used immunofluorescence to show localization of GLUT2 on the apical and basolateral membranes (Steinhoff-Wagner et al., 2014). Their data showed that the apical:basolateral distribution of GLUT2 was positive (Steinhoff-Wagner et al., 2014), indicating greater abundance of GLUT2 on the apical membrane. A follow-up study demonstrated that feeding colostrum for 4 d after birth decreased basolateral GLUT2 fluorescence and increased apical GLUT2 fluorescence, suggesting an increase in GLUT2 translocation in small intestinal enterocytes of calves (Steinhoff-Wagner et al., 2015).

However, in sheep, jejunal BBMV did not express GLUT2, whereas jejunal CCM did (Klinger et al., 2016). Brush-border membrane vesicles can potentially be contaminated with increased basolateral enrichment, indicated by increased Na^+/K^+-ATPase activity or abundance compared with the homogenate. Contamination of BBMV with the basolateral membrane could artificially increase GLUT2 abundance estimates in the apical membrane (Röder et al., 2014). Activity of SGLT1 is typically assessed using BBMV preparations and measuring glucose uptake in the presence or absence of Na^+. Bauer et al. (2001a) measured Na^+-independent glucose uptake in BBMV from cattle and found that at 200 µM luminal glucose, Na^+-independent glucose uptake only contributed to 3% of total glucose uptake by BBMV. These data indicate that Na^+-independent uptake activity at 200 µM luminal glucose in the apical membrane is unlikely to be a major route of glucose absorption in growing beef steers. Solvent drag (Pappenheimer and Reiss, 1987), a phenomena where glucose is paracellularly absorbed across intracellular junctions, is also not thought to be a major route of glucose absorption under physiological conditions because passive diffusion of glucose is small in cattle (Krehbiel et al., 1996). Further evaluation is needed across different physiological states, intakes, and luminal glucose concentrations to better understand the contribution of GLUT2 to apical glucose transport in ruminants.

Studies evaluating effects of nutrition on GLUT2 mRNA expression or protein abundance have not been consistent in ruminants. Abomasal infusion of partially hydrolyzed starch tended to increase ileal *GLUT2* mRNA expression in steers (Liao et al., 2010). Duodenal or jejunal *GLUT2* mRNA expression and protein abundance in BBMV or CCM were not influenced by feeding diets with differing starch concentrations to lactating

dairy cows (Lohrenz et al., 2011). Klinger et al. (2016) found that jejunal GLUT2 abundance was greater for lactating ewes compared with dried-off ewes. Dietary fructose supplementation to neonatal calves did not influence *GLUT2* mRNA expression in the duodenum, jejunum, or ileum (Trotta et al., 2020e).

2.7.4 *Portal appearance of glucose*

There is a disproportional relationship between intestinal carbohydrate disappearance and portal glucose appearance in cattle. In mature ruminants, limited amounts of glucose appear in portal blood (Schambye, 1951) which indicate that microbial fermentation and/or visceral metabolism of glucose are substantial. Short-chain fatty acid concentrations in digesta are typically used to evaluate the fermentability of a given diet or nutrient. Reductions in ileal pH and increased short-chain fatty acid concentrations in ileal digesta could suggest microbial activity in the small intestine (Ørskov et al., 1972; Mayes and Ørskov, 1974; Kreikemeier et al., 1991). In general, small intestinal short-chain fatty acid concentrations are far less than large intestinal concentrations in cattle (Kreikemeier et al., 1991) and pigs (Franklin et al., 2002). Huntington and Reynolds (1986) abomasally infused glucose or raw corn starch in lactating dairy cows and beef heifers and measured net nutrient flux across the portal-drained viscera (PDV). 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 corn starch appeared 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 abomasally 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 corn starch that disappears in the small intestine resulted in net portal glucose absorption. In a similar study, Holstein steers were abomasally infused with water, glucose, corn dextrin, or corn starch at 66 g/h (Kreikemeier and Harmon, 1995). A total of 73% of glucose, 60% of corn dextrin, and 57% of corn starch that disappeared in the small intestine could be accounted for as net portal glucose flux. 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 starch flow does result in an increase in net portal glucose flux; however, there is a large amount of glucose that is utilized by splanchnic tissues. When corrected for visceral metabolism, glucose uptake by the PDV was 77% of supply (Harmon et al., 2001).

In beef steers, abomasal raw corn starch infusions with casein increased portal glucose appearance by 0.38 g per gram of casein infused (Taniguchi et al., 1995). However, when corn starch was infused ruminally and casein was infused abomasally, net portal glucose flux did not differ (Taniguchi et al., 1995). The amino acids Gln, Glu, and Pro are

found in the largest abundance in casein (Lapierre et al., 2012) and Glu is the primary substrate used by duodenal enterocytes for energy metabolism in beef cattle (El-Kadi et al., 2009). Because abomasal casein infusion with ruminal raw corn starch infusion did not increase net portal glucose flux, these data suggest that increases in net portal glucose flux in response to casein are because of increased intestinal starch hydrolysis and/or greater intestinal glucose transport rather than shifts in PDV metabolism (Oba et al., 2004).

Compared with 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 (Mayes and Ørskov, 1974; Kreikemeier et al., 1991; Kristensen et al., 2005; Gilbert et al., 2015). Gilbert et al. (2015) concluded that fermentation is the primary contributor to starch disappearance in the small intestine rather than enzymatic hydrolysis to glucose in milk-fed calves. However, cellulose and hemicellulose digestibility in the small intestine is small, indicating that microbial activity on carbohydrates in the small intestinal lumen is probably not a major contributor to intestinal carbohydrate disappearance in functional ruminants (MacRae and Armstrong, 1969; Beever et al., 1972; Sniffen et al., 1992). Whether or not starch is digested hydrolytically or microbially in the small intestine, research has shown that energetic advantages can be gained with increased starch digestion in the small intestine (Harmon and McLeod, 2001). Despite limitations in intestinal glucose absorption resulting from insufficient transport, microbial fermentation, or visceral metabolism, adequate amounts

of glucose can be supplied to peripheral tissues via hepatic gluconeogenesis, even in high-producing dairy cows (Aschenbach et al., 2010).

2.8 Conclusions

The interface between nutrient supply and gastrointestinal function are important because digestive and absorptive function in the small intestine are coordinated by luminal nutrient flows. Partially hydrolyzed carbohydrates, microbial-N and nucleosides, and biohydrogenated lipids flowing to the small intestine may alter responses of the pancreas and intestine to luminal nutrient flows. Practical solutions to increase the extent of small intestinal starch disappearance are challenging, yet improvements in small intestinal starch digestibility with extensive grain processing warrant further attention. Ruminant carbohydrase activities of the pancreas and small intestine appear to respond differently to diet and luminal nutrient supply compared with nonruminants. Alignment of the sucrase-isomaltase primary sequence of multiple species suggests that the absent region in ruminant species is related to the absence of intestinal sucrase activity in ruminants and, thus, constitutes a major limitation in ruminant intestinal assimilation of starch compared with nonruminant species. Mechanisms of adaptation of glucose transporters to substrate are apparently less sensitive in cattle compared with small ruminants. Future research efforts in these areas will aid our efforts to optimize feeding strategies that increase the efficiency of meat and milk production by increasing our understanding of how starch is digested, and glucose absorbed, in the ruminant small intestine.

Table 2.1 Selected studies evaluating the effects of dietary or postruminal carbohydrate supply on carbohydrate transporter uptake activity, protein abundance, and mRNA expression in cattle and small ruminants.

Item	Small Ruminants				
	(Shirazi-Beechey et al., 1991)	(Dyer et al., 1994)	(Bauer et al., 2001b)	(Mabjeesh et al., 2003)	(Klinger et al., 2013)
Species	Lambs	Sheep	Sheep	Lambs	Goats
Diet	Pelleted	Roughage	85% fescue hay	Wheat hay + cracked corn	Hay/Corn/Wheat
Source	Duodenal	Duodenal	Ruminal/abomasal	Abomasal	Dietary
Nutrient	Glucose	Glucose/Galactose/ Fructose	PHS	Casein	-
Length	4 d	4 d	7 d	10 d	21 d
Amount	8.1 g/d	8.1 g/d	6 g/h	35 g/d	600 g/d
Transporter	SGLT1	SGLT1	SGLT1	SGLT1	SGLT1
Parameter	Abundance, activity	Abundance, activity	Activity	Abundance, activity	Abundance, activity
Duodenum [1]	↑Abundance and activity	↑Abundance and activity	-	↑Abundance, →activity	-
Jejunum [1]	-	-	↑Activity	↓Abundance, ↑activity	→ Abundance,↑activity
Ileum [1]	-	-	-	→ Abundance, activity	-
Item	Cattle				
	(Bauer et al., 2001b)	(Rodriguez et al., 2004)	(Guimaraes et al., 2007)	(Liao et al., 2010)	(Trotta et al., 2020e)
Species	Steers	Steers	Steers	Steers	Calves
Diet	85% fescue hay	90% orchardgrass	90% alfalfa cubes	Alfalfa cubes	Milk replacer
Source	Ruminal/abomasal	Abomasal	Abomasal	Ruminal/abomasal	Dietary
Nutrient	PHS	PHS or Glucose	PHS + Casein	PHS	Fructose
Length	7 d	35 d	10 d	14 d	28 d

Amount	40 g/h	776 g PHS/d, 886 g glucose/d	352 g/d starch, 52.8 g/d casein	800 g/d	92 g/d
Transporter	SGLT1	SGLT1	SGLT1	SGLT1/GLUT2/ GLUT5	SGLT1/GLUT2/ GLUT5
Parameter	Activity	Abundance, activity	Abundance, activity	mRNA expression	mRNA expression
Duodenum [1]	-	→	→	↑SGLT1 (RS)	→
Jejunum [1]	↑Activity	→	→	→	→
Ileum [1]	-	→	→	↑SGLT1, GLUT2 (AS)	→

[1] Response: ↑ = increase; ↓ = decrease; → = no change; - = not evaluated. Abbreviations: AS = abomasal starch; GLUT2 = glucose transporter 2; GLUT5 = glucose transporter 5; PHS = partially hydrolyzed starch; RS = ruminal starch; and SGLT1 = sodium-dependent glucose cotransporter-1.

Figure 2.1 Relationship between the amount of raw corn starch postruminally infused per hour per kilogram of body weight and small intestinal starch disappearance as a percentage of duodenal flow in cattle. Adapted using data from selected studies. (Kreikemeier et al., 1991; Kreikemeier and Harmon, 1995; Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016; Trotta et al., 2020b; Acharya et al., 2023).

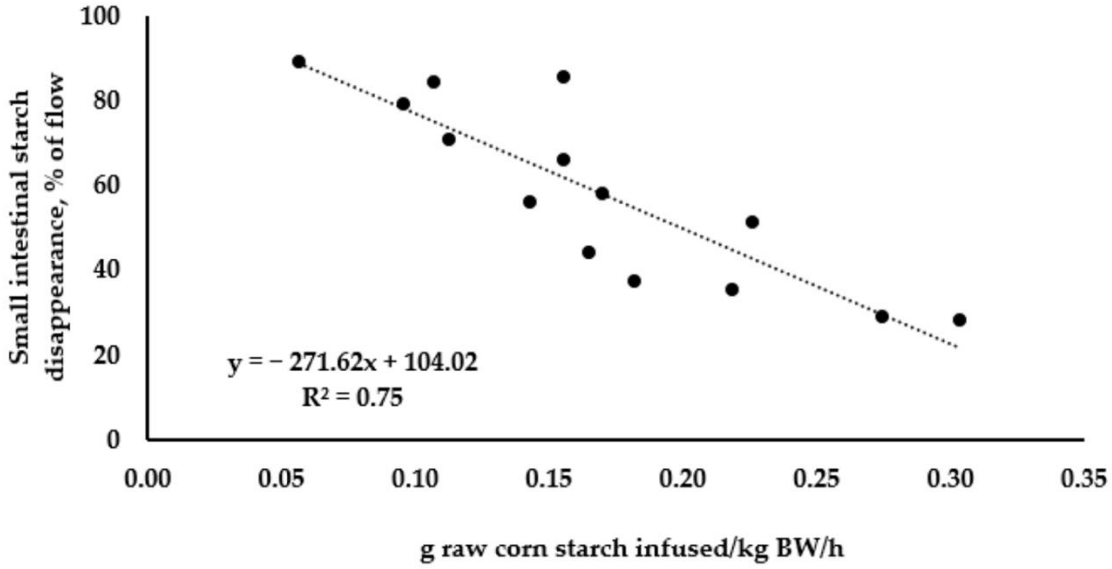


Figure 2.2 (A) Effects of abomasal infusion of 4 g partially hydrolyzed starch (PHS)/kg BW and 0.6 g casein/kg BW infusion on pancreatic α-amylase activity relative to BW in Holstein calves; adapted from Swanson et al. (2002a). (B) Effects of duodenal infusion of 9 g raw corn starch (RS)/kg BW with 0.66 g glutamic acid or 2.4 g casein/kg BW on pancreatic α-amylase activity relative to BW in beef steers; adapted from Trotta et al. (2020c).

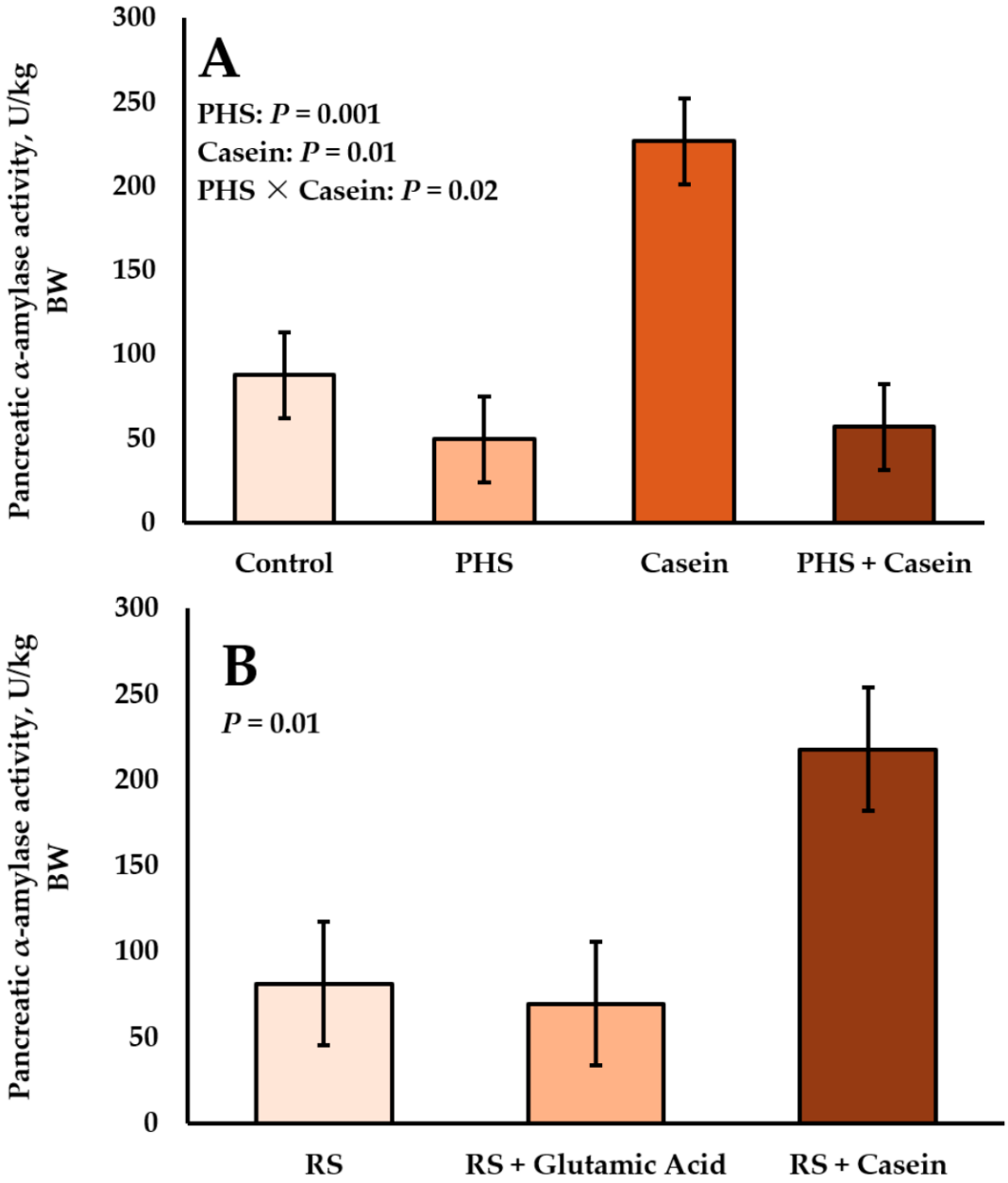
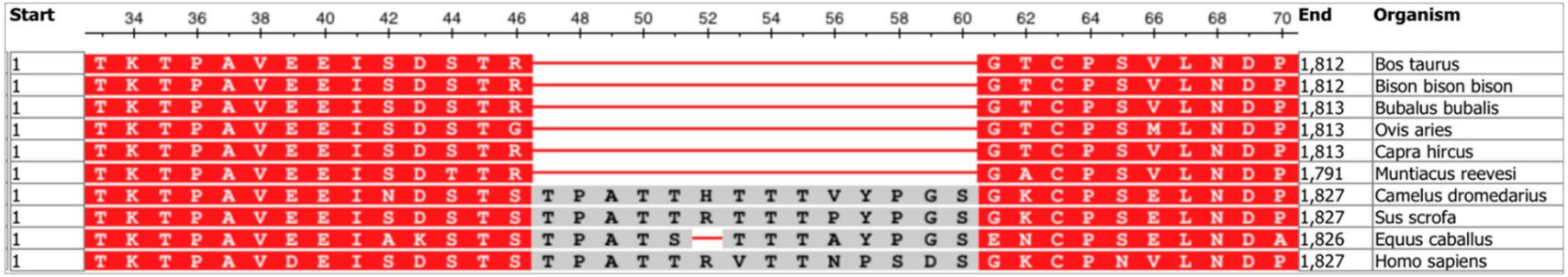


Figure 2.3 Multiple sequence alignment of the sucrase-isomaltase stalk region (amino acids 33–70) for selected ruminant species.



CHAPTER 3. INFLUENCE OF AIR EQUILIBRATION TIME, SAMPLING TECHNIQUES, AND STORAGE TEMPERATURE ON ENZYMATIC STARCH AVAILABILITY OF STEAM-FLAKED CORN

3.1 Introduction

Corn is the primary grain source used in commercial receiving and finishing diets for beef cattle and steam-flaking is the primary grain processing method recommended by consulting nutritionists to improve starch availability, nutrient utilization, and the overall feeding value of corn (Zinn et al., 2002; Samuelson et al., 2016). Enzymatic starch availability is a measurement that is often used to evaluate the degree of starch gelatinization and it is thought that enzymatic starch availability reflects the rate of ruminal starch fermentation (Schwandt et al., 2016). To standardize the steam-flaking process and ensure manufacturing consistency, enzymatic starch availability is routinely assayed by commercial feedlots as a quality control method (Schwandt et al., 2016).

Schwandt et al. (2016) reported that starch availability averaged 50.6% but ranged from 37.0% to 65.0% across 49 steam-flakers from 17 commercial feedlots. Variation in steam-flaked corn starch availability can occur due to factors including moisture content (Sindt et al., 2006; Schwandt et al., 2016; Horton et al., 2020), flake density (Zinn, 1990; Theurer et al., 1999; Sindt et al., 2006; Schwandt et al., 2016), starch retrogradation (Ward and Galyean, 1999; McMeniman and Galyean, 2007), roll diameter (Schwandt et al., 2016), and steam conditioning time (Horton et al., 2020). Flake density has a negative linear relationship with enzymatic starch availability (Schwandt et al., 2016). Moreover, flake density influences the particle size distribution of steam-flaked corn, which influences nutrient composition of each sieved fraction (Hales et al., 2010).

Sampling and handling techniques are also thought to contribute to the variation in estimates of starch availability (Schwandt et al., 2016). Post-flaking sampling and handling techniques that involve collection of flakes, segregation of flakes and fines, and conditions influencing starch availability during transport to the laboratory are not well-described. Thus, sampling procedures can vary among feedyard personnel which can lead to inconsistent estimates of starch availability. The objectives of this study were to evaluate factors (sample handling, sifted portions of steam-flaked corn, air equilibration time, moisture, storage temperature) influencing enzymatic starch availability of steam-flaked corn. Characterizing effects of sifting, air equilibration time, and storage temperature and how that influences starch availability is necessary to improve techniques that reduce the variation of starch availability estimates, thereby developing repeatable methodology for feedyard personnel.

3.2 Materials and methods

No animals were used in the conduct of this study.

3.2.1 *Steam-flakers*

Samples of steam-flaked corn were obtained from 11 steam-flakers (n = 10 for Exp. 1) that were located at five commercial feedyards in Northwest Kansas and Southwest Nebraska. Descriptions of the steam-flakers (experimental units) that were sampled during the conduct of this study are contained in Table 3.1. Each steam-flaker (roll diameter × length; 61.0 cm × 142 cm rolls, n = 4; 45.7 cm × 91.4 cm rolls, n = 4; 50.8 cm × 91.4 cm rolls, n = 3) was used as a blocking factor because previous research has demonstrated that roll diameter can influence starch availability of steam-flaked corn (Schwandt et al., 2016).

All steam-flakers contained round-bottom V-cut roll corrugations and 5.51 corrugations/cm. Roll temperature (97.4 ± 1.83ºC) and roll use time (1249 ± 725 h; hours of active flaking since roll recorrugation) were recorded before the sampling for Exp. 1. Steam-chest dimensions, transition height, and the number of steam ports were also measured and reported.

For sampling, all steam-flakers were pre-adjusted to produce a targeted 335 g/L (26 lb/bu) flake density. All steam-flakers were operating for at least 2-h so equilibration could be achieved. Within each experiment, all samples were collected within 90 min. The ambient temperature and relative humidity in the room for sample preparation were 26.1 ± 3.32ºC and 51.2 ± 6.29%, respectively.

3.2.2 *Experiment 1*

Ten steam-flakers were used to evaluate the effects of air equilibration time on enzymatic starch availability of steam-flaked corn. One steam-flaker from Feedyard #3 was not sampled due to inoperability on the day of sampling. One bulk sample of steam-flaked corn was collected from each steam-flaker using a metal bucket (15.2 cm diameter × 6.35 cm height) on a handle that was extended under the rolls to collect fresh flakes. The bulk sample (341 ± 10.7 g/L) was transferred to a standard testing sieve (30.5 cm diameter, 4-mm screen; Advantech Manufacturing, Inc., New Berlin, WI) fitted above a collection pan. The steam-flaked corn was sieved gently for 20 s and the small particles that passed-through the sifting sieve to the bottom collection pan (< 4-mm; sifted fines) were discarded. The large particles retained in the top portion of the sieve (> 4-mm; sifted flakes) were divided into four subsamples using a 147 mL aluminum scoop and each subsample was

placed in a sealed polycarbonate bottle. Bottles were immediately flash-frozen in ethanol super-cooled with solid CO_2 and then stored at -20ºC.

The four subsamples were then randomly assigned to one of four treatments: 1) control samples were transferred into a sealed plastic bag at 23ºC for 3-d, 2) samples were dumped onto a tabletop and air equilibrated for 240 min and then placed into a plastic bag at 23ºC for 3-d (air equilibrated), 3) samples were placed into aluminum pans and into a forced-air oven at 55ºC for 24-h (oven dry), and 4) the final subsample was freeze-dried. The 3-d storage time was chosen to simulate shipping and handling time to a commercial laboratory.

3.2.3 *Experiment 2*

Samples were collected from 11 steam-flakers described above to evaluate the effects of air equilibration time (0, 15, 30, 60, 120, or 240 min) on enzymatic starch availability of steam-flaked corn. Prior to sampling, sieves and collection pans were pre-weighed and then stacked as described in Exp. 1. Then, we placed a No. 26 density cup (Quart Cup 26; Seedburo Equipment Co., Chicago, IL) in the center of the upper sieve. Six consecutive, independent bulk samples of steam-flaked corn were collected from each steam-flaker using a metal bucket (15.2 cm diameter × 6.35 cm height) on a handle that was extended under the rolls to collect fresh steam-flaked corn. The collected bulk sample was immediately transferred to a No. 26 density testing cup so that the sample overflowed the total volume capacity. and then the excess was scraped off using a zig-zag motion with a No. 65 strike-off stick.

We then measured flake density of the steam-flaked corn sample (fresh flake density) after scraping off the excess using a zig-zag motion with a No. 65 strike-off stick. The contents of the testing cup were then dumped into the upper portion of the sieve. After sieving the steam-flaked corn gently for 20 s, the top and bottom sieves were weighed, and a second flake density measurement (sifted flake density) was recorded for the large particles retained in the upper portion of the 4-mm sieve. The small particles that passed-through the sifting sieve to the bottom collection pan were discarded. The proportion of sifted fines was 11.7 ± 2.88%. After each air equilibration period, we recorded a third density measurement (air equilibrated flake density) on the particles retained on the 4-mm screen. After that, sifted flakes were placed into plastic bags for 3-d at 23ºC to simulate shipping time to a commercial laboratory.

3.2.4 *Experiment 3*

Samples were collected from the same 11 steam-flakers to evaluate the effects of air equilibration time and sifting on enzymatic starch availability of steam-flaked corn. Fresh bulk samples of steam-flaked corn were collected from each steam-flaker as described previously. Samples were either not sifted (flakes + fines) or sifted gently for 20 s with a standard testing sieve to collect the particles retained in the top-portion (> 4-mm; sifted flakes). Flakes + fines and sifted flakes were allowed to air equilibrate for either 0 min or 240 min at room temperature. At the end of the air equilibration period, samples were placed into plastic bags for 3-d at 23ºC to simulate shipping time to a commercial laboratory.

3.2.5 *Experiment 4*

Samples were collected from the same 11 steam-flakers to evaluate the effects of air equilibration time and particle size separation on enzymatic starch availability of steam-flaked corn. Fresh bulk samples of steam-flaked corn were collected from each steam-flaker as described previously. Samples were sifted gently for 20 s with a standard testing sieve to collect the particles retained in the top-portion (> 4-mm; sifted flakes) and in the bottom collecting pan (< 4-mm; sifted fines). Sifted flakes and sifted fines were air equilibrated for either 0 min or 240 min at room temperature. At the end of the air equilibration period, samples were placed into plastic bags for 3-d at 23ºC to simulate shipping time to a commercial laboratory.

3.2.6 *Experiment 5*

Samples were collected from the same 11 steam-flakers to evaluate the effects of air equilibration time and storage temperature on enzymatic starch availability of steam-flaked corn. Fresh bulk samples of steam-flaked corn were collected from each steam-flaker as described previously. Samples were not sifted (flakes + fines) and air equilibrated for either 0 min or 240 min at room temperature. Samples were placed into foil bags and heat-sealed to prevent moisture loss. Foil bags were stored at two different temperatures for 3-d: either room temperature (23ºC) or 55ºC in a forced-air drying oven. The oven temperature setting (55ºC) was chosen because the temperature of the core of the steam-flaked corn pile can remain > 55ºC for more than 17 h (Sindt, 2004; Drouillard and Reinhardt, 2006). Additionally, temperatures during shipping could potentially reach this level during the summer.

3.2.7 *Dry matter and nutrient composition*

Samples were placed into aluminum pans and dried in a forced air oven at 55ºC for 24-h to determine dry matter (**DM**). Dried samples were ground to pass through a 1-mm screen using a cyclone sample mill (Model 3010-014; UDY Corporation, Fort Collins, CO). Samples from Exp. 4 were analyzed for nutrient composition including crude protein (AOAC, 2002; 990.03), soluble crude protein (Roe and Sniffen, 1990), ether extract (945.16), acid-hydrolyzed fat (922.06), neutral and acid detergent fiber (Van Soest et al., 1991), and minerals (990.08).

3.2.8 *Available starch, total starch, and starch availability*

Ground samples were weighed (1.00 ± 0.0368 g) into 225 mL polycarbonate bottles (Item #72475; Qorpak, Bridgeville, PA). The bottle was combined with 75 mL of pre-warmed acetate buffer (8.2 g/L sodium acetate; 8.2 mL/L glacial acetic acid; pH = 4.50), 2.5 mL of amyloglucosidase solution (195 U/mL; AMIGASE GA 400L; Centerchem, Inc., Norwalk, CT), and two stainless steel magnets (1.27 cm × 7.62 cm; Silver Star AlniMAX II, Sundown Industries Co., Plainview, NY). For total starch samples, the bottles were initially incubated in a 100ºC shaking water bath for 60 min to gelatinize the starch and then cooled in a 40ºC water bath before addition of amyloglucosidase. Then, available and total starch samples were incubated in a 40ºC shaking water bath for 60 min to facilitate enzymatic hydrolysis. Bottles were hand-swirled every 15 min during incubation. After completion of the hydrolysis incubation, a 1-mL aliquot of the media was transferred to a 1.5-mL centrifuge tube, placed in a 90ºC water bath for 3 min to terminate the reaction, and then cooled in an ice-water bath. Tubes were then frozen at -20ºC. Samples were

thawed and free glucose concentration was measured using the glucose oxidase electrode on a YSI Series 2900 biochemistry analyzer (YSI Inc., Yellow Springs, OH). An enzyme blank was used to correct for endogenous glucose concentrations. Available starch and total starch concentrations were calculated by converting free glucose to anhydroglucose, as it occurs in starch (McCleary et al., 1994). Starch availability was calculated by dividing available starch by total starch and multiplying by 100. Ground sifted flake samples corresponding to 270 g/L (21 lb/bu; 83.7 ± 3.57% starch availability) and 399 g/L (31 lb/bu; 43.3 ± 3.71% starch availability) flake densities were used as substrates for control samples.

3.2.9 *Statistical analysis*

All variables were checked for normality using the UNIVARIATE procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). For Exp. 1, the data were analyzed as a randomized complete block design using the GLM procedure of SAS. The model included fixed effects of treatment and flaker as a blocking factor.

For Exp. 2, flake density was analyzed as a split-plot design using the MIXED procedure of SAS. The model included fixed effects of flaker (block), air equilibration time, density measurement, and the time × measurement interaction. Fresh flake density (initial measurement) was included in the model as a covariate. The whole plot error was specified as the flaker × time interaction in the random statement. Starch availability data were analyzed as a randomized complete block design using the GLM procedure of SAS for fixed effects of air equilibration time and flaker (block). The IML procedure was used to generate polynomial contrast coefficients (linear and quadratic) to account for unequal spacing between treatments.

For Exp. 3, the data were analyzed as a randomized complete block design using the GLM procedure of SAS. The model included fixed effects of flaker (block), air equilibration time, sifting, and the air equilibration time × sifting interaction. For Exp. 4, the data were analyzed as a randomized complete block design for fixed effects of flaker (block), air equilibration time, sifted portion, and the air equilibration time × sifted portion interaction. For Exp. 5, the data were analyzed as a randomized complete block design for fixed effects of flaker (block), air equilibration time, storage temperature, and the air equilibration time × storage temperature interaction. Least squares means and their standard errors were computed for each fixed effect included in the models. Pairwise differences of least squares means were separated using the Tukey-Kramer adjustment, protected by a significant F-test. Results were considered significant if $P \leq 0.05$.

3.3 Results

3.3.1 *Experiment 1*

Oven-drying decreased ($P < 0.01$) available starch concentration and starch availability of sifted flakes compared to other sampling handling methods (Table 3.2). The total starch concentration of sifted flakes was not influenced by the sample handling method.

3.3.2 *Experiment 2*

There was an air equilibration time × density measurement interaction ($P = 0.04$) for flake density (Table 3.3). Sifting samples decreased ($P = 0.04$) flake density compared to fresh density measurement. Furthermore, air equilibration for 60, 120, or 240 min decreased ($P = 0.04$) flake density compared to the sifted density measurement. Increasing

air equilibration time linearly decreased ($P < 0.01$) moisture content (Table 3.4). Available starch, total starch, and starch availability of sifted flakes were not influenced by increasing air equilibration time.

3.3.3 *Experiment 3*

There was no sifting × air equilibration time interaction for any variable measured (Table 3.5). Sifted flakes contained lesser ($P < 0.01$) moisture compared to flakes + fines. Air equilibration for 240 min decreased ($P < 0.01$) moisture content. Sifted flakes had greater ($P < 0.01$) proportions of available starch and total starch and greater ($P < 0.01$) starch availability than flakes + fines. Air equilibration time did not influence available or total starch. Air equilibration for 240 min increased ($P = 0.02$) starch availability in both sifted flakes and flakes + fines.

3.3.4 *Experiment 4*

There was an air equilibration time × sifted portion interaction ($P < 0.01$) for moisture content (Table 3.6). The interaction occurred because initial moisture was greater ($P < 0.01$) for sifted fines compared to sifted flakes. But after 240 min of air equilibration, final moisture was lesser ($P < 0.01$) in sifted fines compared to final moisture in sifted flakes. Sifted flakes contained greater ($P < 0.01$) concentrations of available starch and total starch compared to sifted fines. Air equilibration for 240 min increased ($P = 0.01$) the concentration of available starch for both sifted flakes and sifted fines. There was an air equilibration time × sifted portion interaction for starch availability because air equilibration time increased ($P < 0.01$) starch availability of sifted fines but did not influence starch availability of sifted flakes.

There was no air equilibration time × sifted portion interaction for any nutrient (data not shown). Available starch and total starch concentrations were greater ($P < 0.01$) for sifted flakes compared to sifted fines (Table 3.7). Concentrations of crude protein, soluble crude protein, neutral and acid detergent fiber, ether extract, and acid-hydrolyzed fat were greater ($P < 0.01$) for sifted fines compared to sifted flakes. Concentrations of Ca, P, K, Mg, S, Fe, Zn, Mg, and Cu were greater ($P < 0.01$) for sifted fines compared to sifted flakes.

3.3.5 *Experiment 5*

There was no air equilibration time × storage temperature interaction for any variable measured (Table 3.8). Moisture content was greater ($P = 0.03$) in samples that were stored in heat-sealed foil bags at 55ºC for 3-d. Air equilibration for 240 min decreased ($P < 0.01$) moisture content. Storing samples in heat-sealed foil bags for 3-d at 55ºC decreased ($P < 0.01$) available starch concentration and starch availability by 41.5% and 40.7%, respectively. Air equilibration for 240 min increased ($P < 0.02$) available starch concentration and starch availability by 13.8% and 14.7%, respectively. Total starch concentration was not influenced by storage temperature or air equilibration time.

3.4 Discussion

Schwandt et al. (2016) reported that starch availability averaged 50.6% but ranged from 37.0% to 65.0% across 49 steam-flakers from 17 commercial feedlots. Interestingly, the average starch availability of steam-flaked corn reported by commercial feedlots (50.6%) (Schwandt et al., 2016) was 18.2% less than the starch availability recommendations by consulting feedlot nutritionists (59.8%) (Samuelson et al., 2016).

Factors such as flake density, steaming time, and moisture content can explain a large proportion of the variation of starch availability estimates. However, there is little information describing how samples of steam-flaked corn should be processed and therefore, considerable variation can occur from differences in post-flaking sample processing methods. Thus, the objective of this study was to identify factors post-flaking that influence measures of starch availability of steam-flaked corn. Defining how sifting, air equilibration time, and storage temperature influence starch availability is necessary to improve sample handling techniques in order to reduce variation in estimates of starch availability.

Because there was a linear decrease in moisture content with increasing air equilibration time, we expected that air equilibration time would increase starch availability. Moisture content and cooled flake density have previously been shown to influence enzymatic starch availability (Schwandt et al., 2016). Additionally, air equilibration of sifted flakes for 60, 120, or 240 min decreased flake density by ≥ 14 g/L (1 lb/bu), which would be expected to increase starch availability. However, this is a relatively minor change in flake density compared to the 50 g/L (3.6 lb/bu) decrease in flake density due to sifting. In contrast to our hypothesis, increasing air equilibration time did not influence starch availability of sifted flakes (Exp. 2). As a result of our sampling protocol, the moisture content of sifted flakes was 18.8% (0 min air equilibration) in Exp. 2 which was less than anticipated based on the moisture content of the whole flakes. Therefore in Exp. 3, the effects of sifting and air equilibration time were tested, and the results confirmed that the sifting procedure decreased the moisture content from 21.5% to

19.0%. Concurrently, there were 6.20% and 2.68% increases in starch availability with air equilibration for 240 min for flakes + fines and sifted flakes, respectively.

Thus in Exp. 4, we sought to characterize the fractions produced by sifting and demonstrated that sifted fines contained 26.6% moisture. Because air equilibration of the sifted fines for 240 min decreases moisture content from 26.6% to 9.80%, the rapid loss of moisture likely contributed to the increase in starch availability of flakes + fines after air equilibration. Rapid cooling of steam-flaked corn is an important process before measuring starch availability because available starch concentrations rapidly increase during steaming and crushing, and then plateau with rapid cooling (McAllister et al., 2006). The importance of air equilibration to the flakes + fines samples was further demonstrated in Exp. 5, where starch availability increased with air equilibration, regardless of storage temperature. Sifting steam-flaked corn samples with a 4-mm sieve may be a useful strategy to reduce variation in the estimates of starch availability due to removal of the fines.

Furthermore, the nutrient composition of sifted fines differed for every nutrient measured when compared to sifted flakes in the current study. Available and total starch concentrations were 2.4- and 1.8-fold greater for sifted flakes compared to sifted fines, respectively. Sifted fines contained greater concentrations of fiber, protein, and minerals compared to sifted flakes. These data agree with Hales et al. (2010) who demonstrated that sifted particles <4.76 mm of steam-flaked corn (335 g/L; 26 lb/bu) contained greater concentrations of crude protein, neutral detergent fiber, ether extract, and P. Sifting steam-flaked corn samples also allows for measurement of the proportion of fines, which can decrease with increases in flake density (Hales et al., 2010). Current sampling practices in the feedlot make it difficult to obtain a representative sample of steam-flaked corn;

however, this can be achieved by sifting steam-flaked corn samples and weighing each fraction.

Gelatinization of corn starch through steam-flaking increases enzymatic starch availability, which is generally associated with improved relative feeding value compared to other methods of grain processing (Zinn et al., 2002). However, exposure of steam-flaked corn to high temperatures can cause retrogradation of starch which is often indicated by decreases in starch availability (McAllister et al., 2006). Ward and Galyean (1999) reported that starch availabilities of steam-flaked corn samples obtained directly from under the rolls or from the storage bin were 55.3% and 33.3%, respectively. It has been reported that the core of the pile of flakes can remain > 55ºC for more than 17 h (Sindt, 2004; Drouillard and Reinhardt, 2006). Averaged across air equilibration times, starch availability of flakes + fines that were stored in heat-sealed foil bags for 3-d at 55ºC decreased from 71.6% to 50.9%, presumably due to starch retrogradation. Although multiple studies have demonstrated post-flaking decreases in starch availability during storage, more research is needed to characterize how the length and degree of heat exposure contribute to starch retrogradation.

Although starch retrogradation of steam-flaked corn can occur through heat exposure in the core of the pile, high temperatures during shipping of samples to commercial laboratories could also potentially lead to starch retrogradation. Shipping temperatures could potentially reach temperatures near 55ºC during the summer months in the midwestern United States, particularly for trucked samples. Flakes + fines that were air equilibrated for 240 min had decreased from 73.7% to 57.1% starch availability when stored in heat-sealed foil bags at 55ºC for 3-d. Therefore, air equilibration of steam-flaked

corn does not overcome the effects of high temperatures on starch availability, which leads to dramatically reduced estimates of starch availability.

Sample processing methods from Exp. 1 such as air equilibration, oven drying, and freeze drying all remove moisture from sifted flakes. However, samples that were oven-dried in aluminum pans in Exp. 1 had a lesser starch availability compared to control, air equilibrated, and freeze-dried flakes. It is possible that this effect is an artifact from the oven temperature used in the current study (55ºC). Oven-drying at a lesser temperature, such as 45ºC, might be sufficient to adequately remove moisture from samples without simultaneously decreasing starch availability. Starch retrogradation is caused by a combination of heat, moisture, and shear (Sindt, 2004). We demonstrated that the combination of sifting and air equilibration decreased moisture content from 21.5% to 15.5% (Exp. 3). It is unclear if methods to remove more moisture (by oven-drying or freeze-drying) would help decrease the incidence of starch retrogradation. Research evaluating optimal oven-drying temperatures and the effects of drying samples before shipping on starch availability could be useful to help further refine sample handling methodology.

3.5 Conclusions

Factors such as air equilibration, heat exposure, moisture, and sifting influenced starch availability of steam-flaked corn. Sifting steam-flaked corn samples is a useful strategy to reduce variation and improve starch availability estimates and obtain a measurement of the proportion of fines. Nutrient composition of sifted flakes and sifted fines differed for every macro- and micronutrient measured. Air equilibration for 240 min

increases starch availability of flakes + fines and sifted fines; however, it cannot overcome the negative effects of starch retrogradation caused by subsequent exposure to high temperatures. Exposing samples of steam-flaked corn to high temperatures (55ºC) in heat-sealed foil bags for 3-d decreases enzymatic starch availability, which suggests retrogradation of starch occurred. Adoption of the strategies discussed in the current study can lead to more consistent estimates of starch availability. Improved estimates of enzymatic starch availability of steam-flaked corn and the proportion of fines could potentially lead to improved flaking consistency in commercial feedlots.

Table 3.1 Description of steam-flakers in commercial feedyards sampled to study starch availability.

Feedyard	Roll Size, cm × cm	Roll Temperature, °C	Roll Use Time, h	Chest Dimensions[2], m	Transition Height, m	Steam Ports
1	61.0 × 142	99.2	816	2.06 × 15.2	2.13	4
2	61.0 × 142	96.7	644	3.86 × 13.1	2.13	6
3	61.0 × 142	98.2	846	3.86 × 13.1	2.13	5
3	61.0 × 142	98.1	300	3.86 × 13.1	2.13	5
4	45.7 × 91.4	95.6	1760	1.42 × 0.610 × 6.10	0.305	4
4	45.7 × 91.4	93.3	847	1.42 × 0.610 × 6.10	0.305	4
4	45.7 × 91.4	97.8	1320	1.42 × 0.610 × 6.10	0.305	4
4	45.7 × 91.4	96.1	1001	1.42 × 0.610 × 6.10	0.305	4
5	50.8 × 91.4	98.9	1586	1.37 × 6.10	1.42	4
5	50.8 × 91.4	98.9	1677	1.37 × 6.10	1.42	4
5	50.8 × 91.4	98.9	2938	1.37 × 6.10	1.42	4

[1]All steam-flakers had round-bottom V cut roll corrugations (5.51 corrugations/cm).

[2]Feedyard #4 steam-flakers had rectangular steam-chests. All other steam-flaker chest dimensions were circular.

Table 3.2 Experiment 1: Effects of sample handling method on starch availability of sifted flakes[1].

	Treatment[2]					
Item	Control	Air Equilibrated	Oven Dry	Freeze Dry	SEM[3]	*P*-value
Available starch, % of DM	50.9[a]	51.3[a]	48.5[b]	51.2[a]	0.433	<0.01
Total starch, % of DM	78.2	78.9	78.2	78.2	0.484	0.71
Starch availability, %	65.0[a]	65.0[a]	62.0[b]	65.5[a]	0.467	<0.01

[1]Particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s.

[2]Control = storage in plastic bag at 23°C for 3-d; Air Equilibrated = air equilibration for 240 min at room temperature, then storage in plastic bag at 23°C for 3-d; Oven Dry = forced-air oven drying at 55°C in aluminum pans for 24-h; Freeze Dry = lyophilization.

[3]Standard error of the mean (n = 10).

[a,b]Least squares means with different superscripts within a row differ ($P < 0.05$).

Table 3.3 Experiment 2: Interaction between air equilibration time and density measurement on flake density of steam-flaked corn.

	Density Measurement[1]				*P*-value		
Time, min	Fresh	Sifted	Air Equilibrated	SEM[2]	Time	Measure	Time × Measure
0	343^{a}	292^{bc}	292^{bc}	2.60	0.07	<0.01	0.04
15	343^{a}	292^{bc}	286^{bcde}				
30	344^{a}	292^{bc}	284^{bcde}				
60	343^{a}	293^{bc}	278de				
120	344^{a}	295^{b}	281^{cde}				
240	343^{a}	289^{bc}	274^{e}				

[1]Fresh = first density measurement of steam-flaked corn samples taken directly from under the rolls before sifting; Sifted = second density measurement of samples retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s; Air equilibrated = third density measurement of samples after air equilibration time at room temperature.

[2]Standard error of the mean (n = 33).

[a-e]Least squares means that do not contain at least one of the same letters across rows and columns differ ($P \leq 0.05$).

Table 3.4 Experiment 2: Effects of air equilibration time on moisture and starch availability of sifted flakes.

	Air Equilibration Time, min							*P*-value	
Item	0	15	30	60	120	240	SEM[2]	Linear	Quadratic
Moisture, %	18.8	17.9	17.5	17.8	16.7	13.9	0.546	<0.01	0.46
Available starch, % of DM	50.6	51.9	51.7	50.9	52.4	51.3	1.39	0.84	0.59
Total starch, % of DM	77.8	78.4	78.4	78.2	77.9	78.5	0.355	0.52	0.54
Starch availability, %	65.0	66.1	65.9	65.2	67.2	65.4	1.90	0.92	0.54

[1]Particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s. Sifted flakes were air equilibrated for 0, 15, 30, 60, 120, or 240 min at room temperature.

[2]Standard error of the mean (n = 11).

Table 3.5 Experiment 3: Effects of air equilibration time and sifting on moisture and starch availability of steam-flaked corn.

	Treatment[1]							
	Flakes + Fines		Sifted Flakes			*P*-value		
Item	0 min	240 min	0 min	240 min	SEM[2]	Sifting	Time	Sifting × Time
Moisture, %	21.5	17.0	19.0	15.5	0.368	<0.01	<0.01	0.16
Available starch, % of DM	50.8	53.8	57.4	58.7	1.11	<0.01	0.07	0.45
Total starch, % of DM	73.2	73.1	76.9	76.6	0.772	<0.01	0.76	0.89
Starch availability, %	69.4	73.7	74.7	76.7	1.28	<0.01	0.02	0.37

[1]Flakes + Fines = steam-flaked corn samples obtained from directly under the rolls that were not sifted. Sifted Flakes = particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s. Samples were air equilibrated for either 0 or 240 min at room temperature.

[2]Standard error of the mean (n = 11).

Table 3.6 Experiment 4: Effects of air equilibration of the two sifted portions (sifted flakes vs. sifted fines) on moisture and starch availability of steam-flaked corn.

	Treatment							
	Sifted Flakes[1]		Sifted Fines[2]			*P*-value		
Item	0 min	240 min	0 min	240 min	SEM[3]	Sifted Portion	Time	Sifted Portion × Time
Moisture, %	19.0[b]	15.5[c]	26.6[a]	9.80[d]	0.478	0.06	<0.01	<0.01
Available starch, % of DM	57.4	58.7	21.8	26.2	1.09	<0.01	0.01	0.16
Total starch, % of DM	76.9	76.6	41.8	43.8	1.31	<0.01	0.54	0.38
Starch availability, %	74.7[c]	76.7[c]	52.2[a]	59.9[b]	1.02	<0.01	<0.01	<0.01

[1]Particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s.

[2]Particles retained in the bottom-portion of the sieve (< 4-mm) after sifting steam-flaked corn samples for 20 s.

[3]Standard error of the mean (n = 11).

[a-d]Least squares means with different superscripts within a row differ ($P \leq 0.05$).

Table 3.7 Experiment 4: Least square means for the main effect of sifted portion on the nutrient composition of steam-flaked corn.

Item	Sifted Particle Size[1]		SEM[4]
	Sifted Flakes[2]	Sifted Fines[3]	
Total starch, % of DM[5]	76.8	42.8	0.93
Available starch, % of DM[5]	58.1	24.0	0.77
Crude protein, % of DM	8.22	14.0	0.18
Soluble crude protein, % of DM	0.90	4.06	0.212
Soluble crude protein, % of crude protein	11.0	29.0	1.55
Neutral detergent fiber, % of DM	7.34	18.3	0.435
Acid detergent fiber, % of DM	2.26	7.32	0.270
Ether extract, % of DM	1.98	16.2	0.397
Acid-hydrolyzed fat, % of DM	3.09	17.9	0.415
Ca, % of DM	0.0099	0.019	0.0018
P, % of DM	0.169	1.13	0.028
K, % of DM	0.277	1.16	0.025
Mg, % of DM	0.0573	0.469	0.0113
S, % of DM	0.0973	0.140	0.0017
Fe, mg/kg of DM	16.7	87.6	3.92
Zn, mg/kg of DM	9.8	71.5	1.71
Mn, mg/kg of DM	3.3	21.6	0.53
Cu, mg/kg of DM	0.994	9.18	0.413

[1]$P < 0.01$ for all variables.

[2]Particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s.

[3]Particles retained in the bottom-portion of the sieve (< 4-mm) after sifting steam-flaked corn samples for 20 s.

[4]Standard error of the mean (n = 22).

[5]Interactive LSM are presented in Table 5.

Table 3.8 Experiment 5: Effects of air equilibration time and storage temperature on moisture and starch availability of flakes + fines[1].

	Treatment							
	0 min		240 min			*P*-value		
Item	23°C	55°C	23°C	55°C	SEM[2]	Temp.	Time	Temp. × Time
Moisture, %	21.5	23.1	17.0	18.5	0.675	0.03	<0.01	0.93
Available starch, % of DM	50.8	32.7	53.8	41.2	2.30	<0.01	0.02	0.24
Total starch, % of DM	73.2	73.3	73.1	72.2	0.525	0.42	0.26	0.38
Starch availability, %	69.4	44.6	73.7	57.1	2.98	<0.01	<0.01	0.18

[1]Steam-flaked corn samples obtained from directly under the rolls that were not sifted. Samples were air equilibrated for either 0 or 240 min at room temperature. Samples were stored in heat-sealed foil bags for 3-d at either 23°C or 55°C in a forced-air oven.

[2]Standard error of the mean (n = 11).

CHAPTER 4. FLAKE DENSITY AND STARCH RETROGRADATION INFLUENCE IN SITU RUMINAL DEGRADABILITY CHARACTERISTICS OF STEAM-FLAKED CORN AND PREDICTED STARCH DIGESTIBILITY AND ENERGETIC EFFICIENCY

4.1 Introduction

We previously demonstrated that flake density and starch retrogradation have profound effects on starch availability of steam-flaked corn (Trotta et al., 2021b). Starch availability was nearly 2-fold greater for steam-flaked corn samples that were flaked to a bulk density of 270 g/L (83.7% starch availability) compared with corn flaked to 399 g/L (43.3% starch availability) (Trotta et al., 2021b). Storage of steam-flaked corn at 55°C for 3-d in heat-sealed foil bags decreased starch availability by nearly 40% due to starch retrogradation (Trotta et al., 2021b). In cattle feeding operations, starch retrogradation of steam-flaked corn could occur through prolonged heat exposure in the core of corn piled for storage (McAllister et al., 2006), storage in grain bins (Ward and Galyean, 1999), or by moving steam-flaked corn from under the rolls into storage bins (McMeniman and Galyean, 2007). However, it is unclear how differences in starch availability due to changes in flake density or starch retrogradation affect ruminal solubility and degradability of steam-flaked corn.

Starch availability is calculated as the amount of enzymatically-available starch divided by the total starch content of a feed and therefore, is thought to be reflective of the rate of ruminal starch fermentation (Schwandt et al., 2016). Methods of corn processing that decrease the rate of ruminal starch fermentation without affecting total-tract digestibility may be beneficial for improving feeding outcomes for finishing cattle (Zinn, 1990; Sindt, 2004). A recent summary comparing corn processing methods found that

decreasing the extent of ruminal starch digestibility of steam-flaked corn could potentially lead to increased energetic efficiency (Owens et al., 2016). In contrast, the same is not always true for diets containing whole-shelled or rolled corn because there are greater amounts of crystalline starch which is poorly digested in the small intestine compared to gelatinized starch from steam-flaked corn (Sniffen et al., 1992). Therefore, changes in starch availability of steam-flaked corn due to changes in flake density or starch retrogradation may influence digestion characteristics of finishing cattle, thereby affecting growth performance and feed efficiency. Our objective was to create large differences in starch availability using two different models: 1) increasing flake density (257, 296, 335, 373, 412 g/L) and 2) inducing starch retrogradation by exposing steam-flaked corn to 55°C temperatures in heat-sealed foil bags. We hypothesized that increasing flake density and inducing starch retrogradation would decrease the rate of ruminal DM degradation of steam-flaked corn. We then used prediction equations to estimate how the findings of the current study could potentially influence starch digestibility and energetic efficiency.

4.2 Materials and methods

Animal care and management protocols followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 4th Edition.

4.2.1 *Experiment 1*

Five ruminally-cannulated Holstein × Angus crossbred steers (initial body weight = 390 ± 7.86 kg) were used to determine the effects of increasing flake density on the rate and extent of *in situ* ruminal degradability. Steers were pen-fed a starter ration (Table 4.1) *ad libitum*. The experimental design was a randomized complete block design with rep as

the blocking factor. The rolls of a steam-flaker (61 cm × 142 cm roll size) were adjusted to produce steam-flaked corn corresponding to 257 g/L (20 lb/bu), 296 g/L (23 lb/bu), 335 g/L (26 lb/bu), 373 g/L (29 lb/bu), and 412 g/L (32 lb/bu) flake densities. A No. 26 density cup (Quart Cup 26; Seedburo Equipment Co., Chicago, IL) was placed in the center of the upper sieve and bulk samples were dumped into the density cup so that the sample overflowed the total volume capacity, and then the excess was scraped off using a zig-zag motion with a No. 65 strike-off stick. Flake density was recorded and then the contents of the testing cup were dumped into the upper portion of a standard testing sieves (30.5 cm diameter, 4-mm screen; Advantech Manufacturing, Inc., New Berlin, WI) fitted above collection pans. Steam-flaked corn samples were sieved gently for 20 s and the small particles that passed through the sifting sieve to the bottom collection pan (< 4-mm; sifted fines) were discarded. The large particles retained in the top portion (> 4-mm; sifted flakes) were air equilibrated for 24-h before collection. Air equilibration does not influence ruminal or total-tract digestibility of steam-flaked corn (Barajas and Zinn, 1998).

A preliminary experiment was conducted to determine the influence of sample size [5 g (25 mg/cm^2), 10 g (50 mg/cm^2), or 20 g (100 mg/cm^2)] on *in situ* ruminal DM degradation. Incubated sample size did not influence the rate or extent of ruminal DM degradation (Table 4.2.). Thus, 20.5 ± 1.59 g of whole sifted flakes were weighed into bags (10 cm × 20 cm; 50 μm pore size; R1020 Forage Bag; ANKOM Technology, Macedon, NY). Bags were incubated for 0, 3, 6, 12, 24, 48, 72, and 96-h. A single bag for each treatment was placed into a nylon bag (25 cm × 31 cm; k2107; HomeAide Delicate Wash Bag) at each incubation timepoint for each steer. Two stainless steel magnets (1.27 cm × 7.62 cm; Silver Star AlniMAX II, Sundown Industries Co., Plainview, NY) were added to

each nylon bag to ensure immersion in the ventral rumen. Nylon bags were attached to a steel chain with a breeching snap clip (2710231; Koch Industries, Inc., Minneapolis, MN). The steel chain was secured to the rumen cannula cap by connecting the steel chain to an inverted U-bolt on the inner portion of the cannula cap (#1 Eazy-out Stopper; Bar Diamond, Inc., Parma, ID) with a breeching snap clip. Bags were inserted in reverse order so that all bags were removed from the rumen and rinsed simultaneously. At removal, the bags were removed from the steel chain, quickly immersed in cold water, and placed into an ice-water bath to stop fermentation. Bags were rinsed 5 times in a washing machine with 1-min rinse and 2-min spin cycles (Coblentz et al., 1997). The bags were then placed in a 100ºC forced-air oven for 48-h to determine *in situ* DM disappearance. The experiment was replicated twice across days as recommended by (Vanzant et al., 1998).

4.2.2 *Experiment 2*

The same five ruminally-cannulated steers were used to determine the effects of particle size and storage temperature on the rate and extent of *in situ* ruminal degradability. Steers were fed the same diet as described in Table 4.1. Samples of steam-flaked corn were sampled under the rolls using a metal bucket (15.2 cm diameter × 6.35 cm height) on a handle that was extended under the rolls to collect fresh flakes that were flaked to a density of 335 g/L (26 lb/bu). The experimental design was a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Bulk samples were processed to obtain 3 different particle sizes: flakes + fines (not sifted; > 4-mm and < 4-mm), sifted flakes (large particles retained in the top-portion of the sieve; > 4-mm) and sifted fines (small particles that passed through the sieve to the bottom collection pan; < 4-mm). Samples were placed into foil bags (Mylar Bags; IMPAK Corporation, Los Angeles, CA) and heat-sealed to

prevent moisture loss. Foil bags were stored at either 23ºC or 55ºC in a forced-air drying oven for 3-d. The oven temperature setting (55ºC) was chosen because it has been reported that the temperature of the core of piled steam-flaked corn can remain > 55ºC for more than 17 hours (Sindt, 2004; Drouillard and Reinhardt, 2006). We previously demonstrated that exposure of steam-flaked corn to 55°C for 3-d decreased starch availability by 40% due to starch retrogradation (Trotta et al., 2021b).

After the 3-d period, foil bags were stored at -20°C to preserve samples until the day of ruminal incubation. Each feed (19.6 ± 3.00 g) was weighed into bags, as described previously. Treatments were incubated for 0, 3, 6, 12, 24, 48, 72, and 96-h according to the methods described in Exp. 1. The bags were then placed in a 100ºC forced-air oven for 48-h to determine *in situ* DM disappearance. The experiment was replicated twice across days (Vanzant et al., 1998).

4.2.3 *Nutrient composition*

Bulk samples of the basal diet and treatments from Exp. 1 and 2 were dried at 45°C in a forced-air oven. Dried samples were ground to pass through a 1-mm screen using a cyclone sample mill (Model 3010-014; UDY Corporation, Fort Collins, CO). Samples were analyzed for nutrient composition including crude protein (AOAC, 2002; 990.03), ether extract (945.16), neutral and acid detergent fiber (Van Soest et al., 1991), and minerals (990.08).

Available starch and total starch concentrations were measured according to the methods described by Trotta et al. (2021b). Total starch samples were gelatinized in acetate buffer by heating in a 100ºC shaking water bath for 60 min. Then, amyloglucosidase

solution (195 U/mL; AMIGASE GA 400L; Centerchem, Inc., Norwalk, CT) was added to tubes containing the substrate (available starch) or the gelatinized substrate (total starch) and all tubes were incubated for 60 min at 40ºC to facilitate enzymatic hydrolysis. Free glucose concentration was measured. Starch availability was calculated by dividing available starch by total starch and multiplying by 100.

4.2.4 *Rate and extent of in situ ruminal degradability*

The potential rate and extent of *in situ* DM degradation were determined using the first-order asymptotic model (McDonald, 1981):

$$y = a + b\,(1 - e^{-kd(t - Lt)})$$

where y is the degradation after t hours, a is the soluble fraction, b is the potentially degradable fraction, k_d is the fractional rate of degradation of b, t is the incubation time (h), and Lt is the lag time. Dry matter degradability data from time 0, 3, 6, 12, 24, 48, 72, and 96 h were used to generate the parameters described. Degradation data were fitted to the above nonlinear model using SAS (SAS Institute, Cary, NC) according to the procedures described by Fadel (2004).

In situ ruminal degradability was determined by using the parameters generated for the rate and extent of degradation, as previously described, and modeled with the rate of passage (Ørskov and McDonald, 1979):

$$isRD = a + [\,(bk_d) / (k_d + k_p)\,]$$

where *isRD* is *in situ* ruminal degradability, a is the soluble fraction, b is the potentially degradable fraction, k_d is the fractional rate of degradation of b, and k_p is the rate of passage.

A constant rate of passage was selected at 6% per hour, which is considered to be average for high-starch concentrate feeds (Offner et al., 2003). Degradation coefficients from the generated parameters were converted to percentages by multiplying by 100.

4.2.5 *Predicted ruminal and total-tract starch digestibility and energetic efficiency*

In situ ruminal DM degradability of sifted flakes for Exp. 1 (335 vs. 373 g/L) and Exp. 2 (23ºC vs. 55ºC) were used to estimate *in situ* ruminal starch degradability, total-tract starch digestibility, and energetic efficiency. Only the 335 g/L and 373 g/L flake density treatments were used from Exp. 1 because they are similar to the range of flake densities recommended by consulting nutritionists (320-360 g/L) (Samuelson et al., 2016) and the magnitude of differences between flake densities (38 g/L) is similar to the flake density range difference (40 g/L) reported by Samuelson et al. (2016). Additionally, only sifted flake data from Exp. 2 was used to exclude inclusion of the fines so that all data used in prediction equations were generated using the *in situ* ruminal DM degradability of sifted flakes.

In situ ruminal DM degradability was used to predict *in situ* ruminal starch degradability using the regression equation ($y = 0.124 + 0.96x$) by Offner et al. (2003). *In situ* ruminal starch degradability was then used to estimate total-tract starch digestibility of steam-flaked corn using the regression equation ($y = 0.04x + 96.0$) by Owens et al. (2016). Energetic efficiency was estimated using *in situ* ruminal starch degradability with the equation ($y = -0.2x + 99.33$) by Owens et al. (2016). The rate of passage was evaluated at 4%, 6%, and 8% per hour, which is within the range used by others to determine *in situ* ruminal degradability for concentrate feeds (Offner et al., 2003). The rate of passage was

adjusted within this range to account for potential changes in passage rate that might occur with increasing flake density or starch retrogradation.

4.2.6 *Statistical analysis*

Degradation data were analyzed using the NLIN procedure of SAS for nonlinear segmented models (Fadel, 2004) to estimate the parameters in the equation previously described (McDonald, 1981). All data were analyzed using the GLM procedure of SAS with steer as the experimental unit and rep as the blocking factor. For Exp. 1, the data were analyzed as a randomized complete block design for fixed effects of flake density and block. Linear and quadratic contrast statements were generated. For Exp. 2, the data were analyzed as a randomized complete block design with a 3 × 2 factorial arrangement of treatments for fixed effects of block, particle size, storage temperature, and the particle size × storage temperature interaction. Least squares means and their standard errors were computed for each fixed effect included in the models. Pairwise differences of least squares means were separated using the Tukey-Kramer adjustment, protected by a significant F-test. The CORR procedure was used to evaluate the relationship between starch availability with *in situ* ruminal DM degradability of sifted flakes using data generated from Exp. 1 and Exp. 2. The MEANS procedure of SAS was used to generate means and standard deviations for predicted starch digestibility and predicted energetic efficiency. Treatment (Exp. 1 – 335 g/L vs. 373 g/L; Exp. 2 – 23ºC vs. 55ºC) and passage rate were included in the class statement. Results were considered significant if $P \leq 0.05$.

4.3 Results and discussion

4.3.1 *Influence of flake density on the rate and extent of ruminal degradability*

Numerous studies have evaluated the effects of flake density of grains such as corn, barley, and sorghum on digestion and growth performance in cattle. It is widely accepted that flaking to a lighter density (< 300 g/L) does not result in economically favorable outcomes because of limited improvements in total-tract starch digestibility and feed efficiency, decreased mill production rate, increased mill energy consumption, and increased risk of ruminal acidosis and bloat (Reinhardt et al., 1997; Brown et al., 2000; Drouillard and Reinhardt, 2006). In contrast, heavier flake densities (> 400 g/L) increase mill production rate and decrease mill energy consumption but also can decrease ruminal and/or total-tract digestibility. Consulting feedlot nutritionists recommended flaking corn to an average bulk density of 350 g/L, and the range of responses varied by a small margin (320-360 g/L) (Samuelson et al., 2016). We acknowledge that the 257 g/L and 412 g/L treatments are beyond the range of typical production flake densities; however, their inclusion in the study design was essential to create a successful model for large differences in starch availability.

Decreasing flake density increases starch solubility (Zinn, 1990; Theurer et al., 1999) and increasing starch solubility is generally associated with increasing total-tract starch digestibility in cattle (Zinn et al., 1998). The soluble fraction (*a*) responded quadratically ($P = 0.01$) with the soluble fraction decreasing as flake density increased (Table 4.3). Numerically, the soluble fraction decreased from 44.2% to 25.9% with increasing flake density. Likewise, the potentially degradable fraction (*b*) responded quadratically ($P = 0.02$) with the potentially degradable fraction increasing as flake density

increased. The soluble fraction of 335 g/L flakes in Exp. 1 was similar (39.2%) to the soluble fraction (39.0%) of 348 g/L flakes reported by Buttrey et al. (2016). Changes of the soluble and potentially degradable fractions could be due to increased flake thickness, as well as, a reduction in surface area with increasing flake density. Indeed, *Lt* linearly increased ($P = 0.01$) and the fractional rate of degradation (k_d) linearly decreased ($P < 0.01$) with increasing flake density from 257 to 412 g/L.

Despite changes in the soluble and potentially degradable fractions with increasing flake density, the potential extent ($a + b$) of DM degradation was not influenced. In general, increasing flake density (increasing flake thickness) of steam-flaked corn decreases ruminal and total-tract starch digestibility in cattle (Zinn et al., 2002). In the current study, *in situ* ruminal DM degradability linearly decreased ($P < 0.01$) from 78.9% to 57.3% with increasing flake density (257 to 412 g/L). *In vivo* trials evaluating the influence of increasing flake density have observed similar results where digestibility decreases but, to a lesser magnitude than in the current study. Zinn (1990) found that increasing flake density (300, 360, 420 g/L) in diets containing 75% steam-flaked corn linearly decreased total-tract organic matter (OM), starch, and N digestibility without influencing ruminal digestion of OM or starch. However, in a study by Theurer et al. (1999), increasing flake density from 283 g/L to 438 g/L decreased ruminal starch digestibility by 11%. Sindt et al. (2006) found little difference in ruminal OM or starch digestibility from steam-flaked corn-based diets flaked to 309 or 360 g/L. Increasing flake density (283, 335, 386 g/L) in 60% steam-flaked corn-based diets linearly decreased total-tract starch digestibility and tended to decrease total-tract OM digestibility in steers (Ponce et al., 2013). Discrepancies across studies could be attributed to the differences between *in vivo* and *in situ* digestibility

measures. Observed decreases in ruminal or total-tract digestibility likely resulted from the inclusion of treatments with a flake density > 400 g/L, as this is beyond the range of flake densities typically used in feedlot diets (320-360 g/L) (Samuelson et al., 2016).

Additionally, it should also be noted that the *in vivo* trials referenced (Zinn, 1990; Theurer et al., 1999; Sindt et al., 2006; Ponce et al., 2013) were conducted with steers that consumed less than 3 × NE_m (average energy intake = 2.08 × NE_m), which may have masked influences of passage rate on ruminal digestibility in those studies. Indeed, increasing intake from 2 to 3 times maintenance increased ruminal particulate passage of steam-rolled wheat by 53.5% (Kreikemeier et al., 1990a). Increasing ruminal starch particulate passage with greater intakes may not result in a proportional decrease in the rate of starch digestion, which could alter the site of starch digestion by shifting more starch postruminally.

4.3.2 *Influence of particle size and storage temperature on the rate and extent of ruminal degradability*

Gelatinization of corn starch during the steam-flaking process causes irreversible swelling of the starch granules (Zinn et al., 2002). Reassociation of dispersed starch molecules with exposure to a combination of heat, moisture, and shear can cause starch retrogradation (Zinn et al., 2002; Sindt, 2004). Methods of corn processing that decrease the rate of ruminal fermentation and increase total-tract digestibility could potentially be implemented to optimize feeding outcomes for finishing cattle (Zinn, 1990). Sindt (2004) speculated that retrogradation of starch could potentially be beneficial if it decreased the rate of fermentation, without influencing the extent of ruminal or total-tract starch digestibility. Starch availability was 2.48-, 2.07-, and 1.81-fold greater for flakes + fines,

sifted flakes, and sifted fines samples stored at 23ºC compared to 55ºC in the current study indicating our model for inducing starch retrogradation was successful.

There were particle size × storage temperature interactions (P = 0.04) for the soluble (a) and potentially degradable (b) fractions (Table 4.4). As expected, values for the soluble fraction for all particle sizes were greater (P = 0.04) at 23ºC. The interaction occurred because sifted fines had a greater decrease (P = 0.04) in the soluble fraction with storage at 55ºC compared to the other two particle sizes. For the potentially degradable fraction, the opposite occurred. Values were greater (P = 0.04) at 55ºC vs. 23ºC for all particle sizes but, the sifted fines had a greater increase (P = 0.04) in the potentially degradable fraction with storage at 55ºC compared to the other two particle sizes.

The potential extent (a + b) of *in situ* ruminal DM degradation was not influenced by particle size or storage temperature. Likewise, *in vitro* dry matter disappearance of steam-flaked corn was not influenced by sampling location, despite nearly a 40% difference in starch availability from samples taken from directly under the rolls compared to sampling from a storage bin (Ward and Galyean, 1999). These observations indicate that starch retrogradation does not limit the potential extent of digestibility of steam-flaked corn, which could suggest that total-tract DM digestibility would be similar between treatments.

Storage of steam-flaked corn samples at 55ºC for 3-d decreased (P < 0.01) the fractional rate of degradation by 37.6% across all particle sizes. Lag time was not influenced by particle size or storage temperature. Storage of samples at 55ºC for 3-d decreased (P < 0.01) *in situ* ruminal DM degradability of flakes + fines, sifted flakes and sifted fines by 20.9%, 22.6%, and 14.7%, respectively. Within each particle size, nutrient

profiles were similar at 23ºC vs. 55ºC except for starch availability. Therefore, the decrease in the fractional rate of degradation was due to starch retrogradation.

For *in situ* ruminal DM degradability, sifted fines were greater ($P < 0.01$) than sifted flakes or flakes + fines. This could be due to differences in particle size, as well as, differences in their nutrient profiles. The sifted fines had a particle size < 4-mm and sifted flakes were kept whole. Additionally, sifted fines had numerically greater concentrations of crude protein, neutral and acid detergent fiber, and crude fat compared to sifted flakes which are similar to previous reports (Hales et al., 2010; Trotta et al., 2021b).

4.3.3 *Relationship of starch availability with in situ ruminal DM degradability*

Enzymatic starch availability is thought to reflect the rate of ruminal starch fermentation (Schwandt et al., 2016). Schwandt et al. (2016) reported that starch availability averaged 50.6% but ranged from 37.0% to 65.0% across 49 steam-flakers from 17 commercial feedlots. Moisture content (Sindt et al., 2006; Schwandt et al., 2016; Horton et al., 2020), flake density (Zinn, 1990; Theurer et al., 1999; Sindt et al., 2006; Schwandt et al., 2016), starch retrogradation (Ward and Galyean, 1999; McMeniman and Galyean, 2007), roll diameter (Schwandt et al., 2016), and steam conditioning time (Horton et al., 2020) all contribute to the variation surrounding estimates of starch availability. Post-flaking sampling and handling techniques such as drying method, sifting, air equilibration time, and storage temperature can also influence estimates of starch availability of steam-flaked corn (Trotta et al., 2021b). Flake density has a negative linear relationship with enzymatic starch availability (Schwandt et al., 2016), which we were able to replicate in Exp. 1 by increasing flake density from 283 to 412 g/L.

We found that starch availability of sifted flakes was positively correlated ($P < 0.01$) to *in situ* ruminal DM degradability using data from Exp. 1 and Exp. 2 (Fig. 4.1; $r^2 = 0.97$). The average starch availability of sifted flakes ranged from 28.5% to 87% and each treatment contained one measurement of starch availability from the bulk samples used for *in situ* ruminal incubations. Individual estimates of *in situ* ruminal DM degradability ranged from 40.7% to 86.5%. More research is needed to understand how other forms of grain processing (whole-shelled, rolled, high-moisture) influence the relationship between starch availability and ruminal digestion to better understand how starch availability relates to *in vivo* starch utilization.

4.3.4 *Implications for starch digestibility and energetic efficiency*

In general it has been thought that grain processing methods that increase ruminal digestibility result in increased total-tract digestibility (Theurer, 1986). Combined with the limitations of postruminal starch digestibility, recommendations have largely focused on maximizing total-tract digestibility by choosing grain processing methods that increase ruminal digestibility (Ørskov, 1986; Theurer, 1986; Huntington, 1997). Despite theoretical energetic advantages with small intestinal starch digestion compared to ruminal starch fermentation (Owens et al., 1986; McLeod et al., 2001), practical methods to manipulate the site of starch digestion for improved energetic efficiency are limited (Harmon and McLeod, 2001). Using *in situ* ruminal DM degradability data from the current study, increasing flake density from 335 to 373 g/L (Exp. 1) and starch retrogradation (Exp. 2) decreased predicted *in situ* ruminal starch degradability within a constant passage rate (Table 4.5), suggesting increased postruminal starch flows.

Owens et al. (2016) summarized 57 publications evaluating corn-based diets in feedlot cattle and demonstrated that ruminal starch digestibility is poorly related ($R^2 = 0.10$) to total-tract starch digestibility of steam-flaked corn-based diets because total-tract starch digestibility of steam-flaked corn is typically greater than 98% in finishing cattle (Owens et al., 1986; Huntington, 1997; Owens and Soderlund, 2006). If total-tract starch digestibility does not decrease with increasing flake density or starch retrogradation, energetic advantages may be gained with increased small intestinal flow and digestion of steam-flaked corn. Predicted total-tract starch digestibility of the sifted flakes used in Exp. 1 and Exp. 2 ranged from 98.3% to 99.3% across passage rates of interest.

Limitations to the extent of intestinal starch digestion, such as the chemical and physical nature of corn starch, could potentially decrease energetic efficiency (Owens et al., 1986). However, gelatinization of corn starch via steam-flaking likely increases intestinal starch availability and access for enzymes to hydrolyze starch molecules (Zinn et al., 1995). Increasing flake density or inducing starch retrogradation decreases starch availability; however, differences in starch availability are not a result of the same chemical modifications to the starch granule. Increasing flake density results in less gelatinized starch whereas starch retrogradation is a rearrangement of previously gelatinized starch. Thus, the differences in chemical and physical nature of starch resulting from either increasing flake density or starch retrogradation could potentially affect digestibility characteristics.

Apparent intestinal starch digestibility is greatest with steam-flaked processing compared to dry-rolled and high-moisture corn processing (Owens et al., 1986). The extent of intestinal steam-flaked corn starch digestibility (76%) (Owens and Soderlund, 2006) is

greater than the minimum digestibility coefficient (70%) (Huntington et al., 2006; Owens and Soderlund, 2006) required to increase energetic efficiency compared to ruminal starch fermentation. Strategies to modify the site and extent of steam-flaked corn digestion, possibly through starch retrogradation, may increase intestinal starch digestibility in cattle. Theoretical models predicting the relationship between ruminal starch digestibility and energetic efficiency suggest that decreasing ruminal digestibility of steam-flaked corn should increase energetic efficiency (Owens et al., 2016). Using predicted *in situ* ruminal starch digestibility to estimate energetic efficiency, our calculations suggest that decreasing ruminal starch digestibility within a given passage rate could increase energetic efficiency by up to 3.4%.

There are limitations to the use of prediction equations that need to be addressed. First, the equations used were all generated from linear regression. While relationships between ruminal starch digestibility and energetic efficiency of steam-flaked corn appear to be linear, we would expect decreasing the extent of ruminal starch digestibility would decrease total-tract starch digestibility and energetic efficiency at some point. For example, studies mentioned earlier (Zinn, 1990; Theurer et al., 1999; Sindt et al., 2006; Ponce et al., 2013) demonstrated that feeding steam-flaked corn bulked to a density > 400 g/L can decrease ruminal and/or total-tract digestibility.

Also, ruminal passage rates are dynamic and the magnitude of changes in passage rate can largely dictate digestibility outcomes. If the rate of ruminal passage decreased proportional to the decrease of the fractional rate of degradation, there would be no effects on ruminal starch digestibility *in vivo* and no improvements to energetic efficiency. For example, starch retrogradation decreased the fractional rate of degradation of sifted flakes

by 49.4% in Exp. 2. If the rate of ruminal passage decreased proportionally (i.e. 50%) from 8% to 4% per hour with starch retrogradation, *in situ* ruminal starch digestibility and energetic efficiency would not differ from sifted flakes stored at 23ºC with a passage rate of 8% per hour. The level of intake and forage inclusion could potentially also contribute to changes in ruminal passage rates (Kreikemeier et al., 1990a).

Interactions of steam-flaked corn with other dietary ingredients, the level of steam-flaked corn inclusion, and dry matter intake all contribute to variability in predictions of steam-flaked corn digestibility. Modifications to the site and extent of starch digestion could also potentially influence dry matter intake, ruminal microbial protein synthesis, ruminal pH and fiber digestion, and the incidence of ruminal acidosis (Taylor and Allen, 2005; Alman, 2009) and these factors need to be considered when implementing dietary strategies that influence the site of digestion. Our calculations of starch digestibility and energetic efficiency should only be considered tentative until animal measurements can support or refute these concepts (Owens et al., 2016). More research is needed to determine the effects of increasing flake density and starch retrogradation of steam-flaked corn on passage rate and the site of starch digestion.

4.4 Conclusions

Increasing flake density and starch retrogradation modified the solubility of steam-flaked corn by changing the soluble and potentially degradable fractions. The potential extent of degradation was not influenced by flake density or starch retrogradation. Increasing flake density and starch retrogradation decreased the fractional rate of degradation and *in situ* ruminal DM degradability. Starch availability of sifted flakes was

positively correlated with *in situ* ruminal DM degradability of sifted flakes. Decreases in starch availability and *in situ* ruminal degradability may indicate that increasing flake density or starch retrogradation can increase postruminal starch flows in cattle. More research is needed to determine how flake density and starch retrogradation influences passage rate, the site of digestion, and energetic efficiency to develop flaking recommendations for feedlot producers to optimize growth performance and feed efficiency of cattle.

Table 4.1 Composition of the starter diet fed to steers.[1,2]

Item	
Ingredient composition, DM basis	
Mixed corn and triticale silage, %	45.0
Wet corn distillers' grains with solubles, %	33.4
Steam-flaked corn, %	13.1
Mixed grass hay, %	5.55
Liquid starter supplement, %	2.95
Chemical composition	
Dry matter, %	53.3
Organic matter, % of DM	90.5
Total starch, % of DM	35.8
Neutral detergent fiber, % of DM	32.4
Acid detergent fiber, % of DM	21.9
Crude protein, % of DM	13.3
Ether extract, % of DM	3.80

[1]Contained: 1.49% K, 0.76% Ca, 0.4% P, 0.26% Mg, 0.26% S, 0.24% salt, and 0.033 g lasalocid / kg (Bovatec; Zoetis Inc., Parsippany, NJ).

[2]Contained (per kg of DM): 227 mg Fe, 104 mg Zn, 74.2 mg Mn, 26.0 mg Cu, 2.83 mg I, 0.42 mg Se, 0.41 mg Co, 565 IU vitamin A, 56.4 IU vitamin D, and 2.82 IU vitamin E.

Table 4.2 Effects of incubated sample size on in situ ruminal DM degradability of steam-flaked corn.[1,2]

Item	Treatment, g			SEM	*P*-value
	5	10	20		
Degradation parameters					
a, %	24.3	26.4	27.5	2.05	0.54
b, %	71.3	69.3	68.3	1.79	0.51
a + *b*, %	95.6	95.7	95.8	0.923	0.98
k_d, %/h	8.56	8.70	8.92	1.84	0.99
Lt, h	1.99	2.17	1.87	1.02	0.98
is*RD*, %	67.8	68.2	69.4	2.83	0.93

Abbreviations: DM = dry matter; SEM = standard error of the mean (n = 5); *a* = soluble fraction; *b* = potentially degradable fraction; *a* + *b* = potential extent of degradation; k_d = fractional rate of degradation of *b*; *Lt* = lag time; is*RD* = *in situ* ruminal degradability.

[1]Nutrient analysis: DM = 78.3%; crude protein = 8.3% of DM; neutral detergent fiber = 9.3%; acid detergent fiber = 3.7%; ether extract = 2.1%; total starch = 78.5%; starch availability = 56%.

[2]Samples were incubated in the rumen for 0, 6, 12, 24, 48, 72, or 96 h.

Table 4.3 Nutritional composition and effects of flake density on in situ ruminal DM degradation parameters of steam-flaked corn.

	Flake density, g/L						*P*-value	
Item	257	296	335	373	412	SEM	Linear	Quadratic
Nutrient analysis								
DM, %	87.8	89.4	88.3	88.5	87.4	-	-	-
Crude protein, % of DM	8.3	8.2	8.4	8.3	8.1	-	-	-
NDF, % of DM	8.2	9.0	8.4	9.0	10.1	-	-	-
ADF, % of DM	3.9	3.7	3.6	3.9	4.4	-	-	-
Ether extract, % of DM	1.8	2.0	2.1	2.5	2.7	-	-	-
Total starch, % of DM	80.3	76.0	78.9	77.7	76.0	-	-	-
Starch availability, %	87.0	76.0	66.0	43.0	49.0	-	-	-
Degradation parameters								
a, %	44.2	47.2	39.2	31.5	25.9	1.69	<0.01	0.01
b, %	51.6	47.7	56.4	64.3	68.9	1.82	<0.01	0.02
a + *b*, %	96.0	94.9	95.6	95.7	94.9	0.786	0.69	0.89
k_d, %/h	14.7	10.1	7.82	4.89	5.36	1.76	<0.01	0.16
Lt, h	0.139	0.389	0.897	0.214	1.60	0.315	0.01	0.37
is*RD*, %	78.9	75.6	68.7	60.0	57.3	1.42	<0.01	0.91

Table 4.3 (continued)

Abbreviations: ADF = acid detergent fiber; DM = dry matter; NDF = neutral detergent fiber; SEM = standard error of the mean (n = 10); *a* = soluble fraction; *b* = potentially degradable fraction; *a* + *b* = potential extent of degradation; k_d = fractional rate of degradation of *b*; *Lt* = lag time; is*RD* = *in situ* ruminal degradability.

Table 4.4 Nutritional composition and effects of particle size (PS) and storage temperature on in situ ruminal DM degradability parameters of steam-flaked corn.

	Treatment									
	Flakes + Fines		Sifted Flakes		Sifted Fines			*P*-value		
Item	23°C	55°C	23°C	55°C	23°C	55°C	SEM	PS	Temp.	PS × Temp.
Nutrient analysis										
DM, %	74.7	75.2	77.9	76.5	70.9	68.7	-	-	-	-
Crude protein, % of DM	8.7	8.6	8.5	8.3	13.4	12.7	-	-	-	-
NDF, % of DM	10.0	11.6	9.6	10.4	18.1	20.6	-	-	-	-
ADF, % of DM	4.1	4.7	3.8	3.6	9.9	8.8	-	-	-	-
Ether extract, % of DM	3.3	3.4	2.3	2.1	13.9	12.9	-	-	-	-
Total starch, % of DM	74.4	74.5	78.4	76.7	50.8	52.2	-	-	-	-
Starch availability, %	52.0	21.0	59.0	28.5	49.0	27.0	-	-	-	-
Degradation parameters										
a, %	26.8^{b}	17.7^{a}	27.3^{b}	16.6^{a}	41.6^{c}	22.4^{ab}	2.04	<0.01	<0.01	0.04
b, %	66.7^{b}	76.3^{cd}	67.5^{bc}	76.8^{d}	53.8^{a}	73.2^{bcd}	2.19	<0.01	<0.01	0.04
a + *b*, %	93.4	94.0	94.8	93.4	95.3	95.6	1.05	0.21	0.80	0.63
k_d, %/h	9.57	5.35	10.9	5.39	9.70	7.95	1.38	0.62	0.01	0.40

Table 4.4 (continued)

Lt, h	0.176	0.399	0.211	0.687	0.464	0.940	0.333	0.46	0.16	0.91
is*RD*, %	65.7	52.0	66.0	51.1	73.9	63.0	1.48	<0.01	<0.01	0.40

Abbreviations: ADF = acid detergent fiber; DM = dry matter; NDF = neutral detergent fiber; SEM = standard error of the mean (n = 10); *a* = soluble fraction; *b* = potentially degradable fraction; *a* + *b* = potential extent of degradation; k_d = fractional rate of degradation of *b*; *Lt* = lag time; is*RD* = *in situ* ruminal degradability.

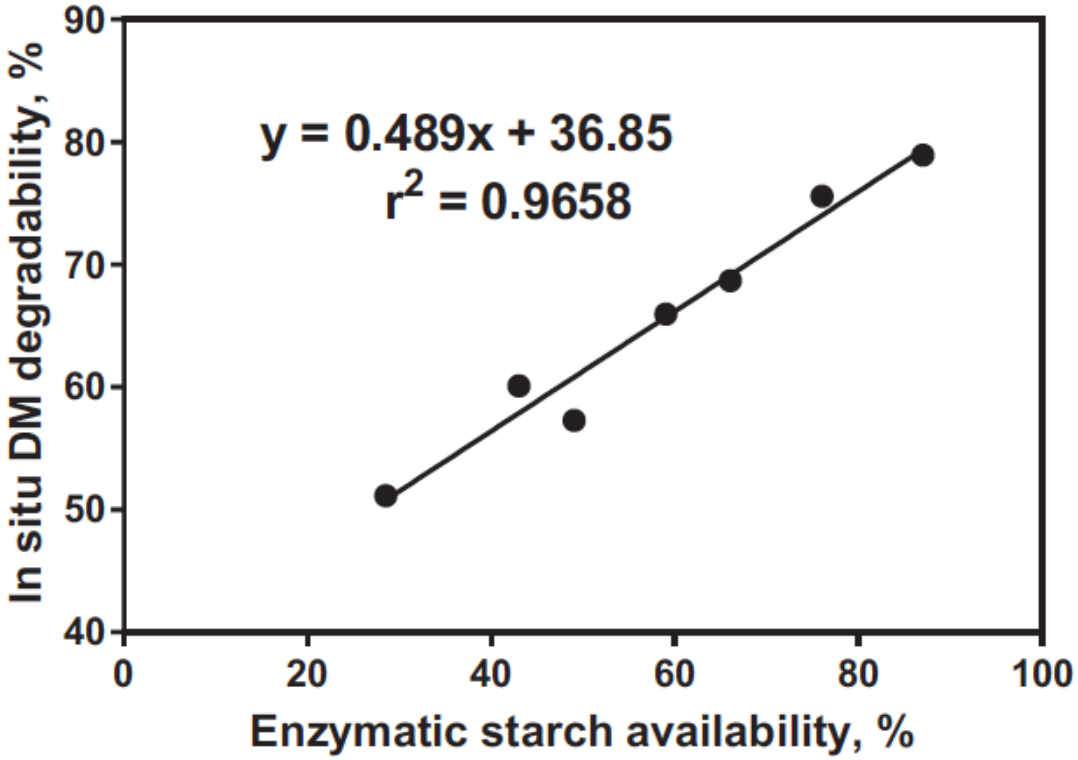
[a-d] Least square means with different superscripts within a row differ ($P \leq 0.05$).

Table 4.5 Means ± standard deviations for effects of flake density (Exp. 1) and starch retrogradation (Exp. 2) on predicted in situ ruminal starch degradability, total-tract starch digestibility, and energetic efficiency of sifted flakes with varying rates of passage.

	Passage rate					
	4% per hour		6% per hour		8% per hour	
Experiment 1	335 g/L	373 g/L	335 g/L	373 g/L	335 g/L	373 g/L
isRD$_{DM}$, %	74.0 ± 3.38	66.5 ± 2.22	68.7 ± 4.13	60.0 ± 2.22	64.8 ± 4.52	55.7 ± 2.17
Predicted is*RD*$_{starch}$, %	83.4 ± 3.25	76.3 ± 2.13	78.3 ± 3.96	70.1 ± 2.13	74.7 ± 4.34	65.8 ± 2.08
Predicted *TTD*$_{starch}$, %	99.3 ± 0.130	99.1 ± 0.085	99.1 ± 0.159	98.8 ± 0.085	99.0 ± 0.174	98.6 ± 0.083
Predicted *EE*, %	82.6 ± 0.65	84.1 ± 0.427	83.7 ± 0.794	85.3 ± 0.426	84.4 ± 0.869	86.2 ± 0.416
	Passage rate					
	4% per hour		6% per hour		8% per hour	
Experiment 2	23°C	55°C	23°C	55°C	23°C	55°C
isRD$_{DM}$, %	72.1 ± 7.17	58.7 ± 7.25	66.0 ± 8.74	51.1 ± 7.91	61.4 ± 9.65	46.0 ± 8.20
Predicted is*RD*$_{starch}$, %	81.6 ± 6.88	68.7 ± 6.96	75.7 ± 8.39	61.5 ± 7.59	71.4 ± 9.27	56.5 ± 7.87
Predicted *TTD*$_{starch}$, %	99.3 ± 0.275	98.7 ± 0.279	99.0 ± 0.336	98.5 ± 0.304	98.9 ± 0.371	98.3 ± 0.315
Predicted *EE*, %	83.0 ± 1.38	85.6 ± 1.38	84.2 ± 1.68	87.0 ± 1.52	85.1 ± 1.85	88.0 ± 1.57

Abbreviations: DM = dry matter; SEM = standard error of the mean (n = 10); is*RD*$_{DM}$ = *in situ* ruminal dry matter degradability; *isRD*$_{starch}$ = predicted *in situ* starch degradability; *TTD*$_{starch}$ = predicted total-tract starch digestibility; *EE* = predicted energetic efficiency.

Figure 4.1 Relationship between enzymatic starch availability and in situ ruminal dry matter degradability of sifted flakes used in Exp. 1 and Exp. 2.



CHAPTER 5. CORN PROCESSING, FLAKE DENSITY, AND STARCH RETROGRADATION INFLUENCE RUMINAL SOLUBILITY OF STARCH, FIBER, PROTEIN, AND MINERALS

5.1 Introduction

In feedlot operations, corn is the primary grain source used for finishing diets for beef cattle and steam-flaking is the primary grain processing method used (Samuelson et al., 2016). Steam-flaking has become a common practice because it improves starch availability, nutrient utilization, and the overall feeding value (Zinn et al., 2002). Additionally, grain processing method, flake density, particle size, and the degree of starch retrogradation influence the soluble fraction of steam-flaked corn.

We previously demonstrated that increasing flake density from 257 g/L to 412 g/L decreased the soluble dry matter (DM) fraction of sifted flakes from 44.2% to 25.9% and increased the potentially degradable DM fraction from 51.6% to 68.9% (Trotta et al., 2021a). The fines that are produced as a by-product of the flaking procedure can make up to 15% of the total steam-flaked corn output on a wet basis, depending on flake density, roll use, and roll corrugations. The fines have a greater soluble DM fraction and lesser potentially degradable fraction compared to flakes (Trotta et al., 2021a). Starch retrogradation decreases the soluble fraction and increases the potentially degradable fraction of both flakes and fines (Trotta et al., 2021a).

Modeling digestion in ruminants is a function of the rate and extent of ruminal degradation and the rate of passage. Heterogeneity of diets, feedstuffs, and nutrients can lead to different rates of degradation and passage within the rumen which must be accounted for when modeling. Because of the unique kinetic properties of different feed particles, digestion models in ruminants typically use a 3-fraction model containing the

soluble fraction, potentially degradable fraction, and the undegraded fraction. The soluble fraction is assumed to either ferment immediately or exit the rumen at a rate equivalent to liquid outflow.

Despite large changes in the soluble and potentially degradable fractions with flake density, particle size, and retrogradation, the potential extent of ruminal DM degradation was not influenced by any of the factors mentioned (Trotta et al., 2021a). With all of this information considered, it remains unclear how flaking procedures that modify the soluble DM fraction influence solubility of individual nutrients. The objective of this experiment was to characterize how corn processing, flake density, particle size, and starch retrogradation influence the soluble fraction of starch, protein, fiber, and minerals.

5.2 Materials and methods

Animal care and management protocols followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, 4th Edition.

5.2.1 *Experiment 1*

Five ruminally-cannulated Holstein × Angus crossbred steers (Initial body weight = 390 ± 7.86 kg) were used to determine the effects of corn processing on nutrient disappearance of the soluble fraction using the *in situ* nylon bag technique. The experimental design was a randomized complete block design with rep as a blocking factor. Steers were pen-fed a starter diet [53.3% DM, 35.8% total starch, 21.9% acid detergent fiber (ADF), 13.3% crude protein (CP)] *ad libitum* (Trotta et al., 2021a). Bulk samples of whole-shelled corn (~5 kg) were collected from a storage bin and either left whole or

processed by grinding with no screen, grinding through a 6-mm screen, or grinding through a 1-mm screen (SM-100 Cutting Mill; Retsch GmbH, Haan, Germany).

Samples of each corn treatment were weighed (92.7 ± 6.48 g) into nylon bags (10 cm × 20 cm; 50 μm pore size; R1020 Forage Bag; ANKOM Technology, Macedon, NY). One nylon bag for each treatment was placed into a wash bag (25 cm × 31 cm; k2107; HomeAide Delicate Wash Bag) for each steer. The wash bag was immersed in the ventral rumen and swirled for approximately 10 seconds to ensure complete exposure to ruminal fluid. This process corresponds to the 0-h measurement of an *in situ* digestibility experiment which is used to calculate the soluble fraction. Nylon bags were quickly immersed in cold water and placed into an ice-water bath to stop fermentation. Nylon bags were rinsed 5 times in a washing machine with 1-min rinse and 2-min spin cycles (Coblentz et al., 1997). The bags were then placed in a 55ºC forced-air oven for 72-h to determine *in situ* nutrient disappearance. The experiment was replicated twice across days as recommended by Vanzant et al. (1998).

5.2.2 *Experiment 2*

The same five steers were used to evaluate the effects of flake density on soluble fraction disappearance of sifted flakes and sifted fines. The experimental design was a randomized complete block design with rep as a blocking factor. The same source of whole-shelled corn used in Exp. 1 was used to produce steam-flaked corn treatments in Exp. 2. The rolls of a steam-flaker (61 cm × 142 cm roll size) were adjusted to produce steam-flaked corn corresponding to 309 g/L (24 lb/bu), 335 g/L (26 lb/bu), 360 g/L (28 lb/bu), and 386 g/L (30 lb/bu) flake densities. Bulk samples of steam-flaked corn were collected using a metal bucket (15.2 cm diameter × 6.35 cm height) on a handle that was

extended under the rolls to collect fresh flakes. The collected bulk samples were transferred to standard testing sieves (30.5 cm diameter, 4-mm screen; Advantech Manufacturing, Inc., New Berlin, WI) fitted above a collection pan. Steam-flaked corn was sieved gently for 20 s. The large particles retained in the top portion (> 4-mm; sifted flakes) and the small particles that passed-through the sifting sieve to the bottom collection pan (< 4-mm; sifted fines) were air equilibrated for 24-h and then stored in plastic bags. Each treatment (79.6 ± 6.74 g) was weighed into nylon bags and one bag for each treatment was placed into a wash bag for each steer, as described in Exp. 1. Ruminal incubation, rinsing, and drying procedures were conducted as previously described in Exp. 1. The experiment was replicated twice across days as recommended by Vanzant et al. (1998).

5.2.3 *Experiment 3*

The same five ruminally-cannulated steers were used to determine the effects of starch retrogradation on nutrient disappearance of the soluble fraction using the *in situ* nylon bag technique. The experimental design was a randomized complete block design with rep as a blocking factor. The same source of whole-shelled corn used in Exp. 1 was used to produce steam-flaked corn treatments in Exp. 3. The rolls of a steam-flaker (61 cm × 142 cm roll size) were adjusted to produce steam-flaked corn corresponding to 335 g/L (26 lb/bu). Bulk samples were not sifted (flakes + fines; > 4-mm and < 4-mm) and then placed into foil bags (Mylar Bags; IMPAK Corporation, Los Angeles, CA) and heat-sealed to prevent moisture loss. Foil bags were stored at either 23ºC or 55ºC in a forced-air drying oven for 3-d. The oven temperature setting (55ºC) was chosen because it has been reported that the temperature of the core of piled steam-flaked corn can remain > 55ºC for more than 17-h (Sindt, 2004; Drouillard and Reinhardt, 2006). We previously demonstrated that

exposure of steam-flaked corn to 55°C for 3-d decreased starch availability from 69.4% to 44.6% (Trotta et al., 2021b) and *in situ* ruminal DM degradability (Trotta et al., 2021a) due to starch retrogradation. Flakes + fines (85.6 ± 6.32 g) were weighed into nylon bags and one bag for each treatment was placed into a wash bag for each steer, as described in Exp. 1. Ruminal incubation, rinsing, and drying procedures were conducted as previously described in Exp. 1. The experiment was replicated twice across days as recommended by Vanzant et al. (1998).

5.2.4 *Nutrient analysis*

Dried feed samples and feed residues from the ruminal nylon bag incubations were ground to pass through a 1-mm screen using a cyclone sample mill (Model 3010-014; UDY Corporation, Fort Collins, CO). Samples were analyzed for nutrient composition including acid-hydrolyzed fat (AHF; AOAC, 2012; 922.06), available starch and total starch (Trotta et al., 2021b), CP (AOAC, 2012; 990.03), and NDF and ADF (Van Soest et al., 1991). Starch availability was calculated by dividing available starch by total starch and multiplying by 100. Samples were prepared for mineral analyses by pre-digesting samples in HNO_3 followed by H_2O_2 digestion with HCl (Huang and Schulte, 1985; Mills and Jones, 1997) and analyzed using inductively-coupled plasma emission spectroscopy (AOAC, 2012; methods 990.08 and 968.08). Nutrient concentrations for each corn treatment are presented in Table 5.1. The nutrient concentration in each feed sample was used to calculate the initial amount weighed into each bag. The nutrient concentration of each feed residue was used to calculate the final amount of each nutrient remaining. Nutrient disappearance (%) was calculated as the difference between initial nutrient amount (g) and final nutrient amount (g) divided by the initial nutrient amount and multiplied by 100. Nutrient

disappearance during the 0-h incubation represents the soluble fraction of feeds that is estimated using first-order digestion kinetic models.

5.2.5 *Statistical analysis*

All variables were checked for normality using the UNIVARIATE procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). For Exp. 1, the data were analyzed using the GLM procedure of SAS as a randomized complete block design for effects of corn processing and rep (blocking factor). To determine the effect of grinding, a contrast statement was analyzed for whole-shelled corn vs. no screen, 6-mm screen, and 1-mm screen. To determine the effect of grinding with a screen, a contrast statement was analyzed for no screen vs. 6-mm screen and 1-mm screen. To determine the effect of screen size, a contrast statement was analyzed for 6-mm screen vs. 1-mm screen.

For Exp. 2, sifted flakes and sifted fines data were analyzed separately using the GLM procedure of SAS as a randomized complete block design for effects of flake density and rep. Linear and quadratic contrast statements were generated.

For Exp. 3, data were analyzed as a randomized complete block design using the GLM procedure of SAS for fixed effects of rep and starch retrogradation. Least squares means and their standard errors were computed for each fixed effect included in the models. Pairwise differences of least squares means were separated using the Tukey-Kramer adjustment, protected by a significant F-test. Results were considered significant if $P \leq 0.05$. Tendencies were declared when $0.05 < P \leq 0.10$.

5.3 Results

5.3.1 *Experiment 1*

Whole shelled corn had lesser ($P < 0.01$) ruminal solubility of all nutrients measured compared with ground corn (Table 5.2). Corn ground with a screen (6-mm and 1-mm) had greater ($P < 0.01$) ruminal solubility of all nutrients measured compared with corn ground with no screen. Corn ground through a 1-mm screen had greater ($P \leq 0.05$) ruminal solubility of DM, total starch, CP, ADF, AHF, P, Mg, K, S, Zn, Fe, and Mn compared with corn ground through a 6-mm screen. The size of the screen used to grind corn (6-mm vs. 1-mm) did not influence ($P = 0.38$) the soluble fraction of NDF.

5.3.2 *Experiment 2*

The mass of sifted flakes after sieving increased linearly ($P < 0.01$) with increasing flake density (Table 5.3). Sifted fines mass responded quadratically ($P = 0.01$) where increasing flake density from 309 to 360 g/L decreased sifted fines mass and then increased from 360 to 386 g/L. The proportion of sifted fines responded quadratically ($P < 0.01$) where it decreased from 309 to 335 g/L and then plateaued.

With sifted flakes, increasing flake density linearly decreased ($P \leq 0.02$) the soluble fraction of DM, total starch, CP, ADF, AHF, P, K, S, and Zn (Table 5.4). The NDF soluble fraction increased from 309 to 335 g/L, remained similar to 360 g/L, and then decreased from 360 to 386 g/L ($P = 0.02$). The soluble Mg and Mn fractions decreased ($P = 0.04$) from 309 to 360 g/L and then increased from 360 to 386 g/L.

With sifted fines, increasing flake density did not influence the soluble fractions of total starch, CP, NDF, or Zn (Table 5.5). The soluble DM fraction of sifted fines tended to

decrease ($P = 0.06$) linearly with increasing flake density. Increasing flake density resulted in a linear increase ($P < 0.01$) in the soluble ADF fraction but, the soluble AHF fraction decreased ($P < 0.01$). Quadratic responses for P, Mg, K, S, and Mn of the sifted fines decreased ($P < 0.01$) and then increased as flake density increased to 386 g/L. The soluble fraction of Fe increased ($P = 0.02$) from 309 g/L to 360 g/L and then decreased from 360 g/L to 386 g/L.

5.3.3 *Experiment 3*

Inducing starch retrogradation by storing flakes + fines in heat-sealed foil bags at 55ºC for 3-d decreased ($P < 0.04$) the soluble fractions of DM, total starch, CP, NDF, P, Mg, K, S, and Fe (Table 5.6). Storage temperature did not influence the soluble fraction of ADF, AHF, Zn, or Mn.

5.4 Discussion

5.4.1 *Corn processing*

The treatments used in Exp. 1 were designed to produce corn fractions that represent common feeds fed to finishing cattle. Grinding with no screen produced a fraction that resembled coarsely cracked corn. Grinding with a 6-mm screen produced a fraction that resembled coarsely ground corn and grinding with a 1-mm screen produced a fraction that resembled finely ground corn. A possible limitation with the *in situ* ruminal digestibility procedure is that mastication is avoided by placing samples directly into the rumen. It is plausible to assume that feeds with greater particle sizes may be masticated to a greater extent which may alter *in vivo* degradability characteristics compared with use of the *in situ* procedure.

Processing corn by grinding, grinding with the use of a screen, and decreasing screen size all influenced the soluble fractions of different nutrients of corn. The soluble DM and starch fractions responded similarly because starch is the principal component of corn (total starch ≥ 73.2% of DM) and thus, relationships between ruminal DM and starch digestibility are similar for high-starch feeds (Offner et al., 2003). Interestingly, with each increase in the degree of processing, the soluble starch fraction increased by 8.2, 13.3, and 15.8 percentage units relative to whole-shelled corn. However, with each increase in the degree of processing the relative increase was at a decreasing efficiency. With each increase in the degree of processing, the starch soluble fraction increased by 8.9-fold, 2.5-fold, and 1.7-fold. These data suggest that grinding corn without a screen is the most efficient method to increase starch solubility; but, fine grinding can achieve the largest increase in ruminal starch solubility.

Many studies that had evaluated effects of decreasing corn particle size in finishing beef cattle diets had replaced the entire corn portion of the diet with finely ground corn (Corona et al., 2006; Loe et al., 2006; Macken et al., 2006; Swanson et al., 2014). In general, previous studies did not find improvements in average daily gain or gain:feed when the entire corn portion of the diet was replaced by finely-ground corn (Corona et al., 2006; Loe et al., 2006; Macken et al., 2006; Swanson et al., 2014). In the current study, the soluble DM fraction of finely ground corn was similar to the soluble DM fraction of corn steam-flaked to a density of 360 g/L (Buttrey et al., 2016; Trotta et al., 2021a). Because the soluble DM fraction of finely ground corn is similar to that of corn steam-flaked to 360 g/L, future research should compare these two feeds directly to evaluate whether or not ruminal degradability characteristics are similar. If so, fine grinding corn could potentially

improve nutrient utilization similar to that of steam-flaked corn when included in finishing diets at low inclusion levels and/or when included in diets with other forms of processed grains. Grinding coarseness, dietary inclusion level, interactions with other dietary components, roughage inclusion level, dry matter intake, and the rate of ruminal passage could all be important factors when deciphering impacts of fine ground corn inclusion in finishing cattle diets.

5.4.2 *Flake density*

As flake density increased, the proportion of sifted fines decreased, which is in agreement with previous findings by Hales et al. (2010). In the current study, increasing flake density from 309 g/L to 386 g/L decreased the soluble DM fraction of sifted flakes from 48.6% to 35.6%. Similarly, we previously demonstrated that increasing flake density from 257 g/L to 412 g/L decreased the soluble DM fraction of sifted flakes from 44.2% to 25.9% (Trotta et al., 2021a). In general, increasing flake density decreased the soluble fraction of most nutrients measured in the current study. As flake density decreases, the extent of gelatinization, starch availability, and ruminal disappearance increases (Trotta et al., 2021a). However, it is widely accepted that flaking to a lighter density (< 300 g/L) does not result in economically favorable outcomes because of limited improvements in total-tract starch digestibility and feed efficiency, decreased mill production rate, increased mill energy consumption, and increased risk of ruminal acidosis and bloat (Reinhardt et al., 1997; Brown et al., 2000; Drouillard and Reinhardt, 2006). In contrast, flaking to a heavier density (> 400 g/L) can increase mill production rate and decrease mill energy consumption but also decrease ruminal and/or total-tract digestibility (Zinn, 1990; Theurer et al., 1999). Therefore, consulting feedlot nutritionists recommended flaking corn to an intermediate

bulk density of 350 g/L (Samuelson et al., 2016) to improve starch digestibility while maintaining a feasible mill production rate and low to moderate risk of digestive disturbances.

In agreement with above, Zinn et al. (2002) suggested that steam-flaking not only increases starch digestibility compared to other grain processing methods, but also increases digestibility of nonstarch OM. Moreover, Zinn et al. (1995) noted that the increase in nonstarch OM digestibility was proportional to the increase in starch digestibility. These observations were most notable when comparing steam-flaked corn digestibility with other forms of grain processing. In the current study, our data supports this concept and expands our current understanding by demonstrating that decreasing flake density increases ruminal solubility of several nonstarch OM components and minerals.

5.4.3 *Particle size: Flakes vs. fines*

During the flaking process, starch is gelatinized and other nutrients are typically segregated into the fines. Improper sampling of steam-flaked corn can lead to under- or over-representation of specific nutrients (Corona et al., 2006; Hales et al., 2010). The flakes and fines have drastically different nutrient profiles (Hales et al., 2010; Trotta et al., 2021b) where the fines contain greater proportions of fiber, protein, fat and minerals. This is most likely because different nutrients are stored in different anatomical locations of the corn kernel which can become segregated during the flaking procedure. The endosperm contains large proportions of starch which is gelatinized to produce flakes and the minerals from the germ segregate into the fines. Differences in nutrient profiles and particle size between flakes and fines influence their degradability characteristics in the rumen (Trotta et al., 2021a). Although not directly compared in the current study, sifted fines had a greater

soluble fraction for most macronutrients while the sifted flakes had a greater soluble fraction for most minerals. However, because sifted fines contained greater proportion of most minerals, the amount that disappeared (g) was greater compared to sifted flakes. Ruminal solubility of sifted flakes was dramatically affected by changes in flake density, whereas changes in solubility were much smaller with sifted fines. These data show that it is critical to obtain similar fractions when sampling steam-flaked corn for laboratory analysis. If incorrect sampling of proportions occur, the wrong interpretation/conclusion will be drawn relative to flaker quality control and/or laboratory analytical error.

5.4.4 *Starch retrogradation*

In cattle feeding operations, starch retrogradation of steam-flaked corn could occur through prolonged heat exposure in the core of corn piled for storage (McAllister et al., 2006), storage in grain bins (Ward and Galyean, 1999), or by method of moving steam-flaked corn from under the rolls into storage bins (McMeniman and Galyean, 2007). Storage of steam-flaked corn at 55°C for 3-d in heat-sealed foil bags decreased starch availability from 69.4% to 44.6% due to starch retrogradation (Trotta et al., 2021b). Furthermore, inducing starch retrogradation of flakes + fines decreased *in situ* ruminal DM degradability from 65.7% to 52.0% (Trotta et al., 2021a). However, starch retrogradation decreases the soluble fraction and rate of ruminal degradation without influencing the potential extent of degradation (Trotta et al., 2021a). If starch retrogradation decreases the soluble fraction or the rate of ruminal degradation while maintaining total-tract digestibility, it is plausible that the site of digestion could be shifted from the rumen to the small intestine to improve the efficiency of total-tract starch digestion in feedlot cattle.

Proximate analyses from our previous study (Trotta et al., 2021b) and the current study show that the nutrient composition of steam-flaked corn stored at 23ºC or 55ºC is similar, with the exception of starch availability. In the current study, inducing starch retrogradation decreased the soluble fraction of DM, starch, CP, ADF, and minerals. These data suggest that starch retrogradation could decrease ruminal digestibility of nonstarch components. It is important to note that the total starch content of flakes + fines was ≥ 72.3% and thus, reducing the digestibility of nonstarch components in retrograded steam-flaked corn may not be of practical concern because these nutrients do not contribute to a substantial portion of the animal's intake of CP, fiber, or mineral. More information is needed to understand how starch retrogradation of steam-flaked corn influences the site and extent of starch and nonstarch OM digestibility, growth, and feed efficiency.

5.5 Conclusions

Corn processing, flake density, and starch retrogradation influenced the solubility of nutrients in the rumen. Processing corn by grinding, grinding with a screen, and decreasing screen size can increase the soluble fraction of starch, nonstarch OM, and minerals. Increasing flake density linearly decreases the solubility of DM and starch of sifted flakes; however, the soluble DM and starch fractions of sifted fines were not influenced by flake density. The soluble fraction of macronutrients were greater for sifted fines compared to sifted flakes. Storage of steam-flaked corn in heat-sealed foil bags for 3-d at 55ºC to induce starch retrogradation decreased the soluble fraction of most nutrients. These data will be useful for understanding the various factors influencing the soluble fraction of processed corn when modeling.

Table 5.1 Nutrient composition of corn treatments used in experiments 1, 2, and 3.

	Nutrient[1]													
Item	DM %	Total starch % DM	SA %	CP % DM	NDF % DM	ADF % DM	AHF % DM	P % DM	Mg % DM	K % DM	S % DM	Zn ppm	Fe ppm	Mn ppm
Exp. 1[2]														
WSC	94.1	74.4	9	8.6	10.9	3.7	4.5	0.27	0.10	0.33	0.10	17	20	6
NS	92.2	73.9	9	8.2	10.9	2.4	4.8	0.26	0.11	0.33	0.10	16	20	6
6S	91.8	73.2	9	8.2	10.9	3.6	4.6	0.25	0.10	0.32	0.10	17	22	6
1S	92.7	73.3	8	8.6	10.0	3.8	4.9	0.25	0.10	0.32	0.10	16	20	5
Exp. 2														
Flakes														
309 g/L	87.1	78.8	77	8.1	6.7	3.4	3.0	0.14	0.05	0.21	0.09	9	16	3
335 g/L	86.4	79.1	68	8.2	7.8	3.3	3.7	0.19	0.06	0.25	0.10	12	19	4
360 g/L	85.9	80.0	60	8.3	8.2	4.1	2.9	0.18	0.06	0.25	0.09	11	20	4
386 g/L	85.7	79.4	51	7.9	8.1	4.6	3.2	0.20	0.08	0.26	0.10	13	20	5
Fines														
309 g/L	84.1	39.8	47	13.0	24.3	11.5	15.5	1.18	0.50	1.26	0.14	69	71	23
335 g/L	86.7	37.7	48	13.9	23.3	12.5	18.3	1.26	0.53	1.20	0.15	78	103	23

Table 5.1 (continued)

360 g/L	84.0	40.5	49	14.1	24.2	13.3	18.2	1.20	0.50	1.19	0.14	72	180	23
386 g/L	83.1	41.3	48	13.0	22.4	12.9	17.2	1.32	0.54	1.33	0.16	76	86	24
Exp. 3[3]														
23ºC	90.0	73.6	55	10.1	10.3	4.8	7.6	0.40	0.17	0.49	0.1	23	33	8
55ºC	89.8	72.3	41	9.9	10.5	5.6	6.9	0.35	0.14	0.43	0.1	23	31	8

[1]Abbreviations: DM = dry matter; SA = starch availability; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; AHF = acid-hydrolyzed fat.

[2]Abbreviations: WSC = whole shelled corn; NS = ground corn (no screen); 6S = ground corn (6-mm screen); 1S = ground corn (1-mm screen).

[3]Samples were stored for 3-d in heat-sealed foil bags at either 23ºC or 55ºC to induce starch retrogradation.

Table 5.2 Experiment 1: Effects of corn processing on the soluble fraction (%) of dry matter, starch, crude protein, fat, fiber, and minerals.

Nutrient disappearance, %	Whole shelled corn	Ground corn			SEM[1]	Contrast *P*-value[2]		
		No screen	6-mm screen	1-mm screen		Grinding	Screen	Screen size
Dry matter	0.00	7.75	22.0	38.1	0.559	<0.01	<0.01	<0.01
Total starch	1.04	9.20	22.5	38.3	0.813	<0.01	<0.01	<0.01
Crude protein	-0.384	-1.12	12.1	30.6	0.765	<0.01	<0.01	<0.01
Neutral detergent fiber	3.60	12.4	34.0	36.9	2.26	<0.01	<0.01	0.38
Acid detergent fiber	3.17	5.63	28.9	41.2	2.85	<0.01	<0.01	<0.01
Acid hydrolyzed fat	12.9	22.5	36.1	53.7	6.2	<0.01	<0.01	0.05
P	-0.792	2.86	27.3	63.4	1.39	<0.01	<0.01	<0.01
Mg	-6.00	7.85	21.2	56.7	1.62	<0.01	<0.01	<0.01
K	6.63	24.3	54.4	85.5	0.904	<0.01	<0.01	<0.01
S	-0.0322	5.95	18.2	30.7	1.14	<0.01	<0.01	<0.01
Zn	4.10	2.07	30.7	60.2	1.63	<0.01	<0.01	<0.01
Fe	7.99	8.40	43.6	63.8	6.38	<0.01	<0.01	0.03
Mn	8.31	14.0	35.0	56.8	2.09	<0.01	<0.01	<0.01

[1]SEM = standard error of the mean (n = 10).

Table 5.2 (continued)

[2]Grinding = whole shelled corn vs. others; Screen = no screen vs. 6-mm screen and 1-mm screen; Screen size = 6-mm screen vs. 1-mm screen.

Table 5.3 Experiment 2: Effects of flake density on sifted flakes and sifted fines mass and the proportion of sifted fines.[1]

	Flake density, g/L					*P*-value	
Item	309	335	360	386	SEM[2]	Linear	Quadratic
Sifted flakes mass, g	535	561	590	617	19.3	<0.01	0.99
Sifted fines mass, g	67.0	60.9	57.1	64.5	2.48	0.33	0.01
Sifted fines, % of sample	11.2	9.79	8.85	9.48	0.330	<0.01	<0.01

[1]Sifted flakes = particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s; sifted fines = particles retained in the bottom-portion of the sieve (< 4-mm) after sifting steam-flaked corn samples for 20 s.

[2]SEM = standard error of the mean (n = 10).

Table 5.4 Experiment 2: Effects of flake density on in situ ruminal solubility (%) of dry matter, starch, crude protein, fat, fiber, and minerals of sifted flakes.[1]

	Flake density, g/L					*P*-value	
Nutrient disappearance, %	309	335	360	386	SEM[2]	Linear	Quadratic
Dry matter	48.6	44.4	41.9	35.6	1.23	<0.01	0.39
Total starch	53.0	48.5	48.8	42.9	1.34	<0.01	0.61
Crude protein	27.0	21.5	20.0	12.2	1.54	<0.01	0.45
Neutral detergent fiber	-4.91	5.38	6.19	-0.918	3.57	0.43	0.02
Acid detergent fiber	26.7	17.8	31.5	36.9	2.26	<0.01	<0.01
Acid hydrolyzed fat	46.6	50.1	25.8	26.5	3.87	<0.01	0.72
P	60.4	60.3	49.3	50.6	2.10	<0.01	0.75
Mg	41.5	38.0	23.8	39.7	3.36	0.20	<0.01
K	90.2	88.7	85.7	83.6	0.712	<0.01	0.73
S	25.9	28.4	16.7	19.8	1.68	<0.01	0.88
Zn	35.6	35.3	19.3	29.4	3.13	0.02	0.11
Fe	33.4	23.7	31.3	33.5	5.33	0.74	0.27
Mn	36.7	36.1	27.2	39.4	3.03	0.95	0.04

[1]Particles retained in the top-portion of the sieve (> 4-mm) after sifting steam-flaked corn samples for 20 s.

[2]SEM = standard error of the mean (n = 10).

Table 5.5 Experiment 2: Effects of flake density on in situ ruminal solubility (%) of dry matter, starch, crude protein, fat, fiber, and minerals of sifted fines.[1]

	Flake density, g/L					*P*-value	
Nutrient disappearance, %	309	335	360	386	SEM[2]	Linear	Quadratic
Dry matter	43.6	42.0	40.3	41.6	0.893	0.06	0.13
Total starch	55.9	53.3	57.4	55.0	1.42	0.82	0.93
Crude protein	24.8	26.9	24.5	24.4	1.13	0.50	0.35
Neutral detergent fiber	33.3	29.0	33.6	36.0	3.21	0.39	0.30
Acid detergent fiber	32.8	26.7	40.3	41.3	2.84	<0.01	0.21
Acid hydrolyzed fat	34.7	42.3	35.2	30.0	1.67	<0.01	<0.01
P	42.8	36.4	33.7	40.1	1.28	0.11	<0.01
Mg	32.5	24.8	20.7	28.4	1.56	0.02	<0.01
K	78.5	72.7	72.8	75.9	0.635	0.01	<0.01
S	28.9	29.3	25.1	30.6	1.04	0.04	<0.01
Zn	18.6	15.6	12.6	17.8	2.49	0.63	0.10
Fe	9.27	22.7	49.0	23.7	5.39	<0.01	0.01
Mn	19.5	10.6	10.7	18.5	3.35	0.84	0.02

[1]Particles retained in the bottom-portion of the sieve (< 4-mm) after sifting steam-flaked corn samples for 20 s.

[2]SEM = standard error of the mean (n = 10).

Table 5.6 Experiment 3: Effects of starch retrogradation on in situ ruminal solubility (%) of dry matter, starch, crude protein, fat, fiber, and minerals of flakes + fines (335 g/L).[1]

	3-d storage temperature			
Nutrient disappearance, %	23ºC	55ºC	SEM	*P*-value
Dry matter	39.7	28.7	0.663	<0.01
Total starch	40.8	28.7	0.974	<0.01
Crude protein	31.1	23.2	0.74	<0.01
Neutral detergent fiber	24.2	18.1	1.95	0.04
Acid detergent fiber	38.9	43.5	1.88	0.11
Acid hydrolyzed fat	67.2	64.6	2.48	0.46
P	73.9	64.8	2.04	<0.01
Mg	69.6	56.7	2.51	<0.01
K	92.3	88.4	0.654	<0.01
S	28.4	20.2	0.937	<0.01
Zn	56.1	52.3	2.86	0.36
Fe	49.3	40.9	1.76	<0.01
Mn	54.9	47.4	3.15	0.11

[1]Steam-flaked corn samples obtained from directly under the rolls that were not sifted.

[2]SEM = standard error of the mean (n = 10).

CHAPTER 6. INFLUENCE OF SUPPLEMENTAL SOURCES AND LEVELS OF ANTI-COCCIDIAL COMPOUNDS AND SAPONINS ON *IN VITRO* AND *IN VIVO* RUMINAL FERMENTATION AND METHANE PRODUCTION OF STEERS FED A HIGH-CONCENTRATE DIET

6.1 Introduction

Recent studies have demonstrated that *Yucca schidigera* extract (YSE) increased the average daily gain and feed efficiency of finishing cattle when included in the diet at up to 4 g/d (De Sousa et al., 2019; Rett et al., 2020). In ruminants, isolated saponins or saponins from YSE inclusion have been shown to decrease CH_4 production both *in vitro* (Pen et al., 2006; Xu et al., 2010) and *in vivo* (Lila et al., 2005; Wang et al., 2009a). Potential mechanisms by which YSE decreases CH_4 production include altering ruminal microbial populations, inhibiting ruminal H_2 production, or decreasing feed intake or ruminal digestibility. Lila et al. (2005) found that feeding sarsaponin to steers at 0.5% or 1% of the diet (11.2 or 22.4 g/d) on a DM basis decreased whole-body CH_4 production by up to 12.7% which was associated with increased ruminal propionate proportion and decreased ruminal NH_3-N and protozoa concentrations. Plasma glucose concentrations were increased after feeding sarsaponin, suggesting that shifting ruminal metabolism to favor propionate production resulted in increased gluconeogenesis. Those authors also found that dietary sarsaponin inclusion decreased total-tract DM and neutral detergent fiber digestibility (Lila et al., 2005).

In general, the effects of YSE on ruminal fermentation, growth performance, and feed efficiency appear to be similar to finishing cattle diets containing monensin. Monensin also functions as an anti-coccidial compound and although YSE may have anti-coccidial activity (Rambozzi et al., 2011), replacing monensin in cattle diets with YSE alone may not be adequate to control coccidia outbreaks. Decoquinate (DCQ) is a non-antibiotic feed additive approved for use in the control of coccidiosis in cattle. Feeding DCQ has been shown to have minimal effects

on ruminal fermentation characteristics (Harmon et al., 1987) but may improve average daily gain and feed efficiency of beef cattle (Rust et al., 1981; Fox, 1983). The objectives of the current study were to evaluate the combination of YSE with DCQ on CH_4 production of steers using indirect calorimetry and ruminal fermentation characteristics. We hypothesized that feeding YSE in combination with DCQ would decrease whole-body CH_4 production, and increase ruminal propionate proportion, without influencing the rate or extent of ruminal DM degradability. We further evaluated sources and levels of anti-coccidial compounds and saponins individually on *in vitro* ruminal fermentation and CH_4 production.

6.2 Materials and methods

All animal procedures were approved by the University of Kentucky Animal Care and Use Committee.

6.2.1 *Experiment 1*

Eight Holstein steers [initial body weight (BW) = 309 ± 28.0 kg] were used to determine the effects of DCQ + YSE supplementation on *in vivo* CH_4 production. Steers were housed in individual pens (3 m × 3 m) in the Intensive Research Building of the University of Kentucky C. Oran Little Research Center in Versailles, KY. The experimental design was a randomized crossover design with two periods. Steers were fed either the basal diet (control) or the basal diet supplemented with 0.5 mg of DCQ per kg of BW (Deccox; Zoetis, Parsippany, NJ) + 3.33 mg of YSE per kg of BW (30% solids; Micro-Aid Feed Grade Concentrate; DPI Global, Porterville, CA) (DCQ + YSE). The basal high-concentrate diet (Table 6.1) was formulated to supply 2 × the net energy required for maintenance (NE_m) and to exceed requirements for ruminally degradable protein, metabolizable protein, vitamins, and minerals (NASEM, 2016). Rations were fed once

daily at 0800. Finely ground corn (454 g) was mixed with treatments and fed 15 minutes before the morning feeding each day to ensure complete consumption of the offered dose of DCQ + YSE. Steers had ad libitum access to water throughout the experiment. Periods were 10 d in length including a 7-d adaptation to treatments, followed by a 3-d collection period. Respiratory gas exchange was measured over three consecutive 24-h periods from day 8 to day 10. After completion of the collection period, steers were switched to the opposite treatment to begin the next adaptation period.

6.2.1.1 Feed analysis

Samples of the basal diet were collected weekly for nutrient analysis including DM, crude protein, neutral and acid detergent fiber, and minerals. Diet samples were dried at 60°C in a forced-air oven (NFTA 2.2.2.5.) and then ground to pass a 1-mm screen using a Wiley mill. Dry matter content was determined by oven-drying for 3 h at 105°C. Nitrogen content was analyzed by combustion (AOAC, 2006; method 990.03) using a CN628 Carbon/Nitrogen Determinator (Leco Corporation, St. Joseph, MI). Crude protein was calculated by multiplying N concentration × 6.25. Acid detergent fiber and neutral detergent fiber concentrations were determined using the filter bag technique (ANKOM Technology Methods 14 and 15, respectively). Mineral concentrations (Ca and P) were determined using inductively coupled plasma spectroscopy.

6.2.1.2 Respiratory gas exchange

Steers were fed treatments for 7 d in individual pens before being transferred to metabolism stalls (1.2 m × 2.4 m) for the measurement of respiratory gas exchange using indirect calorimetry. The design of the head-box-style respiration chambers was previously described by Koontz et al. (2010). Each respiration chamber was fitted with a waterer, feeder, and air-conditioning unit to

maintain consistent temperature (21ºC) and relative humidity (35%). Before use, each gas analyzer was calibrated with a commercial gas standard (19.900% O_2, 0.700% CO_2, 0.0650% CH_4). Recovery of O_2 (105 ± 7.1%) and CO_2 (102 ± 6.8%) for each respiration chamber was determined by combusting a known amount of propane (119 ± 8.2 g) over a 120-min period.

Air flow from each respiration chamber was determined using an individual mass flow meter (Columbus Instruments, Columbus, OH). Air flow was maintained at 600 L/min during measures of respiratory gas exchange. Inspired and expired respiratory gases were analyzed for O_2 concentration by paramagnetic detection (Columbus Instruments) and CO_2 (Columbus Instruments) and CH_4 (VIA-510; Horiba Ltd., Kyoto, Japan) concentrations were measured using infrared gas analyzers. Respiratory gas measurements were recorded in 9-min intervals using Oxymax software (Columbus Instruments, Columbus, OH). The respiratory quotient was calculated as the liters of CO_2 produced divided by the liters of O_2 consumed.

6.2.2 *Experiment 2*

Four ruminally-cannulated Holstein steers (initial body weight = 469 ± 22.1 kg) were used to determine the effects of DCQ + YSE supplementation on ruminal fermentation, *in situ* ruminal degradability, and liquid passage rate. The experimental design was a randomized crossover design with two periods. Steers were fed either the basal diet (control) or the basal diet supplemented with 0.5 mg of DCQ per kg of BW + 3.33 mg of YSE per kg of BW (DCQ + YSE). Steers were housed in individual pens (3 m × 3 m), fed the same diet as described in Exp. 1 once daily, and had ad libitum access to water. Treatments were mixed with finely ground corn as described in Exp. 1. Periods were 14 d in length including 7 d for adaptation to treatments and 7 d for sample collection. The *in situ* degradability experiment was conducted from day 8 to day 12, followed by the

collection of ruminal fluid on day 14. After completion of the collection period, steers were switched to the opposite treatment to begin the next adaptation period.

6.2.2.1 In situ ruminal degradability

From day 8 to day 12, *in situ* ruminal DM degradability of the basal diet was measured using methods previously described (Trotta et al., 2021a). Twenty grams of the basal diet ground to pass a 2-mm screen was weighed into nylon bags (10 cm × 20 cm; 50 μm pore size; R1020 Forage Bag; ANKOM Technology, Macedon, NY). Nylon bags were incubated in the rumen for 0, 3, 6, 9, 12, 24, 36, 48, 72, and 96-h. One nylon bag for each incubation timepoint was placed into a zipped wash bag (25 cm × 31 cm; k2107; HomeAide Delicate Wash Bag) that was suspended in the ventral rumen of each steer. Two stainless steel magnets (1.27 cm × 7.62 cm; Silver Star AlniMAX II, Sundown Industries Co., Plainview, NY) were added to each wash bag to ensure immersion in the ventral rumen. Wash bags were attached to a steel chain with a breeching snap clip (2710231; Koch Industries, Inc., Minneapolis, MN). The steel chain was secured to the rumen cannula cap by connecting the steel chain to an inverted U-bolt on the inner portion of the cannula cap (#1 Eazy-out Stopper; Bar Diamond, Inc., Parma, ID) with a breeching snap clip. Wash bags were inserted in reverse order so that all bags were removed from the rumen and rinsed simultaneously. At removal, the wash bags were removed from the steel chain and placed into an ice-water bath to stop fermentation. Nylon bags were removed from the wash bag and rinsed 5 times in a washing machine with 1-min rinse and 2-min spin cycles (Coblentz et al., 1997). The nylon bags were then dried in a 100ºC forced-air oven for 48 h to determine *in situ* DM disappearance.

The potential rate and extent of *in situ* DM degradation were determined using the first-order asymptotic model (McDonald, 1981):

$$y = a + b\,(1 - e^{-kd(t - Lt)})$$

where y is the degradation after t hours, a is the soluble fraction, b is the potentially degradable fraction, k_d is the fractional rate of degradation of b, t is the incubation time (h), and Lt is the lag time (h). Dry matter degradability data from time 0, 3, 6, 9, 12, 24, 36, 48, 72, and 96 h were fitted to the above nonlinear model using SAS according to the procedures described by Fadel (2004) to generate the parameters described.

In situ ruminal degradability was determined by using the parameters generated for the rate and extent of degradation, as previously described, and modeled with the rate of passage (Ørskov and McDonald, 1979):

$$isRD = a + [\ (bk_d) / (k_d + k_p)\ ]$$

where *isRD* is *in situ* ruminal degradability, a is the soluble fraction, b is the potentially degradable fraction, k_d is the fractional rate of degradation of b, and k_p is the fractional rate of liquid passage. The fractional rate of liquid passage measured on day 14 was used for k_p. Degradation coefficients from the generated parameters were converted to percentages by multiplying coefficients by 100.

6.2.2.2 Ruminal fermentation and liquid passage

On day 14, steers were administered 500 mL of CrEDTA solution (Binnerts et al., 1968) through the rumen cannula 2 h after the morning feeding to evaluate ruminal liquid passage rate. Approximately 150 mL of ruminal contents were collected from the mid-ventral region of each steer immediately before administration of the dose (0 h) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h after dosing. Ruminal contents were squeezed through four layers of cheesecloth and 5 mL of squeezed ruminal fluid was combined with 0.5 mL of 500 g/L metaphosphoric acid and 0.5 mL of 85 mM 2-ethylbutyrate and frozen at -20ºC to facilitate protein precipitation. Samples were

thawed and then centrifuged at 20,000 × *g* for 20 min at 4ºC, transferred to autosampler vials, and analyzed for volatile fatty acid (VFA) concentrations (Erwin et al., 1961; Ottenstein and Bartley, 1971) using a gas chromatograph equipped with a flame ionization detector (Hewlett-Packard 6890 Plus GC, Wilmington, DE), fitted with a Supelco 25326 Nukol fused silica capillary column (Supelco Inc., Bellefonte, PA). Ruminal NH_3-N concentration was analyzed using the glutamate dehydrogenase procedure (Kun and Kearney, 1974) adapted to a Konelab 20XTi Clinical Analyzer (ThermoFisher Scientific Inc., Beverly, MA). Ruminal L(+)-lactate concentration was measured using the L(+)-lactate dehydrogenase procedure (Gutmann and Wahlefeld, 1974; Engel and Jones, 1978) adapted to a multi-mode plate reader (Synergy HTX; BioTek Instruments Inc., Winooski, VT).

Chromium concentrations for each sample were determined using atomic absorption spectroscopy (Aanalyst 200; PerkinElmer Inc., Waltham, MA) at a wavelength of 357.87 nm. Baseline concentrations of Cr (0 h) were used to correct the concentrations measured at each time point. The concentration of Cr after dosing and fractional clearance rate of Cr was determined by calculation of the exponential decay rate for Cr using the NLIN procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) and the following equation:

$$Cr_t = Cr_0 \times e^{-kt}$$

where Cr_t represented Cr concentration at a given time, Cr_0 represented Cr concentration at time 0-h, k represented the fractional rate of Cr clearance which is assumed to be equivalent to the fractional rate of liquid passage (k_p), and t represented time in hours (Allen et al., 2000; Resende Júnior et al., 2006). Liquid retention time in the rumen was calculated as the absolute value of 1/k. Rumen liquid volume (L) was determined by dividing the amount of Cr dosed by the amount of Cr present at time zero (C_0). Liquid flow rate (L/h) was calculated as k_p × rumen liquid volume.

6.2.3 *Experiment 3 and 4*

6.2.3.1 Experiment 3

The objectives of this experiment were to determine the effects of different coccidiostat sources and levels on *in vitro* methane production and ruminal fermentation. The experimental design was a randomized complete block design with 7 treatments in a 3 × 2 + 1 factorial arrangement. Three sources of anti-coccidial compounds were tested: monensin (Rumensin 90; Elanco Animal Health Incorporated, Greenfield, IN), DCQ (Deccox; Zoetis Inc., Parsippany, NJ), and amprolium (Corid 9.6% Oral Solution; Huvepharma Inc., Peachtree City, GA). There were two levels for anti-coccidial sources: 1X (based on current feeding recommendations) and 10X (10-fold greater than the 1X dose). The 1X and 10X treatment levels were 30 and 300 mg/kg substrate for monensin, respectively. The 1X and 10X treatment levels were 25 and 250 mg/kg substrate for DCQ, respectively. The 1X and 10X treatment levels were 500 and 5000 mg/kg substrate for amprolium, respectively. Treatment concentrations were corrected for active ingredient percentages and DM content. The basal substrate without any feed additives (0X) was included as a negative control.

Treatments were added to the fermentation vessels according to the proportions described in Table 6.2. To aid in the accuracy of treatment dispersal, treatment premixes (Rumensin 90 and Deccox) were mixed with the basal substrate. Monensin was prepared as a 10% (w/w) mixture of Rumensin 90 with the basal substrate. Decoquinate was prepared as 10% and 50% (w/w) mixtures of Deccox with the basal substrate for the 1X and 10X treatments, respectively. Amprolium was prepared as a 6.4% or 64% (v/v) solution of Corid 9.6% Oral Solution diluted in water for the 1X and 10X treatments, respectively. All vessels received 500 mg of the basal substrate or basal

substrate mixed with treatment so that all flasks contained the same substrate volume. All vessels received 2 mL of water or added treatment diluted in water so that all flasks contained the same liquid volume. There were 2 replicate fermentation vessels for each treatment and the experiment was replicated on four separate days.

6.2.3.2 Experiment 4

The objectives of this experiment were to determine the effects of different saponin sources and levels on *in vitro* methane production and ruminal fermentation. The experimental design was a randomized complete block design with 7 treatments in a 2 × 3 + 1 factorial arrangement of treatments. Two liquid sources of saponins containing 50% solids were tested: YSE (Micro-Aid Liquid; DPI Global, Porterville, CA) and *Quillaja saponaria* extract (QSE; Phytogenic Patch Plus Triple P; DPI Global, Porterville, CA). There were three levels for each saponin source: 1X, 10X, and 20X. The 1X, 10X, and 20X treatments were 100 mg/kg substrate, 1000 mg/kg substrate, and 2000 mg/kg substrate, respectively. Treatments were corrected for active ingredient percentages of the premix. The basal substrate without any feed additive (0X) was included as a negative control.

Treatments were added to the fermentation vessels according to the proportions described in Table 6.2. Saponin treatments were prepared as 0.25%, 2.5%, or 5% (v/v) solutions diluted in water for the 1X, 10X, and 20X treatments, respectively. All vessels received 2 mL of water or added treatment diluted in water so that all flasks contain the same liquid volume. There were 2 replicate fermentation vessels for each treatment and the experiment was replicated on four separate days.

6.2.3.3 Fermentation preparation

The same four ruminally-cannulated Holstein steers (initial BW = 554 ± 30.9 kg) used in Exp. 2 were housed outdoors in dry lot pens (2.4 m × 14.6 m). Steers were fed the same high-concentrate diet used in Exp. 1 and 2 and the diet was fed ad libitum once per day. Ruminal contents (500 g) were collected 4 h after feeding from each steer and combined into an insulated container (YETI Rambler One Gallon Water Jug; Yeti Holdings, Inc., Austin, TX) for transport to the laboratory. Ruminal contents were blended under CO_2 headspace for 30 s and then were squeezed through four layers of cheesecloth. Squeezed ruminal fluid (600 mL) was combined with buffer, macro-, and micro-mineral, and reducing solutions (total = 2700 mL) that were prepared as described by Goering and Van Soest (1970). Fermentation vessels used in the current study were 250-mL coated glass bottles (Cat. #7056; ANKOM Technology, Macedon, NY). Samples of the basal diet were dried at 55°C and ground to pass a 2-mm screen for use as the substrate for the *in vitro* ruminal fermentation. Substrates (501 ± 0.281 mg) were pre-weighed into fermentation vessels and combined with 2 mL of water or treatment diluted in water to aid in the dispersal of the buffered inoculum. The buffered inoculum (103.2 ± 2.52 g) was then added to each fermentation vessel. Fermentation vessels were gassed with CO_2 for 20 s, capped with RF1 gas production modules (ANKOM Technology, Macedon, NY), and placed into a 39°C water bath to equilibrate. After all fermentation vessels were added to the water bath, valves of the gas production modules were opened simultaneously to release any accumulated pressure. Then, valves were closed and cumulative gas pressure was measured in 5-min intervals over a 24-h incubation period.

6.2.3.4 Sample collection and analysis

At the completion of the 24-h incubation period, vessels were removed from the water bath and transferred to an ice bath to stop the fermentation. Valves of the RFI gas production modules

were opened and gas samples were collected with a syringe, and stored in serum tubes (BD Vacutainer; Beckton, Dickinson and Company, Franklin Lakes, NJ). Gas samples were analyzed for methane concentration using a gas chromatograph equipped with a flame ionization detector (Hewlett-Packard 6890 Plus GC, Wilmington, DE), fitted with a Supelco 12382 stainless steel 40/60 Carboxen 1000 packed column (Supelco Inc., Bellefonte, PA). The RFI gas production modules were removed and a 1-mL aliquot of the fermentation media was prepared and analyzed for VFA and NH_3-N concentrations as described in experiment 2.

6.2.3.5 In vitro gas production

Cumulative gas pressure in the vessel was corrected for atmospheric pressure and then converted into standard atmospheric pressure using the ideal gas law. Cumulative gas pressure was converted to moles of gas produced and then to milliliters of gas produced using Avogadro's law. Cumulative gas production was corrected for the gas volume in the head-space of each fermentation vessel. The gas volume in the head-space of each fermentation vessel was calculated as the total volume of the vessel (308.2 ± 5.22 mL) minus the sum of the volume of the buffered inoculum and substrate (103.8 ± 2.47 mL). Cumulative gas production was fitted to the exponential model described by Pitt et al. (1999) using GraphPad Prism 5 (Dotmatics, Boston, MA):

$$F(t) = 1 - e^{-r(t-\lambda)}$$

where $F(t)$ is cumulative gas production, r is the rate of gas production, t is the time in hours, and λ is the lag time in hours. The rate of gas production was converted to a percentage by multiplying by 100. Methane production was determined by multiplying the methane concentration of gas samples by cumulative gas production after 2 -h of fermentation.

6.2.4 *Statistical analysis*

For Exp. 1, variance-covariance structures for the repeated measures statement were assessed for fit using Bayesian information criterion for antedependence 1, autoregressive 1, compound symmetry, simple, and unstructured including steer as the subject. Whole-body O_2 consumption, CO_2 production, and CH_4 production were analyzed using the repeated measures statement of the MIXED procedure of SAS for fixed effects of rep, day, treatment, period, and their interactions.

For Exp. 2, ruminal Cr concentrations were fitted to the nonlinear equation previously described using the NLIN procedure of SAS. *In situ* ruminal degradation data were analyzed using the NLIN procedure of SAS to estimate the parameters of the equation previously described. Degradation parameters and liquid passage characteristics were analyzed using the GLM procedure of SAS for fixed effects of period, treatment, and the period × treatment interaction. Variance-covariance structures were tested for ruminal VFA, ammonia-N, and L(+)-lactate concentrations as described in Exp. 1. Concentrations of ruminal fermentation end-products were analyzed using the repeated measures statement of the MIXED procedure of SAS for fixed effects of rep, period, time, treatment, and the time × treatment interaction. The initial metabolite concentration (0-h) was included in the model statement as a covariate for ruminal metabolites.

For Exp. 3, gas production kinetics and fermentation end-products were analyzed using the GLM procedure of SAS for fixed effects of replicate and treatment. The IML procedure was used to generate orthogonal contrast coefficients to adjust for unequal spacing between treatment levels. Contrast statements were used to determine differences between treatments. To determine the effect of anti-coccidial supplementation, a contrast was analyzed for basal substrate vs. others. To determine the effect of ionophore supplementation (antibiotic vs. non-antibiotic anti-coccidials), a

contrast was analyzed for monensin vs. DCQ and amprolium. To determine the effect of non-antibiotic anti-coccidial source, a contrast was analyzed for DCQ vs. amprolium. For each anti-coccidial source (monensin, DCQ, amprolium), a linear polynomial contrast (0X, 1X, 10X) was analyzed to determine the effects of supplementation level.

For Exp. 4, gas production kinetics and fermentation end-products were analyzed using the GLM procedure of SAS for fixed effects of replicate and treatment. The IML procedure was used to generate orthogonal contrast coefficients to adjust for unequal spacing between treatment levels. Contrast statements were used to determine differences between treatments. To determine the effect of saponin supplementation, a contrast was analyzed for basal substrate vs. others. To determine the effect of saponin source, a contrast was analyzed for YSE vs. QSE. For each saponin source, linear and quadratic polynomial contrasts (0X, 1X, 10X, 20X) were analyzed to determine the effects of supplementation level.

All data were checked for normality using the Shapiro-Wilk test of the UNIVARIATE procedure of SAS. Pairwise differences of least squares means were separated using the Tukey-Kramer adjustment, protected by a significant F-test. Results were considered significant if $P \leq 0.05$.

6.3 Results

6.3.1 *Experiment 1*

Supplementation of DCQ + YSE did not influence whole-body O_2 consumption, CO_2 production, or CH_4 production of finishing steers (Table 6.3). The respiratory quotient was not different between dietary treatment groups.

6.3.2 *Experiment 2*

Supplementation of DCQ + YSE did not influence the soluble or potentially degradable DM fractions of the basal diet (Table 6.4). The potential rate and extent of DM degradation were not influenced by DCQ + YSE supplementation. *In situ* ruminal DM degradability of the basal diet was not affected by dietary treatments. The fractional rate of liquid passage, ruminal liquid retention time, rumen liquid volume, and ruminal liquid outflow were not influenced by DCQ + YSE supplementation.

The combination of DCQ + YSE supplementation increased (P = 0.03) ruminal L(+)-lactate concentration. Ruminal NH_3 and total VFA concentrations were not influenced by DCQ + YSE supplementation. The ruminal acetate molar proportion and acetate:propionate were not changed by DCQ + YSE supplementation. Supplementation of DCQ + YSE tended to increase (P = 0.09) ruminal propionate proportion. Molar proportions of isobutyrate and butyrate were not influenced by DCQ + YSE supplementation. Supplementation of DCQ + YSE decreased (P = 0.01) ruminal isovalerate proportion and increased (P < 0.01) ruminal valerate proportion.

6.3.3 *Experiment 3*

Increasing monensin inclusion linearly increased (P < 0.01) pH of the fermentation media after 24-h of incubation (Table 6.6). The inclusion of anti-coccidial compounds increased (P = 0.02) gas production after 24 h but, gas production was greater (P < 0.01) for DCQ and amprolium compared with monensin. Gas production decreased (P < 0.01) linearly with increasing monensin inclusion, increased (P < 0.01) linearly with DCQ inclusion, and tended to increase (P = 0.07) linearly with amprolium inclusion. Monensin inclusion produced a faster (P < 0.01) rate of gas production compared with DCQ and amprolium. Increasing monensin inclusion linearly increased

the rate of gas production. Increasing DCQ and amprolium inclusion linearly decreased ($P < 0.01$) and tended to decrease ($P = 0.07$) the rate of gas production. Monensin inclusion decreased ($P < 0.01$) CH_4 percentage and production compared with DCQ and amprolium. Increasing monensin linearly decreased ($P < 0.01$) CH_4 percentage and production. Ammonia concentration linearly decreased ($P = 0.04$) with increasing DCQ inclusion.

Total VFA concentrations were lower ($P = 0.05$) for monensin compared with DCQ and amprolium. Acetate molar proportion linearly decreased ($P = 0.02$) with increasing levels of DCQ. Propionate proportion was greater ($P < 0.01$) for all treatments containing anti-coccidial compounds compared to the basal substrate. However, the propionate proportion was greater ($P < 0.01$) when monensin was included in the *in vitro* fermentation compared with DCQ and amprolium. Increasing monensin and decoquinate inclusion linearly increased ($P < 0.01$) the molar proportion of propionate. Isobutyrate and butyrate molar proportions were greater ($P \leq 0.04$) for non-antibiotic anti-coccidial compounds compared with monensin. Increasing monensin inclusion linearly decreased ($P < 0.01$) butyrate proportion and tended to decrease ($P = 0.08$) isobutyrate proportion linearly. Isobutyrate proportion tended to increase ($P = 0.08$) linearly with increasing inclusion of DCQ. The isovalerate proportion was greater ($P < 0.01$) with monensin inclusion compared with DCQ and amprolium because increasing monensin inclusion linearly increased ($P < 0.01$) isovalerate proportion. Valerate proportion linearly decreased ($P = 0.04$) with monensin inclusion and linearly increased ($P = 0.04$) with amprolium inclusion. Anti-coccidial compound inclusion decreased ($P < 0.01$) the acetate:propionate, with the acetate:propionate being reduced ($P < 0.01$) to the greatest extent by monensin. Increasing levels of DCQ and monensin linearly decreased ($P < 0.01$) the acetate:propionate.

6.3.4 *Experiment 4*

The pH of the fermentation media after 24-h of incubation was greater ($P < 0.01$) with QSE inclusion compared with YSE (Table 6.7). Gas production after 24 h was greater ($P < 0.01$) for YSE compared with QSE. Increasing levels of YSE inclusion linearly increased ($P = 0.02$) gas production after 24 h. The rate of gas production was greater ($P < 0.01$) for QSE compared with YSE. Increasing levels of QSE inclusion linearly increased ($P < 0.01$) the rate of gas production. Methane percentage and production were not influenced by saponin inclusion. Ammonia and total VFA concentrations were not influenced by saponin inclusion.

The inclusion of saponins decreased ($P = 0.04$) acetate proportion. Increasing YSE and QSE inclusion decreased ($P < 0.01$) and tended to decrease ($P = 0.06$) acetate proportion linearly, respectively. Increasing YSE and QSE inclusion linearly increased ($P < 0.01$) propionate proportion. Increasing YSE and QSE inclusion linearly decreased ($P \leq 0.04$) the molar proportions of isobutyrate, butyrate, and isovalerate. Butyrate proportion was greater ($P = 0.03$) in fermentation media with YSE inclusion compared with QSE. Increasing YSE and QSE inclusion linearly increased ($P < 0.01$) valerate proportion; however, valerate proportion was greater ($P < 0.01$) with QSE inclusion. Increasing YSE and QSE inclusion linearly decreased ($P < 0.01$) the acetate:propionate.

6.4 Discussion

Ruminal methanogenesis is a potential pathway for the elimination of H_2 and the regeneration of NAD^+ for glycolysis (Moss et al., 2000). Methane mitigation strategies could largely be classified into three main groups: animal and feed management, diet formulation, and rumen manipulation (Arndt et al., 2022). High-concentrate diets containing moderate to large

proportions of starch are fed to increase the energy density of the diet to allow for more efficient growth and improved product quality. When high-concentrate diets are fed to beef cattle, the metabolizable energy to digestible energy ratio increases compared to feeding high-roughage diets (Hales et al., 2022). This is largely attributed to proportionally lower CH_4 energy losses when feeding high-concentrate diets (Hales et al., 2022). Although feeding high-concentrate diets results in less CH_4 production per unit of fermentable organic matter compared with high-roughage diets, the challenge remains to develop strategies to further decrease enteric CH_4 emissions from feedlot cattle. Monensin has historically been supplemented in finishing cattle diets to improve average daily gain and feed efficiency (Duffield et al., 2012). Dietary monensin inclusion leads to energetic advantages by modifying ruminal microbial populations which result in increased propionate proportion and decreased CH_4 production (Thornton and Owens, 1981). There is rising interest globally to develop alternative approaches to improve growth performance and reduce CH_4 emissions of beef cattle without the use of antibiotic feed additives. The objectives of this experiment were to evaluate sources and levels of non-antibiotic anti-coccidial compounds and saponin sources on *in vivo* and *in vitro* ruminal fermentation and methane production.

6.4.1 *Effects of DCQ + YSE on in vivo ruminal fermentation and methane production*

Previous research had demonstrated that YSE supplementation decreased *in vitro* methane production across low-, medium-, and high-forage inclusion substrates (Xu et al., 2010). In the current study, supplementation of DCQ + YSE for up to 10 d did not influence whole-body CH_4 production of steers. Because YSE supplementation had previously resulted in reduced CH_4 production *in vitro* (Xu et al., 2010), we assumed that after 7 d of adaptation, there would be adequate ruminal turnover to observe effects on CH_4 production. It is possible that longer adaptation to dietary treatments was necessary to observe effects on CH_4 production. Also, sources

and levels of DCQ and YSE fed, concentration of saponins, and/or types of saponins present in YSE could potentially alter responses in methane production.

Reductions in CH_4 production could potentially be associated with increases or decreases in economically-important variables in beef cattle. For example, decreased CH_4 production could be due to decreased intake, decreased digestibility, or decreased VFA production and therefore, could negatively affect production outcomes. Methane mitigation strategies that modify ruminal microbial populations to decrease protozoa, favor greater propionate production, and increase energy retention could be beneficial for both productive and environmental outcomes. Feed intake was controlled by limiting energy intake to 2 × NE_m in experiments 1 and 2. Results from the *in situ* degradability experiment demonstrated that the rate and extent of DM degradation and *in situ* ruminal DM degradability of the basal diet was not affected by DCQ + YSE supplementation. Likewise, ruminal liquid passage kinetics were not affected by DCQ + YSE supplementation.

Changes in end-products of ruminal fermentation in experiment 2 may suggest that supplementation of DCQ + YSE modified ruminal fermentation similarly when compared to previous studies. In general, supplementation of YSE typically results in decreased ruminal NH_3-N concentration, increased ruminal propionate proportion, and decreased ruminal protozoa concentration (Zúñiga-Serrano et al., 2022). Although not statistically significant, ruminal NH_3-N concentration was numerically reduced by 9.15% with DCQ + YSE supplementation. Ruminal propionate proportion tended to increase with DCQ + YSE supplementation. Although there was a slight increase in the propionate proportion, DCQ + YSE supplementation did not influence the acetate proportion or the acetate:propionate. It should be noted that the acetate:propionate was 1.43 and 1.41 for control and DCQ + YSE treatments, respectively. The decreased acetate proportion and increased propionate proportion resulting in a decreased acetate:propionate is closely

correlated with decreased CH_4 production (Ørskov et al., 1968). It is possible that the current diet composition and feeding level was propiogenic, limiting the opportunity for YSE to shift H_2 sinks. A recent review pointed out that the positive effects of YSE supplementation are not always observed when included in diets for cattle (Zúñiga-Serrano et al., 2022). Sources of saponin-containing extracts, plant saponin composition, dietary inclusion levels, manufacturing processes, and interactions with dietary components are some factors that could potentially contribute to inconsistencies across studies.

6.4.2 *Effects of monensin on in vitro ruminal fermentation and methane production*

A recent meta-analysis reported that monensin supplementation decreased CH_4 production in beef steers and dairy cows (Ranga Niroshan Appuhamy et al., 2013). Of the anti-coccidial and saponin sources tested, only monensin decreased *in vitro* CH_4 production in the current study. Monensin decreased total VFA concentrations and gas production in the current study, which is similar to the findings of others using *in vitro* gas production systems (Kim et al., 2014). However, decreased total VFA concentration is not typically observed when cattle are fed monensin (Clary et al., 1993). Rather, monensin can alter ruminal microbial populations by decreasing protozoa and gram-positive bacteria which could result in less acetate, butyrate, CH_4, lactate, and NH_3-N production (Hino and Russell, 1987; Mendoza et al., 1993). Consistent with numerous previous *in vitro* and *in vivo* studies, monensin inclusion decreased the acetate:propionate and increased molar propionate proportion. Decreased isobutyrate and valerate proportions with monensin inclusion are likely due to anti-microbial effects of monensin on proteolytic and/or amino acid-fermenting bacteria (Russell and Martin, 1984), suggesting reduced amino acid degradation (Ogunade et al., 2018). Decreased butyrate proportion with monensin inclusion was also found previously when a concentrate substrate was used (Kim et al., 2014). In the current study, monensin did not influence

NH_3-N concentration which contrasts with several studies reporting that monensin decreased NH_3-N concentration (Ricke et al., 1984; Russell and Martin, 1984; Kim et al., 2014). This could be due to the excess N available from the *in vitro* buffer solutions, as well as, the basal substrate. Overall, monensin modified *in vitro* ruminal fermentation and CH_4 production consistent with several previous experiments.

6.4.3 *Effects of non-antibiotic anti-coccidial compounds on in vitro ruminal fermentation and methane production*

In contrast to monensin, non-antibiotic anti-coccidial compounds had minimal impacts on *in vitro* CH_4 production and ruminal fermentation. Feeding increasing levels of DCQ did not influence total-tract DM digestibility of steers fed a high-concentrate diet (Harmon et al., 1987). In contrast, results from the current experiment demonstrated that increasing levels of DCQ linearly increased *in vitro* gas production. However, this is likely due to changes in the fermentability of the Deccox premix which replaced the basal substrate (2.1% and 20.1% inclusion rate for 1X and 10X treatments) in the current experiment. It is possible that some of the increase in propionate proportion in experiment 2 with DCQ + YSE supplementation could have been due to both DCQ and YSE, as increasing decoquinate linearly increased *in vitro* propionate proportion in experiment 3. Whether or not those effects are due to DCQ or the premix itself remains to be determined. Similar to previous *in vivo* findings, DCQ had little or no influence on characteristics of ruminal fermentation including VFA profiles or CH_4 production in the current study. Like DCQ, amprolium inclusion had minimal effects on *in vitro* ruminal fermentation in the current study.

6.4.4 *Effects of YSE and QSE on in vitro ruminal fermentation and methane production*

Previous research had demonstrated that YSE inclusion decreased *in vitro* CH_4 production across 10%, 50%, and 100% forage-based diets (Xu et al., 2010). However, increasing YSE or

QSE did not influence *in vitro* CH_4 production in the current experiment. In the current study, increasing levels of saponins linearly decreased acetate proportion and increased propionate proportion, resulting in decreased acetate:propionate. Changes in molar propionate proportion with an absence of a change in *in vitro* CH_4 production in experiment 4 are similar to the results found in experiments 1 and 2, where the combination of DCQ + YSE increased propionate without influencing CH_4 production. Zúñiga-Serrano et al. (2022) proposed that YSE may modify ruminal fermentation through several mechanisms which can lead to downstream effects on enteric CH_4 production. *Yucca schidigera* extract or saponins from YSE can decrease ruminal cellulolytic bacteria and fungi (Wang et al., 2000), decrease methanogenic archaea (Narvaez et al., 2013), and decrease ruminal protozoa (Hristov et al., 1999; Lila et al., 2005; Pen et al., 2006). Reduced ruminal NH_3-N, decreased acetate:propionate, and decreased fiber degradation are associated with decreased CH_4 production with YSE inclusion (Zúñiga-Serrano et al., 2022). Others have found that YSE and QSE decrease CH_4 production but at much greater concentrations than those used in the current experiment (Pen et al., 2006). Sources of saponin-containing extracts, plant saponin composition, dietary inclusion levels, manufacturing processes, and interactions with dietary components are some factors that could potentially contribute to inconsistencies across studies.

6.5 Conclusions

Supplementation of DCQ + YSE for 7 to 10 days did not influence O_2 consumption, CO_2 production, or CH_4 production in steers consuming a high-concentrate diet at 2 × NE_m. Supplementation of DCQ + YSE did not influence the rate or extent of ruminal DM degradation of the basal finishing diet or liquid passage kinetics. Supplementation of DCQ + YSE did not influence total VFA concentrations but tended to increase ruminal propionate proportion. Increasing levels of monensin decreased *in vitro* CH_4 production, acetate:propionate, isovalerate

and valerate proportions. Decoquinate and amprolium had minimal effects on *in vitro* ruminal fermentation. Increasing YSE or QSE inclusion increased propionate proportion but was not accompanied by a reduction in *in vitro* CH_4 production. Further research is necessary to identify alternative compounds for the simultaneous reduction of CH_4 emissions and control of coccidiosis in feedlot cattle diets.

Table 6.1 Composition of the finishing diet fed to steers in experiments 1-4.

Item	
Ingredient composition, DM basis	
Cracked corn, %	27.5
High-moisture corn, %	27.5
Dried corn distillers' grains with solubles, %	25.0
Corn silage, %	10.0
Finely ground corn, %	6.95
Limestone, %	1.92
Trace mineral premix, %[1]	0.50
Urea, %	0.36
Choice white grease, %	0.25
Vitamin A, D, & E premix, %[2]	0.02
Chemical composition	
Dry matter, %	71.5
Crude protein, % of DM	14.5
Neutral detergent fiber, % of DM	12.1
Acid detergent fiber, % of DM	6.7
Ca, % of DM	0.73
P, % of DM	0.45
Net energy for maintenance, Mcal/kg[3]	1.95
Net energy for gain, Mcal/kg[3]	1.30

[1]Contained: 56.34% Cl, 36.53% Na, 1.2% S, 0.06% Ca, 9.29 g Fe/kg, 5.52 g Zn/kg, 4.79 g Mn/kg, 1.84 g Cu/kg, 120 mg I/kg, 68.9 mg Co/kg, and 18.5 mg Se/kg on a DM basis.

[2]Composed of vitamin A acetate (1,814 kIU/kg), D-activated animal sterol (source of vitamin D_3; 363 kIU/kg), vitamin E supplement (227 IU/kg), roughage products, calcium carbonate, and mineral oil.

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

Table 6.2 Description of treatments used in experiments 3 and 4.

Source	Level	Amt. basal substrate	Amt. mix substrate[1]	Amt. liquid added[2]
Exp. 3				
Basal substrate (control)	-	500 mg	-	2 mL of water
Monensin	1X	462.5 mg	37.5 mg of 10% Rumensin 90 mix	2 mL of water
Monensin	10X	125 mg	375 mg of 10% Rumensin 90 mix	2 mL of water
Decoquinate	1X	396 mg	104 mg of 10% Deccox mix	2 mL of water
Decoquinate	10X	292 mg	208 mg of 50% Deccox mix	2 mL of water
Amprolium	1X	500 mg	-	2 mL of 6.4% Corid 9.6% Oral solution
Amprolium	10X	500 mg	-	2 mL of 64% Corid 9.6% Oral solution
Exp. 4				
Basal substrate (control)	-	500 mg	-	2 mL of water
Yucca schidigera extract	1X	500 mg	-	2 mL of 0.25% Micro-Aid Liquid solution
Yucca schidigera extract	10X	500 mg	-	2 mL of 2.5% Micro-Aid Liquid solution
Yucca schidigera extract	20X	500 mg	-	2 mL of 5% Micro-Aid Liquid solution
Quillaja saponaria extract	1X	500 mg	-	2 mL of 0.25% Phytogenic Patch Plus solution
Quillaja saponaria extract	10X	500 mg	-	2 mL of 2.5% Phytogenic Patch Plus solution
Quillaja saponaria extract	20X	500 mg	-	2 mL of 5% Phytogenic Patch Plus solution

[1]Anti-coccidial compound premixes were mixed with the basal substrate on a weight by weight basis.

[2]Liquid saponin treatments were mixed with water on a volume by volume basis.

Table 6.3 Experiment 1: Effects of decoquinate and *Yucca schidigera* extract supplementation (DCQ + YSE) on whole-body O_2 consumption, CO_2 production, and CH_4 production of steers limit-fed a high-concentrate diet at 2 × NE_m.

Item	Treatment		SEM[1]	*P*-value
	Control	DCQ + YSE		
O_2 consumption				
L/d	2963	2931	66.9	0.74
L/kg BW/d	8.95	8.90	0.193	0.85
L/kg $BW^{0.75}$/d	38.2	37.9	0.813	0.82
CO_2 production				
L/d	3009	2964	68.7	0.65
L/kg BW/d	9.09	9.00	0.197	0.75
L/kg $BW^{0.75}$/d	38.7	38.3	0.831	0.72
CH_4 production				
L/d	98.6	94.3	6.43	0.64
L/kg BW/d	0.294	0.281	0.0172	0.60
L/kg $BW^{0.75}$/d	1.26	1.20	0.0754	0.61
Respiratory quotient	1.01	1.01	0.00433	0.64

Abbreviations: BW = body weight; $BW^{0.75}$ = metabolic body weight.

[1]Standard error of the mean (n = 8).

Table 6.4 Experiment 2: Effects of decoquinate and *Yucca schidigera* extract supplementation (DCQ + YSE) on *in situ* ruminal dry matter degradation kinetics and liquid passage rate of steers limit-fed a high-concentrate diet at 2 × NE_m.

Item	Treatment		SEM[1]	*P*-value
	Control	DCQ + YSE		
Soluble fraction, %	53.1	51.6	0.775	0.24
Potentially degradable fraction, %	29.9	31.9	1.34	0.35
Potential extent of degradation, %	83.0	83.4	0.982	0.74
Lag time, h	4.24	2.72	2.81	0.72
Fractional rate of degradation, % per hour	3.08	3.22	0.489	0.85
Fractional rate of liquid passage, % per hour	5.52	5.23	0.546	0.72
In situ ruminal degradability, %	63.6	63.7	0.964	0.99
Liquid retention time, h	18.5	19.9	1.83	0.61
Rumen liquid volume, L	30.0	29.2	6.46	0.94
Rumen liquid outflow, L/h	1.60	1.46	0.305	0.77

[1]Standard error of the mean (n = 4).

Table 6.5 Experiment 2: Effects of decoquinate and *Yucca schidigera* extract supplementation (DCQ + YSE) on ruminal fermentation characteristics of steers limit-fed a high-concentrate diet at 2 × NE_m.

	Treatment			*P*-value		
Item	Control	DCQ + YSE	SEM[1]	Trt	Time	Time × Trt
L(+)-Lactate, mM	0.545	1.32	0.250	0.03	<0.01	0.19
NH_3, mM	6.67	6.06	0.289	0.14	<0.01	0.82
Total VFA, mM	125	119	3.81	0.33	<0.01	0.93
mol/100 mol						
Acetate	46.8	45.8	0.494	0.19	<0.01	0.99
Propionate	33.7	34.9	0.510	0.09	0.37	0.99
Isobutyrate	0.524	0.468	0.0607	0.12	<0.01	0.99
Butyrate	10.8	10.1	0.356	0.18	0.02	0.96
Isovalerate	2.12	1.80	0.0823	0.01	<0.01	0.99
Valerate	5.57	7.43	0.239	<0.01	<0.01	0.89
Acetate:propionate	1.43	1.41	0.0314	0.64	<0.01	0.34

[1]Standard error of the mean (n = 4).

Table 6.6 Experiment 3: Effects of sources and levels of anti-coccidial compounds on *in vitro* methane production and ruminal fermentation.

	Treatment								Contrast *P*-value					
	Basal	Monensin		Decoquinate		Amprolium				Source		Level		
Item	substrate (0X)	1X	10X	1X	10X	1X	10X	SEM[1]	ACC	Ionophore	DCQ vs. AMP	Lin. MON	Lin. DCQ	Lin. AMP
pH	6.78	6.81	6.87	6.84	6.83	6.85	6.83	0.0145	<0.01	0.92	0.80	<0.01	0.20	0.34
Gas production, mL	116	111	93.5	115	122	115	120	1.37	0.02	<0.01	0.42	<0.01	<0.01	0.07
Rate, %/h	13.7	15.0	16.0	14.0	11.6	13.8	12.7	0.422	0.80	<0.01	0.30	<0.01	<0.01	0.07
CH_4, %	7.83	7.43	5.79	7.75	7.92	7.76	7.68	0.252	0.11	<0.01	0.65	<0.01	0.74	0.66
CH_4, mL	9.14	8.46	5.51	9.09	9.84	9.02	9.27	0.299	0.07	<0.01	0.30	<0.01	0.11	0.60
NH_3, mM	27.5	27.3	25.7	27.2	25.3	27.2	26.3	0.833	0.25	0.97	0.58	0.19	0.04	0.19
Total VFA, mM	61.5	57.0	60.7	62.5	64.5	60.7	64.8	2.48	0.92	0.05	0.73	0.64	0.24	0.34
mol/100 mol														
Acetate	41.6	41.3	41.5	41.3	40.8	41.1	41.0	0.314	0.24	0.21	0.99	0.93	0.02	0.17
Propionate	18.0	18.8	21.1	18.5	18.6	18.6	18.4	0.235	<0.01	<0.01	0.80	<0.01	<0.01	0.32
Isobutyrate	5.28	5.15	4.58	5.25	5.38	5.21	5.11	0.208	0.47	0.04	0.47	0.08	0.08	0.16
Butyrate	12.6	12.1	10.4	12.4	12.6	12.4	12.6	0.234	0.04	<0.01	0.99	<0.01	0.64	0.40
Isovalerate	5.43	5.52	5.68	5.40	5.35	5.45	5.45	0.0453	0.39	<0.01	0.10	0.01	0.12	0.85
Valerate	5.61	5.73	5.40	5.59	5.64	5.64	5.76	0.0650	0.82	0.14	0.15	0.04	0.78	0.04
Acetate:propionate	1.92	1.82	1.58	1.85	1.83	1.84	1.86	0.0185	<0.01	<0.01	0.79	<0.01	<0.01	0.19

Abbreviations: ACC = anti-coccidial compound; AMP = amprolium; DCQ = decoquinate; MON = monensin.

Table 6.6 (continued)

[1]Standard error of the mean (n = 8).

[2]Contrasts: ACC = Basal substrate vs. others; Ionophore (antibiotic vs. non-antibiotic anti-coccidials) = monensin vs. decoquinate and amprolium; DCQ vs. AMP = decoquinate vs. amprolium.

Table 6.7 Experiment 4: Effects of saponin sources and levels on *in vitro* methane production and ruminal fermentation.

	Treatment								Contrast *P*-value					
		Yucca schidigera extract			Quillaja saponaria extract						Level			
Item	Basal substrate (0X)	1X	10X	20X	1X	10X	20X	SEM[1]	Saponin	Source	Lin. YSE	Quad. YSE	Lin. QSE	Quad. QSE
pH	6.88	6.88	6.88	6.87	6.90	6.90	6.89	0.0082	0.20	<0.01	0.67	0.34	0.75	0.16
Gas production, mL	114	116	116	120	111	108	114	2.15	0.96	<0.01	0.02	0.43	0.88	0.08
Rate, %/h	12.9	12.7	12.8	12.9	12.5	13.9	15.4	0.453	0.39	<0.01	0.89	0.82	<0.01	0.80
CH_4, %	7.67	7.71	7.24	7.25	7.49	7.47	7.58	0.234	0.39	0.56	0.13	0.44	0.91	0.61
CH_4, mL	8.78	8.97	8.34	8.72	8.30	8.03	8.53	0.313	0.38	0.13	0.59	0.28	0.75	0.13
NH_3, mM	27.2	25.9	27.1	26.8	27.4	28.4	26.8	0.928	0.91	0.22	0.76	0.83	0.86	0.22
Total VFA, mM	66.8	64.3	67.6	69.7	63.3	67.3	67.3	2.20	0.93	0.52	0.11	0.81	0.42	0.81
mol/100 mol														
Acetate	47.4	47.3	47.1	46.5	46.5	47.0	46.7	0.237	0.04	0.26	<0.01	0.31	0.06	0.51
Propionate	24.0	24.2	24.9	25.2	24.6	24.9	25.6	0.240	<0.01	0.17	<0.01	0.30	<0.01	0.96
Isobutyrate	2.03	2.03	1.99	1.95	2.04	2.02	1.94	0.0155	0.03	0.46	<0.01	0.87	<0.01	0.11
Butyrate	14.9	14.8	14.4	14.6	14.7	14.4	14.0	0.131	<0.01	0.03	0.04	0.07	<0.01	0.73
Isovalerate	4.72	4.70	4.63	4.55	4.71	4.69	4.55	0.0427	0.07	0.47	<0.01	0.85	<0.01	0.27
Valerate	6.94	6.95	6.96	7.23	6.98	7.29	7.32	0.0691	0.01	<0.01	<0.01	0.09	<0.01	0.07

Table 6.7 (continued)

Acetate:propionate	2.01	2.00	1.92	1.87	1.95	1.91	1.84	0.0269	<0.01	0.15	<0.01	0.51	<0.01	0.75

Abbreviations: YSE = Yucca schidigera extract; QSE = Quillaja saponaria extract.

[1]Standard error of the mean (n = 8).

[2]Contrasts: Saponin = Basal substrate vs. others; Source = Yucca schidigera extract vs. Quillaja saponaria extract.

CHAPTER 7. STIMULATION OF PANCREATIC AND SMALL INTESTINAL GROWTH WITH POSTRUMINAL CASEIN INFUSION AND EXOGENOUS GLUCAGON-LIKE PEPTIDE 2 RESULTS IN INCREASED CARBOHYDRASE ACTIVITY IN CATTLE

7.1 Introduction

In North American beef cattle and dairy cattle production systems, grain-based diets containing moderate to large proportions of starch are typically fed to increase the net energy concentration of the diet to allow for more efficient growth and improved product quality. Depending on grain source and processing methods, up to 40% of dietary starch intake can escape ruminal fermentation and flow to the small intestine for potential enzymatic digestion (Ørskov, 1986). Enzymatic digestion of starch in the small intestine can be energetically more efficient than ruminal fermentation of starch because absorption and oxidation of glucose provides more energy to the host than the production and oxidation of short-chain fatty acids (Black, 1971). However, summaries have indicated that the extents of small intestinal starch digestibility (55% in beef cattle and 60% in dairy cows) (Owens et al., 1986; Moharrery et al., 2014) is inadequate to achieve potential energetic advantages compared with ruminal starch fermentation (Huntington et al., 2006).

Small intestinal starch digestion in ruminants could potentially be limited by inadequate production of pancreatic and/or small intestinal carbohydrases (Owens et al., 1986; Brake and Swanson, 2018). Nonruminants have greater activities of pancreatic α-amylase and small intestinal sucrase-isomaltase, and maltase-glucoamylase compared with cattle and sheep (Walker, 1959; Siddons, 1968; Toofanian et al., 1974). In nonruminants, digestive enzyme activity typically increases proportionally to substrate supply (Brannon, 1990; Harmon, 1993). However, pancreatic α-amylase activity increases with luminal protein flow (Wang and Taniguchi, 1998; Swanson et

al., 2002a; Richards et al., 2003; Swanson et al., 2003; Swanson et al., 2004a; Trotta et al., 2020c) and decreases with luminal carbohydrate flow (Walker and Harmon, 1995; Wang and Taniguchi, 1998; Swanson et al., 2002a; Swanson et al., 2002b; Swanson et al., 2004a) in ruminants. Small intestinal α-glucosidase activity may respond to changes in dietary energy intake and luminal substrate flow in ruminants (Kreikemeier et al., 1990b; Rodriguez et al., 2004; Górka et al., 2017; Trotta et al., 2020c; Trotta et al., 2020d), suggesting that regulation of pancreatic and small intestinal carbohydrases is complex and perhaps uncoordinated in response to luminal nutrient flows (Harmon and Swanson, 2020). Due to the complexity of coordinating carbohydrase activity with luminal nutrient flows, the development of an *in vivo* animal model is necessary to elucidate the effects of increasing endogenous activity of pancreatic α-amylase or small intestinal α-glucosidases or both on small intestinal starch digestion in ruminants.

Postruminal infusion of casein has been shown to increase pancreatic mass (Swanson et al., 2002a), pancreatic α-amylase activity and secretion (Wang and Taniguchi, 1998; Swanson et al., 2002a; Richards et al., 2003; Swanson et al., 2003; Swanson et al., 2004a; Trotta et al., 2020c), small intestinal starch disappearance (Richards et al., 2002; Mabjeesh et al., 2003; Mendoza and Britton, 2003; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016; Acharya et al., 2023), and net portal glucose absorption (Taniguchi et al., 1995) in ruminants. Exogenous glucagon-like peptide 2 (GLP-2) has been shown to increase small intestinal mucosal growth by increasing villus height and crypt depth by increasing cell proliferation and decreasing cell proteolysis and apoptosis (Drucker et al., 1996; Brubaker et al., 1997; Tsai et al., 1997a; Tsai et al., 1997b; Burrin et al., 2000; Burrin et al., 2005; Taylor-Edwards et al., 2011). In neonatal pigs, exogenous GLP-2 increased sucrase-isomaltase and maltase-glucoamylase mRNA expression, as well as, maltase and sucrase activity (Petersen et al., 2002). Pancreatic α-amylase is synthesized in pancreatic

acinar cells and small intestinal α-glucosidases are localized to the brush border membrane of small intestinal enterocytes. Because pancreatic acinar cells and small intestinal enterocytes are the predominant cell types (> 80%) of the pancreas and small intestinal mucosa (de Santa Barbara et al., 2003; Gorelick et al., 2018), it was hypothesized that stimulation of pancreatic and small intestinal mucosal growth would result in increased carbohydrase activity. The objectives of this experiment were to use postruminal casein infusion and exogenous GLP-2 administration, known stimulants of pancreatic and small intestinal function, to evaluate limitations of small intestinal starch digestion by determining their effects on tissue growth, carbohydrase activity, and postruminal starch disappearance in cattle.

7.2 Materials and methods

All surgical, animal care, and experimental protocols were approved by the University of Kentucky Animal Care and Use Committee (2020-3479).

7.2.1 *Animals, diet, and surgery*

Twenty-four Holstein steers (initial shrunk BW = 240 ± 22.2 kg) were initially housed in individual pens (3 m × 3 m) in the Intensive Research Building of the University of Kentucky C. Oran Little Agricultural Research Center in Versailles, KY. Before surgery, steers were deprived of feed (36 h) and water (12 h), fitted with temporary jugular vein catheters (Mila International Inc., Florence, KY), and received a subcutaneous injection of ceftiofur (6.6 mg/kg of BW) and intravenous injection of flunixin meglumine (1 mg/kg of BW). Steers were sedated by intravenous injection of xylazine (0.088 mg/kg of BW) with ketamine hydrochloride (1.76 mg/kg of BW), intubated, and anesthesia was maintained with isoflurane in O_2 using a ventilator in left lateral recumbency. The depth of anesthesia was monitored through observations of eye reflex and heart

rate throughout the surgery. Steers were surgically fitted with infusion catheters in the abomasum that were constructed of Tygon tubing (6.35-mm internal diameter; Saint-Gobain North America, Malvern, PA) (McLeod et al., 2007). Flunixin meglumine was administered intravenously 24-h post-surgery and steers were monitored for abnormal feed intake, fecal consistency, behavior, and mobility for 7 d, and rectal temperatures were monitored for 3 d post-surgery.

Steers were limit-fed 5.65 ± 0.468 kg of an alfalfa hay cube-based diet on a DM basis (Table 7.1). The diet was formulated to supply 1.33 times the net energy required for maintenance and exceed requirements for vitamins, minerals, ruminally degradable protein, and metabolizable protein for a steer gaining 0.455 kg/d (NASEM, 2016). Rations were provided twice daily at 0800 and 2000 h in equal portions. Steers were adapted to the basal diet for at least 7 d before the start of the treatment period.

Diet samples were collected for nutrient analysis including DM, crude protein, neutral and acid detergent fiber, starch, and minerals. Diet samples were partially dried at 60°C for 4 h in a forced-air oven (NFTA 2.2.1.1.) and then ground to pass a 1-mm screen using a Wiley mill. Dry matter content was determined by oven-drying for 3 h at 105°C (NFTA 2.1.4.). Nitrogen content was analyzed by combustion (AOAC, 2006) method 990.03 using a CN628 Carbon/Nitrogen Determinator (Leco Corporation, St. Joseph, MI). Crude protein was calculated by multiplying N concentration × 6.25. Acid detergent fiber and neutral detergent fiber concentrations were determined using the filter bag technique (ANKOM Technology Methods 14 and 15, respectively). Samples were prepared for total starch analysis (YSI Life Sciences Application Note 222LS-02) by gelatinizing the sample in water with an autoclave, followed by enzymatic hydrolysis with glucoamylase in acetate buffer. Free glucose concentration was measured using the glucose oxidase electrode of a YSI Series 2950 D-1 Biochemistry Analyzer (YSI Inc., Yellow Springs,

OH) and then multiplied by 0.9 to convert to anhydroglucose, as it occurs in starch (McCleary et al., 1994). Mineral concentrations (Ca and P) were determined using microwave digestion and inductively coupled plasma-optical emission spectroscopy.

7.2.2 *Experimental design*

Steers were transferred to metabolism stalls (1.2 m × 2.4 m) for the 7-d treatment period. Due to limited stall space and infusion apparatus, steers were stratified by BW into 6 replicate blocks. Steers were then randomly assigned to one of four treatments within each replicate block. The experimental design was a randomized complete block design with a 2 × 2 factorial arrangement of treatments. All steers were abomasally infused with 3.94 ± 0.245 g raw corn starch · kg BW^{-1} · d^{-1}. Along with raw corn starch, steers were abomasally infused with either water or 10% wt:wt sodium caseinate solution to provide 0 or 1.30 ± 0.299 g sodium caseinate · kg BW^{-1} · d^{-1}. Additionally, steers received subcutaneous injections in two equal portions daily of either: 5 g/L bovine serum albumin (BSA) diluted in 9 g/L NaCl as a vehicle or 100 µg GLP-2 in vehicle · kg BW^{-1} · d^{-1}. This resulted in four treatments: 1) abomasal water + vehicle injection (control), 2) abomasal water + GLP-2 injection (GLP-2), 3) abomasal 10% sodium caseinate solution + vehicle injection (casein), and 4) abomasal 10% sodium caseinate solution + GLP-2 injection (casein + GLP-2). All steers received abomasal infusion and subcutaneous injection treatments for 7 d. The 7-d treatment period was chosen because postruminal casein infusion has been shown to increase small intestinal starch disappearance within 6 d (Brake et al., 2014b) and acute (1 d) or chronic (10 d) exposure to GLP-2 achieved pharmacological plasma concentrations of GLP-2 (Taylor-Edwards et al., 2011).

7.2.3 *Glucagon-like peptide 2 preparation and administration*

Glucagon-like peptide 2 was synthesized (Alan Scientific Inc., Gaithersburg, MD) based on the native bovine sequence (Lopez et al., 1983; Burrin et al., 2003). The purity (96%) was quantified by the manufacturer using high-performance liquid chromatography. The GLP-2 solution was prepared to a final concentration of 5 mg/mL by dissolving GLP-2 in 5 g/L BSA in 9 g/L NaCl solution. Aliquots were frozen at -20°C until use. The dosage of GLP-2 used in the current study has been shown to increase arterial GLP-2 concentrations in cattle previously (Taylor-Edwards et al., 2011). Intestinotrophic effects of GLP-2 are greater when GLP-2 was administered subcutaneously compared with intramuscular or intraperitoneal injections (Tsai et al., 1997b). Therefore, treatments were administered subcutaneously in the neck region in front of the shoulder. Left and right lateral administration sites were alternated for morning and evening injections, respectively.

7.2.4 *Nutrient infusion preparation and techniques*

Raw corn starch (Clinton 185 Corn Starch; Archer Daniels Midland Company, Decatur, IL) was chosen as the starch source to facilitate the greatest limitations in small intestinal starch digestibility, as the action of pancreatic α-amylase and small intestinal α-glucosidases are required for complete hydrolysis to glucose. The chosen amount of infused raw corn starch was predicted to limit the extent of small intestinal starch disappearance (Trotta et al., 2022) and mimic natural amounts of postruminal starch flow in cattle fed high-grain diets (Owens et al., 2016). Additionally, infusion of 986 g of raw corn starch per day equates to 41.1 g/h, which does not cause diarrhea and appreciable amounts (10 to 15 g/h) of infused carbohydrate were expected to pass the ileocecal junction (Kreikemeier et al., 1991). Sodium caseinate (AMCO Proteins,

Burlington, NJ) was chosen as the protein source because of its relatively high solubility in water and because approximately 70-85% of luminal amino acids are transported across the apical membrane as small peptides (Krehbiel and Matthews, 2003). The amount of casein was chosen relative to the amount of corn starch infused (1 part casein: 3 parts corn starch) and because previous experiments have demonstrated increased small intestinal starch disappearance (Richards et al., 2002; Brake et al., 2014a) and pancreatic α-amylase secretion (Richards et al., 2003) at similar levels.

Two containers of infusate suspensions per steer were prepared daily, immediately before infusion, for use over 12-h intervals. Infusate suspensions were maintained through continuous stirring. Infusate suspensions (3.26 ± 0.109 kg) were prepared using the appropriate amount of raw corn starch, 5% (w/w) CrEDTA solution as an indigestible flow marker (Binnerts et al., 1968), and tap water every 12 h for each steer. A 10% (w/w) solution of sodium caseinate (MacLeod et al., 1982) was prepared with warm tap water in an insulated kettle (LEC-40; Legion Industries Inc., Waynesville, GA) using an industrial electric mixer (CDP3330; Baldor Electric Co., Ft. Smith, AR). The solution was mixed for at least 2 h and then was allowed to rest for 8 h to allow for air bubbles to escape (Ardalan et al., 2022). The final solution was stored in 18.9-L buckets at -20°C until use. For casein treatments, the sodium caseinate solution replaced tap water in the infusate suspension. Corn starch and casein solute concentrations and abomasal infusion rates were similar to those reported by Kreikemeier et al. (1991) and Richards et al. (2002). Abomasal infusion treatments were continuously infused (271 ± 9.08 g infusate/h) through Tygon tubing (3.18-mm internal diameter) using a multi-channel peristaltic pump (205U/CA; Watson-Marlow, Falmouth, United Kingdom). The amount infused was determined by the weight of the residual infusate after each 12-h period. The infusate containers were placed on top of a portable shelf (> 2 m height) to

aid in the pumping of the suspension. Infusion tubing was cleaned by pumping dilute NaClO solution through the tubing in between each replication of the experiment.

7.2.5 *Fecal collection and analysis*

On day 7 of the infusion period, feces (~ 300 g) was collected after cattle defecated in response to rectal stimulation at 4, 6, 8, 10, and 12 h after the morning feeding. Immediately after collection, approximately 5 g of wet feces was mixed with 15 mL of nanopure H_2O and fecal pH was measured. Fecal samples were composited equally by wet weight for each steer, split into three portions, and frozen at -20°C until analysis. The first portion of feces was analyzed for DM by weighing 5 g of feces into an aluminum pan and drying the sample in a forced-air oven for 72 h at 55°C. The second portion of feces was analyzed for total starch content according to the methods described by Knudsen (1997). Total starch concentration was calculated by converting free glucose to anhydroglucose, as it occurs in starch (McCleary et al., 1994). The third portion was diluted 10-fold with deionized H_2O, vortexed, and 1-mL aliquots were transferred to 1.7-mL microcentrifuge tubes. Tubes were centrifuged at 20,000 × *g* for 15 min at 4°C and the supernatants were collected. The supernatant was analyzed for glucose concentration using reagent containing hexokinase and glucose-6-phosphate dehydrogenase (Infinity Glucose Liquid Stable Reagent; Cat no. TR15421; Thermo Fisher Scientific Inc., Waltham, MA) (Farrance, 1987) that was adapted for use with a multi-mode plate reader (BioTek Synergy HTX; Agilent Technologies Inc., Santa Clara, CA). The supernatant was combined with 100 µL of 500 g/L metaphosphoric acid and 100 µL of 2-ethylbutyrate and frozen at -20°C to facilitate protein precipitation. Samples were thawed, centrifuged at 20,000 × *g* for 15 min at 4°C, and transferred to autosampler vials for VFA analysis. The gas chromatograph was equipped with a flame ionization detector (Hewlett-Packard 6890 Plus GC, Wilmington, DE) and fitted with a Supelco 25326 Nukol fused silica capillary column

(Supelco Inc., Bellefonte, PA). The supernatant was diluted further and analyzed for Cr using atomic absorption spectroscopy (Aanalyst 200; Perkin Elmer Inc., Waltham, MA) at a wavelength of 357.87 nm.

7.2.6 *Fecal excretion and postruminal starch disappearance*

Fecal flow was calculated using equations described by Kreikemeier and Harmon (1995):

$$1.\ \text{Fecal fluid flow (g/h)} = \frac{\text{[Cr] in abomasal infusate (ppm)}}{\text{[Cr] in feces (ppm)}} \times \text{abomasal infusion rate (g/h)}$$

$$2.\ \text{Total fecal flow (g/h)} = \frac{\text{fecal fluid flow (g/h)}}{(1 - \text{fecal DM})}$$

$$3.\ \text{Fecal DM flow (g/h)} = \text{total fecal flow} - \text{fecal fluid flow}$$

Fecal excretion of glucose and short-chain fatty acids were calculated as fecal fluid flow multiplied by nutrient concentration. Fecal excretion of starch was calculated as fecal DM flow multiplied by nutrient concentration. Apparent postruminal starch disappearance was calculated as the amount of starch abomasally infused minus the amount of starch excreted in the feces divided by the amount of starch abomasally infused and multiplied by 100.

7.2.7 *Tissue collection*

Upon completion of the 7-d infusion period, the infusion tubing was disconnected from abomasal infusion catheters and steers were transported to the University of Kentucky Meats Laboratory within 1 h. Steers were stunned via captive bolt and exsanguinated. After slaughter, the viscera were rapidly removed and separated for individual weights and subsample collection. Tissue masses are reported as fresh weight because previous data have suggested little difference between fresh and dry tissue weights in response to treatments (Swanson et al., 1999; Swanson et

al., 2000b). The pancreas was trimmed of excess adipose tissue, the mass was recorded, and a subsample from the body of the pancreas was flash-frozen in liquid nitrogen at -80°C. 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 2.43-m board (Kreikemeier et al., 1990b). The small intestine was separated into the duodenum (0.1 to 1.1 m caudal to the pyloric sphincter), jejunum (proximal half of non-duodenal small intestine), and ileum (distal half of non-duodenal small intestine) (Liao et al., 2008). Each intestinal section (duodenum, jejunum, ileum) was weighed and sampled (1 m) from the midpoint. Each 1-m segment was cut into three equidistant subsamples, everted, and rinsed in ice-cold saline. The first subsample was cut laterally, scraped with a glass microscope slide, and 200 mg of mucosa was weighed and preserved with RNAlater stabilization solution (Thermo Scientific Inc., Waltham, MA) in RNase-free tubes and stored at -80°C. The second subsample was scraped as described previously and the mucosa was flash-frozen in liquid nitrogen in an aluminum pouch and stored at -80°C for cellularity and enzymatic analyses. The third subsample was weighed, mucosa scraped, and then the isolated mucosa was weighed. The percentage of mucosa was calculated as mass of the mucosa divided by mass of the total subsample and multiplied by 100.

7.2.8 *Pancreatic and small intestinal cellularity*

Pancreatic tissue and duodenal, jejunal, and ileal mucosa (223 ± 18.3 mg) were homogenized (Kinematica Polytron PT 3100; Brinkmann Instruments Inc., Westbury, NY) with 2.80 mL of ice-cold TBS-EDTA buffer (2 M NaCl, 10 mM EDTA, 10 mM Tris; pH = 7.0) for DNA analysis. Pancreatic tissue (264 ± 10.3 mg) and duodenal, jejunal, and ileal mucosa (604 ± 7.56 mg) were homogenized with 2.25 mL or 2.40 mL of 9 g/L NaCl for protein analysis,

respectively. The DNA concentration was measured using the bis-benzimidazole fluorescence procedure with DNA Type I from calf thymus as the standard (Labarca and Paigen, 1980). Protein concentration was measured using the bicinchoninic acid (BCA) procedure (Pierce BCA Protein Assay Kit; Cat no. 23225; Thermo Fisher Scientific Inc., Waltham, MA) with BSA used as the standard (Smith et al., 1985). Protein and DNA assays were adapted for use on a multi-mode plate reader. Changes in DNA concentration were assumed to reflect changes in the number of cells per gram of tissue (index of hypoplasia/hyperplasia) and changes in the protein:DNA were assumed to reflect changes in cell size (index of hypotrophy/hypertrophy).

7.2.9 *Pancreatic α-amylase and small intestinal α-glucosidase activity*

Pancreatic and small intestinal mucosal homogenates used for protein analysis were also used for carbohydrase activity assays. The activity of α-amylase in pancreatic homogenates was determined using the procedure of Wallenfels et al. (1978) that was adapted for the analysis of pancreatic tissue. The α-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 $CaCl_2$; 12.5 mM buffer] with 24 mL of nanopure water and pre-warmed to 39°C in an incubator. Analyses were adapted for use on a multi-mode plate reader at 39°C. One unit (U) of enzyme activity equals 1 μmol of p-nitrophenol produced per minute.

Procedures used for small intestinal α-glucosidase activities in mucosal homogenates were based on those developed by Dahlqvist (1964) for maltase and isomaltase and by Kidder et al. (1972) for glucoamylase, with assay modifications from Siddons (1968) and Kreikemeier et al.

(1990b) for use with intestinal mucosal tissue from cattle. Maltase activity was assayed by incubating 125 μL of the mucosal homogenate with 875 μL of 34 mM maltose in 0.1 M sodium maleate buffer (pH = 5.8) for 60 min at 39°C in a water bath. Isomaltase activity was assayed by incubating 500 μL of the mucosal homogenate with 500 μL of 180 mM isomaltose in 0.1 M sodium maleate buffer (pH = 5.8) for 60 min at 39°C in a water bath. Glucoamylase activity was assayed by incubating 500 μL of the mucosal homogenate with 500 μL of 7.34 mg/dL soluble starch in 0.2 M sodium phosphate buffer (pH = 6.5) for 30 min at 39°C in a water bath. The reactions were terminated by heating microcentrifuge tubes for 2 min in a 99°C water bath, followed by drenching in an ice bath. Tubes were then centrifuged at 4000 × g for 20 min at 4°C. Glucose concentration was measured on a multi-mode plate reader, as described previously. One unit (U) of enzyme activity equals 1 μmol of glucose produced per minute for glucoamylase and 2 μmol of glucose produced per minute for maltase and isomaltase. Blanks of each mucosal homogenate and buffer were quantified to account for endogenous glucose concentrations and subtracted from the total amount of product produced per minute.

All assays were optimized to achieve maximal velocity through the linear concentration range. Enzyme activity data were expressed as U/g tissue, U/g protein, and U or kU for tissue content. Tissue DNA, protein, or enzyme activity was calculated by multiplying the concentration per gram of tissue by the mass of the tissue. The total DNA, protein, or enzyme activity of the small intestinal mucosa was considered the sum of tissue content (duodenal, jejunal, ileal mucosa). Because cattle do not have measurable sucrase activity (Trotta et al., 2022), α-glucosidase activity is equal to the sum of maltase, isomaltase, and glucoamylase activity.

7.2.10 *Statistical analysis*

All variables were checked for normality using the UNIVARIATE procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The data were analyzed as a randomized complete block design with steer as the experimental unit and replicate as the blocking factor. Fecal characteristic data were excluded from analyses for one steer in the casein treatment because of temporary loss of patency in the abomasal infusion catheter during the fecal sampling period. Fecal pH was analyzed using the repeated measures statement of the MIXED procedure of SAS. Antedependence 1, autoregressive 1, compound symmetry, simple, and unstructured variance-covariance structures for the repeated measures statement were assessed for fit using Bayesian information criterion. The model included fixed effects of block, casein, GLP-2, time, and their interactions. All other variables were analyzed for fixed effects of block, casein, GLP-2, and the casein × GLP-2 interaction. Least squares means and their standard errors were computed for each fixed effect in the models. Pairwise comparisons of least squares means were separated using the Tukey-Kramer adjustment, protected by a significant *F*-test. Results were considered significant if $P \leq 0.05$. Tendencies were declared when $0.05 < P \leq 0.10$.

7.3 Results

7.3.1 *Fecal flow, nutrient excretion, and starch disappearance*

Initial BW and BW at slaughter did not differ among treatments (Table 7.2). Infusate and starch infusion rates did not differ among treatments. Postruminal casein infusion decreased ($P \leq 0.04$) fecal pH and fecal DM and starch percentages. Exogenous GLP-2 administration tended to increase ($P = 0.07$) fecal DM. Postruminal casein infusion tended to decrease ($P = 0.07$) total fecal flow and exogenous GLP-2 administration tended to decrease ($P = 0.06$) fecal fluid flow.

Postruminal casein infusion decreased ($P < 0.01$) fecal DM flow and fecal starch excretion. Fecal glucose excretion was not influenced by postruminal casein infusion or exogenous GLP-2 administration. Postruminal casein infusion tended to decrease ($P = 0.09$) fecal VFA excretion. Postruminal casein infusion increased ($P < 0.01$) apparent postruminal starch disappearance.

7.3.2 *Pancreatic mass, cellularity, and α-amylase activity*

Postruminal casein infusion increased ($P = 0.03$) absolute pancreas mass and tended to increase ($P = 0.06$) pancreas mass relative to BW (Table 7.3). Pancreatic DNA concentration, DNA content, protein concentration, and protein:DNA were not influenced by casein or GLP-2. Postruminal casein infusion increased ($P \leq 0.03$) pancreatic protein content, α-amylase activity per gram pancreas, α-amylase activity per gram protein, and total α-amylase activity.

7.3.3 *Small intestinal mass, cellularity, and α-glucosidase activity*

Postruminal casein infusion and exogenous GLP-2 administration increased ($P \leq 0.02$) the absolute mass of the small intestine and the absolute mass of the small intestinal mucosa (Table 7.4). As a proportion of BW, exogenous GLP-2 administration increased ($P < 0.01$) small intestinal mass and small intestinal mucosal mass, and casein tended to increase ($P = 0.07$) small intestinal mass and mucosa mass. Exogenous GLP-2 administration increased ($P < 0.01$) the percentage of small intestinal mucosa relative to the total mass of the small intestine. There was a casein × GLP-2 interaction for small intestinal length because the small intestinal length was greater ($P = 0.05$) for steers in the casein + GLP-2 treatment compared with steers in the control, casein, and GLP-2 treatments. Small intestinal mass:length did not differ among treatments. Deoxyribonucleic acid concentration, protein concentration, and protein:DNA of the small intestinal mucosa did not differ among treatments. Exogenous GLP-2 administration increased ($P = 0.02$) the total DNA and

protein content of the small intestinal mucosa. The total protein content of the small intestinal mucosa was greater ($P = 0.02$) for steers postruminally infused with casein.

Maltase activity per gram small intestinal mucosa and activity per gram protein were greater ($P \leq 0.05$) for GLP-2 steers compared with control, casein, and casein + GLP-2 treatments. Relative to control, GLP-2 and casein + GLP-2 had greater ($P = 0.03$) total maltase activity in the small intestinal mucosa. Isomaltase activity per gram small intestinal mucosa and activity per gram of protein were not different among treatments. Exogenous GLP-2 administration increased ($P < 0.01$) total isomaltase activity of the small intestinal mucosa. Total isomaltase activity of the small intestinal mucosa was greater ($P = 0.05$) for steers postruminally infused with casein. There was an interaction for glucoamylase activity per gram of small intestinal mucosa because glucoamylase activity was greater ($P = 0.04$) for GLP-2 compared to casein + GLP-2. There was an interaction for total glucoamylase activity of the small intestinal mucosa because GLP-2 increased ($P = 0.04$) total glucoamylase activity compared with control but, the combination of casein + GLP-2 did not increase total glucoamylase activity compared with casein. Compared to the control, the total α-glucosidase activity of the small intestinal mucosa was greater ($P = 0.04$) for GLP-2 and casein + GLP-2 treatments.

7.3.4 *Duodenal mass, cellularity, and α-glucosidase activity*

Duodenal mass (g and g/kg of BW) did not differ among treatments (Table 7.5). Duodenal mucosal mass (g and g/kg of BW) tended to increase ($P = 0.10$) with exogenous GLP-2 administration. Duodenal mucosal DNA concentration, total DNA content, and protein concentration were not affected by treatments. Total protein content and protein:DNA of the duodenal mucosa tended to increase ($P = 0.08$) with exogenous GLP-2 administration. Exogenous GLP-2 administration tended to increase ($P \leq 0.08$) duodenal maltase and glucoamylase activities

per gram of duodenal mucosa, but not when expressed per gram of protein. Total maltase and glucoamylase activity in the duodenal mucosa increased ($P \leq 0.04$) with exogenous GLP-2 administration. Isomaltase activity in the duodenal mucosa was not affected by postruminal casein infusion or exogenous GLP-2 administration. Exogenous GLP-2 administration increased ($P \leq 0.05$) α-glucosidase activity per gram of duodenal mucosa and total α-glucosidase activity of the duodenal mucosa.

7.3.5 *Jejunal mass, cellularity, and α-glucosidase activity*

Postruminal casein infusion and exogenous GLP-2 administration increased ($P \leq 0.03$) jejunal mass (g and g/kg of BW) and mass of the jejunal mucosa (Table 7.6). Jejunal DNA concentration was not affected by GLP-2 or casein. Jejunal protein concentration tended to increase ($P = 0.09$) with casein infusion. Postruminal casein infusion and exogenous GLP-2 administration tended to increase ($P \leq 0.10$) the total DNA content of the jejunal mucosa and increase ($P < 0.01$) the total protein content of the jejunal mucosa. The activity of α-glucosidase per gram jejunal mucosa and individual activities of maltase, isomaltase, and glucoamylase were not influenced by GLP-2 or casein. Glucagon-like peptide 2 administration decreased ($P = 0.04$) the protein:DNA of the jejunal mucosa. Exogenous GLP-2 administration increased ($P < 0.01$) total α-glucosidase activity of the jejunal mucosa because of increased ($P \leq 0.01$) total maltase, isomaltase, and glucoamylase activity.

7.3.6 *Ileal mass, cellularity, and α-glucosidase activity*

Exogenous GLP-2 administration increased ($P < 0.01$) ileal mass and ileal mucosal mass (g and g/kg of BW) (Table 7.7). Ileal DNA and protein concentration of the mucosa were not different among treatments. Exogenous GLP-2 administration tended to increase ($P = 0.07$) the

total DNA content of the ileal mucosa and increase ($P < 0.01$) the total protein content of the ileal mucosa. Ileal protein:DNA was not affected by treatments. There were casein × GLP-2 interactions ($P \leq 0.05$) for ileal maltase and glucoamylase activities because exogenous GLP-2 increased ileal maltase and glucoamylase activity for steers infused with water but, not for steers infused with casein. Exogenous GLP-2 increased ($P = 0.04$) total isomaltase activity of the ileal mucosa. There were casein × GLP-2 interactions ($P \leq 0.05$) for ileal α-glucosidase activities because exogenous GLP-2 injections increased ileal α-glucosidase activity for steers infused with water but, not for steers infused with casein.

7.4 Discussion

Small intestinal starch digestion in ruminants is potentially limited by inadequate pancreatic α-amylase and/or small intestinal α-glucosidase activity (Owens et al., 1986; Harmon and Swanson, 2020; Trotta et al., 2022). The purpose of this experiment was to evaluate the potential limitations of small intestinal starch digestibility by using postruminal casein infusion and exogenous GLP-2 administration to stimulate pancreatic and small intestinal mucosal growth. The hypothesis was that increased pancreatic and small intestinal mucosal growth via postruminal casein infusion and exogenous GLP-2 administration would result in increased pancreatic α-amylase activity and increased small intestinal α-glucosidase activities. In turn, achieving different endogenous pancreatic and small intestinal carbohydrase activity profiles could be useful to evaluate the limitations of small intestinal starch hydrolysis. The findings of the current study describe a novel *in vivo* animal model which resulted in four distinct carbohydrase activity phenotypes related to small intestinal starch digestion. This model will be useful to overcome complications of coordinating endogenous carbohydrase activity with luminal nutrient flows in ruminants. Because four distinct carbohydrase activity phenotypes were achieved, future research

can use this *in vivo* model to evaluate the effects of increasing endogenous pancreatic and/or small intestinal carbohydrase activity on small intestinal starch digestion.

In most mammals, pancreatic α-amylase initially breaks down large chains of amylose and amylopectin into starch oligosaccharides and brush-border maltase-glucoamylase and sucrase-isomaltase hydrolyze short-chain oligosaccharides and disaccharides to glucose (Shirazi-Beechey et al., 1995). However, several points of evidence complicate this simplistic overview of small intestinal starch assimilation. Regulation of postruminal digestive enzyme activity in cattle is complex (Swanson et al., 2000a) because there are numerous neural, hormonal, and feedback signaling mechanisms involved in digestive enzyme synthesis and secretion (Liddle, 2018). Comparisons between optimized enzyme activity measurements from tissues in the laboratory with actual enzymatic activities occurring in the small intestine are difficult as the conditions within the lumen of the small intestine differ between individual animals and with changes in nutrition. Changes in carbohydrase activities do not necessarily relate to the quantity of starch digested as other factors, at least at times, could be contributing to changes in luminal starch hydrolysis. A complicating factor in understanding the nutritional effects on mucosal carbohydrase activities is that the proteins (maltase-glucoamylase and sucrase-isomaltase) exhibiting carbohydrase activity have affinity towards more than one substrate. For example, approximately 80% of the apparent maltase activity is derived from sucrase-isomaltase and the remaining 20% is derived from maltase-glucoamylase in humans and mice (Galand, 1989; Lin et al., 2012). In humans, maltase-glucoamylase is the primary protein exhibiting α-glucosidase activity at low substrate concentrations while sucrase-isomaltase is the primary protein exhibiting α-glucosidase activity at high substrate concentrations (Quezada-Calvillo et al., 2007). Others have suggested that the opposite response may occur in ruminants, based of a lower K_m value for isomaltase compared to

maltase in jejunal homogenates from cattle (Smith et al., 2020). The absence of mucosal sucrase activity in ruminants further complicates our understanding of mucosal carbohydrase activity and suggests that regulation of carbohydrase activity and starch digestion in ruminants might be similar to humans with phenotype V of congenital sucrase-isomaltase deficiency (Trotta et al., 2022). Congential sucrase-isomaltase deficiency can originate from impaired trafficking of sucrase-isomaltase to the apical membrane and it has been recently suggested that impaired sucrase-isomaltase trafficking might also impair maltase-glucoamylase trafficking, leading to an overall decrease in the capacity for intestinal maltose digestion (Tannous et al., 2023). Taken together, these findings question the ability of ruminant carbohydrases to adapt to high luminal substrate concentrations, corresponding with data demonstrating that small intestinal starch disappearance (% of intestinal starch flow) decreases with increasing intestinal starch flow (Huntington et al., 2006). Further research is needed to evaluate these concepts to better understand complex relationships between luminal carbohydrate supply, carbohydrase activity, and carbohydrate digestion in ruminants.

In ruminants, pancreatic and small intestinal carbohydrase activities respond to changes in energy intake and changes in tissue mass (Kreikemeier et al., 1990b; Wang et al., 1998; Swanson et al., 2002a; Górka et al., 2017; Trotta et al., 2020d). In addition to changes in tissue mass, possible mechanisms for changes in digestive enzyme activity include changes in transcription, protein synthesis or secretions, or posttranslational modifications (Wang et al., 1998; Swanson et al., 2000a; Swanson et al., 2002a; Cao et al., 2018; Guo et al., 2018b; Guo et al., 2019; Guo et al., 2020). Past research and findings of the current study demonstrate that postruminal casein infusion can increase pancreatic mass (Swanson et al., 2002a) and that exogenous GLP-2 administration can increase small intestinal mass in cattle (Taylor-Edwards et al., 2011). Swanson et al. (2008)

found that increasing levels of dietary protein intake increased pancreatic DNA concentration and tended to decrease pancreatic protein:DNA, suggesting increasing protein intake resulted in pancreatic cellular hypoplasia and hypertrophy. Exogenous GLP-2 increased small intestinal mass and 84% of the increase in small intestinal mass could be accounted for through increases in small intestinal mucosal mass compared with control. This agrees with observations by McLeod and Baldwin (2000) who suggested that 90% of increased small intestinal mass could be accounted for through increases in mucosal mass. Exogenous GLP-2 increased jejunal DNA content by 32.6% and decreased jejunal protein:DNA by 34.3%, which are similar to findings by Taylor-Edwards et al. (2011) and suggest that exogenous GLP-2 stimulated hypertrophic growth of the small intestinal mucosa and that maturation of cells in the small intestinal mucosa may not have been complete.

Because of the pregastric fermentation of nutrients in the rumen, ruminants have substantial differences in carbohydrate, nitrogen, and lipid flows to the duodenum compared with nonruminants (Merchen, 1988; Swanson, 2019). Differences in duodenal digesta composition between ruminants and nonruminants could potentially contribute to changes in digestive and absorptive functions in the small intestine because regulation of digestion and absorption can be coordinated through luminal nutrient flows (Ferraris and Diamond, 1989). In nonruminants, digestive enzyme activity typically increases proportionally to luminal substrate supply (Brannon, 1990). Increasing postruminal carbohydrate supply as raw corn starch (Wang and Taniguchi, 1998), partially hydrolyzed corn starch (Walker and Harmon, 1995; Swanson et al., 2002a; Swanson et al., 2002b; Swanson et al., 2004a), and glucose (Swanson et al., 2002b) had been shown to decrease pancreatic α-amylase activity in ruminants. In contrast, increasing dietary protein intake (Kreikemeier et al., 1990b; Swanson et al., 2008; Lee et al., 2013), postruminal

casein supply (Wang and Taniguchi, 1998; Swanson et al., 2002a; Richards et al., 2003; Swanson et al., 2003; Swanson et al., 2004a; Trotta et al., 2020c), or postruminal amino acid supply as Ile (Liu et al., 2018), Leu (Liu et al., 2015; Cao et al., 2018), Phe (Yu et al., 2013; Liu et al., 2015; Cao et al., 2018), or Trp (Lee et al., 2020) had been shown to increase pancreatic α-amylase activity in ruminants.

In the current study, postruminal casein infusion increased pancreatic α-amylase activity per gram pancreas, activity per gram protein, and total activity. Mechanisms detailing complex interactions between luminal starch and protein on pancreatic α-amylase activity are not well described but may involve gastrointestinal hormones such as cholecystokinin (Swanson et al., 2003; Swanson et al., 2004a; Relling and Reynolds, 2008; Lee et al., 2013; Brake et al., 2014b; Lee et al., 2020), secretin (Lee et al., 2013), ghrelin (Zhang et al., 2001; Dembinski et al., 2005; Relling and Reynolds, 2008; Lee et al., 2013; Shen et al., 2020), insulin (Pierzynowski and Barej, 1984), melatonin (Keomanivong et al., 2016; Lee et al., 2020; Trotta et al., 2021c), as well as, stimuli from neural or feedback mechanisms. Further research is needed to understand how luminal nutrient flow influences signaling mechanisms between gastrointestinal and peripheral tissues to coordinate changes in pancreatic α-amylase activity and secretion.

Understanding the effects of nutrition on small intestinal α-glucosidase activities in ruminants is less clear than pancreatic α-amylase because there are differing responses depending on animal species, substrate, site of infusion, and length of adaptation. In general, studies have demonstrated that increasing the size of the small intestine, either by increasing intestinal mass or length, can result in increased α-glucosidase activity (Kreikemeier et al., 1990b; Wang et al., 1998; Górka et al., 2017). Górka et al. (2017) noted that the length of adaptation to luminal nutrient supply was an important factor affecting the regulation of small intestinal α-glucosidase activity

in cattle. Short-term postruminal infusion (≤ 10 d) of partially hydrolyzed starch (Bauer et al., 2001a; Guimaraes et al., 2007) or casein (Guimaraes et al., 2007) did not influence jejunal maltase activity in cattle. In support of this concept, abomasal infusion of casein for 7 d did not influence maltase, isomaltase, or glucoamylase activity in the duodenum, jejunum, or ileum in the current study. However, long-term postruminal infusion (≥ 35 d) of partially hydrolyzed starch, glucose, or raw corn starch with casein influenced jejunal α-glucosidase activity in cattle (Rodriguez et al., 2004; Trotta et al., 2020c). Abomasal infusion of partially hydrolyzed starch or glucose increased jejunal maltase activity compared with isoenergetic infusion of partially hydrolyzed starch in the rumen or a negative control (water) (Rodriguez et al., 2004). Those authors suggested that increasing luminal carbohydrate flow as starch or glucose increased jejunal maltase activity and that increasing energy intake did not (Rodriguez et al., 2004). Duodenal infusion of casein for 58 d increased jejunal maltase, isomaltase, and glucoamylase activity (Trotta et al., 2020c). Those authors suggested that casein increased luminal substrate flow because casein increased pancreatic α-amylase activity which might have facilitated greater amylose and amylopectin hydrolysis to maltose, isomaltose, and limit-dextrins (Trotta et al., 2020c). This concept was supported by increased ileal flows of ethanol-soluble oligosaccharides when casein was infused postruminally, indicating greater amylolytic activity (Brake et al., 2014a; Brake et al., 2014b; Acharya et al., 2023). These results collectively suggest that ruminant small intestinal α-glucosidase activities adapt to long-term changes in luminal substrate flow and with changes in small intestinal size.

In the current study, GLP-2 and casein + GLP-2 increased total small intestinal α-glucosidase activity by 83.5% compared with control. Total small intestinal mucosal maltase, isomaltase, and glucoamylase activity was 90%, 100%, and 66.7% greater for GLP-2 and casein + GLP-2 steers compared with control. Although a large portion of the increase in total small

intestinal α-glucosidase activity with GLP-2 could be accounted for by increasing mucosal hypertrophy, exogenous GLP-2 also affected α-glucosidase concentrations. Small intestinal maltase, isomaltase, and glucoamylase activity per gram mucosa were 63%, 37%, and 45% greater for GLP-2 steers compared with control, respectively. In neonatal pigs, exogenous GLP-2 administration was shown to increase maltase-glucoamylase and sucrase-isomaltase mRNA expression, as well as, maltase and sucrase activities (Petersen et al., 2002). In ruminants, feeding an artificial sweetener containing saccharin and neohesperidin dihydrochalcone activated the sweet taste receptor T1R2-T1R3, which stimulated endogenous GLP-2 secretion, leading to increased villus growth, SGLT1 protein abundance and Na^+-dependent glucose uptake, and maltase activity (Moran et al., 2014). The results of the current experiment demonstrate that exogenous GLP-2 administration increases small intestinal α-glucosidase activity in cattle without affecting pancreatic α-amylase activity.

Postruminal casein infusion increases small intestinal starch disappearance in cattle (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016; Acharya et al., 2023) and sheep (Mabjeesh et al., 2003; Mendoza and Britton, 2003). In the current study, postruminal casein infusion increased postruminal starch disappearance, decreased fecal DM and starch excretion, and decreased fecal pH, supporting the findings of previous research. Although previous experiments have demonstrated that postruminal casein infusion increases pancreatic α-amylase activity and small intestinal starch disappearance, it does not necessarily imply a cause and effect relationship. Increases in pancreatic α-amylase activity with postruminal casein infusion are not directly proportional to increases in small intestinal starch disappearance (Richards et al., 2002; Richards et al., 2003; Trotta et al., 2020c; Acharya et al., 2023). Direct infusion of exogenous α-amylase activity in the abomasum or small intestinal lumen has not resulted in increased small

intestinal starch disappearance in cattle (Remillard et al., 1990; Westreicher-Kristen et al., 2018; Robbers et al., 2019).

Although small intestinal starch digestion was not measured in the current study, there are factors within the luminal environment that might influence effects of casein on starch hydrolysis including digestion of other nutrients, stimulation of microbial activity, changes in small intestinal passage rate, and/or chemical interactions between starch and protein structures. Decreases in fecal starch excretion with postruminal casein infusion make up approximately 52% of decreases in fecal DM excretion (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b), indicating that postruminal casein infusion also increases postruminal digestibility of protein and fiber (Richards et al., 2002; Mabjeesh et al., 2003; Acharya et al., 2023). It is possible that overall increases in small intestinal DM digestibility alter digesta viscosity in the lumen of the small intestine and/or allow for greater access of carbohydrases to their respective substrates. Although fecal excretion of VFA was small in the current study, decreased fecal pH may suggest that it is possible that postruminal casein supply stimulated microbial fermentation of starch and/or starch digestion products in the small intestine (Gilbert et al., 2015; Harmon and Swanson, 2020; Shen et al., 2020). Previous research has shown that duodenal infusion of casein did not affect small intestinal passage rate in cattle (Brake et al., 2014b) but, feeding casein increased gastric emptying and the rate of passage in the proximal small intestine of pigs (Shen et al., 2020). Additionally, emulsification properties of casein may influence mixing in the intestinal lumen, accessibility for luminal enzymatic hydrolysis, and interactions between protein and starch chemical structures (Chen et al., 2023). A greater understanding of physiological mechanisms contributing to increased carbohydrase activity and small intestinal starch digestion are needed to develop strategies to optimize starch digestibility in ruminants.

7.5 Conclusions

Postruminal infusion of casein increased pancreatic mass and exogenous GLP-2 increased small intestinal mass. The use of postruminal casein infusion and exogenous GLP-2 administration resulted in four distinct carbohydrase activity phenotypes. Relative to control, casein increased pancreatic α-amylase activity, GLP-2 increased small intestinal α-glucosidase activity, and the combination of casein and GLP-2 increased both pancreatic α-amylase activity and small intestinal α-glucosidase activity. This approach provides a novel *in vivo* animal model to selectively increase endogenous carbohydrase activity to overcome complications of coordinating carbohydrase activity with luminal nutrient flows. This model could be useful to understand physiological mechanisms that might improve small intestinal starch assimilation and efficiency of cattle consuming high-grain diets.

Table 7.1 Composition of the basal alfalfa cube-based diet fed to steers.[1,2]

Item	
Ingredient composition, DM basis	
Alfalfa hay cubes, % of DM	82.9
Finely ground corn, % of DM	16.2
Trace mineral premix, % of DM[1]	0.45
Vitamin premix, % of DM[2]	0.45
Chemical composition	
Dry matter, %	89.9
Neutral detergent fiber, % of DM	40.7
Acid detergent fiber, % of DM	33.3
Crude protein, % of DM	15.4
Total starch, % of DM	13.8
Ca, % of DM	1.81
P, % of DM	0.21
Net energy for maintenance, Mcal/kg	1.32
Net energy for gain, Mcal/kg	0.75

[1]Contained (per kg of DM) : 960 g salt, 9.28 g Fe, 5.5 g Zn, 4.79 g Mn, 1.84 g Cu, 115 mg I, 65 mg Co, 18 mg Se.

[2]Contained (per kg of DM): 1818 kIU vitamin A, 364 kIU vitamin D_3, and 227 IU vitamin E.

Table 7.2 Effects of postruminal casein infusion and exogenous GLP-2 administration on fecal flow, fecal nutrient excretion, and postruminal starch disappearance in steers abomasally infused with corn starch.[1]

	Treatment							
	Water		Casein			*P*-value		
Item	BSA	GLP-2	BSA	GLP-2	SEM[1]	Casein	GLP-2	Casein × GLP-2
Initial shrunk BW, kg	240	240	239	242	4.34	0.95	0.68	0.78
BW at slaughter, kg	247	250	253	251	3.85	0.37	0.95	0.59
Abomasal infusion								
Infusate, g/h	263	263	254	258	7.25	0.28	0.74	0.78
Starch infused, g/h	39.9	39.8	38.7	39.0	1.09	0.32	0.89	0.86
Fecal pH	6.23	6.23	6.12	5.94	0.0812	0.04	0.28	0.32
Fecal DM, %	21.8	22.0	18.9	21.1	0.705	<0.01	0.07	0.13
Fecal starch, % of DM	4.32	4.35	0.472	1.52	1.15	<0.01	0.59	0.62
Fecal flow								
Total flow, g/h	134	132	130	125	3.07	0.07	0.19	0.49
Fluid flow, g/h	105	103	106	98.4	2.57	0.43	0.06	0.23
DM flow, g/h	29.1	29.1	24.7	26.5	1.16	<0.01	0.38	0.38
Fecal excretion								
Starch, g/h	1.19	1.33	0.156	0.452	0.337	<0.01	0.47	0.79
Glucose, g/h	0.162	0.158	0.0526	0.229	0.0984	0.83	0.36	0.33

Table 7.2 (continued)

Volatile fatty acids, g/h	0.942	1.06	0.750	0.892	0.101	0.09	0.20	0.87
Postruminal starch disappearance, % of infused	96.9	96.6	99.6	99.0	0.856	<0.01	0.55	0.81

[1]Steers were abomasally infused with 3.94 g raw corn starch/kg BW per day with either water or 10% sodium caseinate solution to provide 1.30 g casein/kg BW per day. Steers received subcutaneous injections of vehicle or 100 ug GLP-2/kg of BW per day.

[2]Standard error of the mean (n = 5 for casein).

[3]Sum of fecal acetate, propionate, and butyrate excretion.

Table 7.3 Effects of postruminal casein infusion and exogenous GLP-2 administration on pancreatic mass, cellularity, and α-amylase activity in steers abomasally infused with corn starch.[1]

	Treatment							
	Water		Casein			*P*-value		
Item	BSA	GLP-2	BSA	GLP-2	SEM[1]	Casein	GLP-2	Casein × GLP-2
Pancreatic mass								
Absolute mass, g	268	262	305	292	13.7	0.03	0.51	0.78
Mass relative to BW, g/kg of BW	1.07	1.06	1.21	1.16	0.0598	0.06	0.61	0.76
Pancreatic cellularity								
DNA concentration, mg/g pancreas	10.1	10.1	9.46	9.29	0.776	0.35	0.94	0.89
Total DNA content, g	2.74	2.64	2.90	2.71	0.256	0.67	0.58	0.86
Protein concentration, mg/g pancreas	106	101	109	111	4.82	0.20	0.72	0.47
Total protein content, g	28.0	26.6	33.3	32.3	2.18	0.02	0.59	0.92
Protein:DNA	10.8	10.8	11.7	12.1	1.24	0.39	0.91	0.88
Pancreatic α-amylase activity								
Activity per gram pancreas, U/g pancreas	96.9	96.5	131	126	12.1	0.02	0.84	0.86
Activity per gram protein, kU/g protein	0.922	0.942	1.19	1.14	0.0938	0.03	0.84	0.68
Total activity, kU	25.8	25.5	39.9	37.0	4.39	0.01	0.72	0.77

[1]Steers were abomasally infused with 3.94 g raw corn starch/kg BW per day with either water or 10% sodium caseinate solution to provide 1.30 g casein/kg BW per day. Steers received subcutaneous injections of vehicle or 100 ug GLP-2/kg of BW per day.

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

Table 7.4 Effects of postruminal casein infusion and exogenous GLP-2 administration on small intestinal mass, cellularity, and α-glucosidase activity in steers abomasally infused with corn starch.[1]

Item	Treatment				SEM[1]	*P*-value		
	Water		Casein					
	BSA	GLP-2	BSA	GLP-2		Casein	GLP-2	Casein × GLP-2
Small intestinal mass								
Absolute mass, kg	4.61	5.51	4.81	6.32	0.176	0.01	<0.01	0.10
Absolute mucosal mass, kg	2.43	3.19	2.61	3.64	0.122	0.02	<0.01	0.29
Mass relative to BW, g/kg of BW	18.7	22.3	19.0	25.1	0.774	0.07	<0.01	0.13
Mucosal mass relative to BW, g/kg of BW	9.82	12.9	10.3	14.4	0.512	0.07	<0.01	0.32
Mucosa, % of absolute mass	52.4	57.8	54.1	57.6	1.08	0.52	<0.01	0.39
Small intestinal length, m	35.8[a]	37.1[a]	36.4[a]	40.3[b]	0.602	<0.01	<0.01	0.05
Small intestinal mass:length, g/cm	1.35	1.48	1.39	1.45	0.0699	0.87	0.19	0.61
Small intestinal cellularity								
DNA concentration, mg/g small intestinal mucosa	11.2	11.6	12.5	11.5	1.19	0.60	0.80	0.58
Total DNA content, g	26.7	36.7	32.4	41.8	3.87	0.18	0.02	0.94
Protein concentration, mg/g small intestinal mucosa	69.6	73.5	73.5	73.7	1.84	0.29	0.28	0.34
Total protein content, g	169	234	193	268	12.2	0.02	<0.01	0.71
Protein:DNA	6.44	6.81	6.00	6.98	0.667	0.84	0.33	0.66

Table 7.4 (continued)

Small intestinal maltase activity								
Activity per gram mucosa, U/g	0.344[a]	0.561[b]	0.369[a]	0.376[ab]	0.0464	0.11	0.03	0.04
Activity per gram protein, U/g protein	4.96[a]	7.64[b]	5.01[a]	5.08[a]	0.622	0.06	0.04	0.05
Total activity, kU	0.825[a]	1.78[c]	0.990[ab]	1.36[bc]	0.125	0.33	<0.01	0.03
Small intestinal isomaltase activity								
Activity per gram mucosa, U/g	0.192	0.263	0.269	0.262	0.0255	0.26	0.23	0.15
Activity per gram protein, U/g protein	2.78	3.57	3.64	3.58	0.373	0.26	0.34	0.27
Total activity, kU	0.452	0.853	0.720	0.952	0.0853	0.05	<0.01	0.34
Small intestinal glucoamylase activity								
Activity per gram mucosa, U/g	0.309[ab]	0.447[b]	0.329[ab]	0.299[a]	0.0366	0.10	0.16	0.04
Activity per gram protein, U/g protein	4.46	6.10	4.50	4.05	0.501	0.06	0.25	0.06
Total activity, kU	0.750[a]	1.42[b]	0.876[a]	1.08[ab]	0.106	0.32	<0.01	0.04
Small intestinal α-glucosidase activity								
Activity per gram mucosa, U/g	0.846[a]	1.27[b]	0.967[ab]	0.937[ab]	0.0910	0.26	0.05	0.02
Activity per gram protein, U/g protein	12.2[a]	17.3[b]	13.2[ab]	12.7[ab]	1.25	0.16	0.08	0.04
Total activity, kU	2.03[a]	4.05[c]	2.59[ab]	3.39[bc]	0.265	0.85	<0.01	0.04

[1]Steers were abomasally infused with 3.94 g raw corn starch/kg BW per day with either water or 10% sodium caseinate solution to provide 1.30 g casein/kg BW per day. Steers received subcutaneous injections of vehicle or 100 ug GLP-2/kg of BW per day.

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

Table 7.5 Effects of postruminal casein infusion and exogenous GLP-2 administration on duodenal mass, cellularity, and α-glucosidase activity in steers abomasally infused with corn starch.[1]

	Treatment							
	Water		Casein			*P*-value		
Item	BSA	GLP-2	BSA	GLP-2	SEM[1]	Casein	GLP-2	Casein × GLP-2
Duodenal mass								
Absolute mass, g	206	212	198	241	14.8	0.49	0.12	0.22
Mass relative to BW, g/kg of BW	0.835	0.859	0.784	0.962	0.0650	0.70	0.14	0.25
Absolute mucosal mass, g	71.2	82.9	75.7	97.4	9.44	0.33	0.10	0.61
Mucosal mass relative to BW, g/kg of BW	0.287	0.338	0.300	0.391	0.0406	0.43	0.10	0.63
Mucosa, % of absolute mass	34.3	39.0	38.0	39.5	2.32	0.38	0.20	0.51
Duodenal cellularity								
DNA concentration, mg/g duodenal mucosa	11.2	9.98	11.4	12.4	0.843	0.14	0.89	0.22
Total DNA content, g	0.815	0.842	0.860	1.22	0.139	0.15	0.19	0.26
Protein concentration, mg/g duodenal mucosa	66.1	72.1	65.4	70.3	3.65	0.74	0.15	0.88
Total protein content, g	4.63	6.08	4.89	7.14	0.993	0.51	0.08	0.69
Protein:DNA	6.01	7.60	5.83	6.10	0.492	0.11	0.08	0.20
Duodenal maltase activity								
Activity per gram mucosa, U/g	0.0795	0.117	0.0754	0.101	0.0157	0.53	0.06	0.71

Table 7.5 (continued)

Activity per gram protein, U/g protein	1.21	1.66	1.16	1.44	0.218	0.56	0.11	0.70
Total activity, U	6.43	9.69	5.69	10.4	1.63	0.99	0.03	0.66
Duodenal isomaltase activity								
Activity per gram mucosa, U/g	0.161	0.179	0.154	0.139	0.0216	0.30	0.95	0.46
Activity per gram protein, U/g protein	2.49	2.45	2.35	2.00	0.366	0.43	0.60	0.67
Total activity, U	11.5	14.9	11.2	13.0	2.34	0.64	0.29	0.74
Duodenal glucoamylase activity								
Activity per gram mucosa, U/g	0.190	0.266	0.209	0.244	0.0296	0.95	0.08	0.50
Activity per gram protein, U/g protein	2.97	3.69	3.20	3.48	0.448	0.98	0.28	0.64
Total activity, U	13.7	22.9	15.8	23.3	3.64	0.74	0.04	0.81
Duodenal α-glucosidase activity								
Activity per gram mucosa, U/g	0.430	0.561	0.438	0.483	0.0403	0.40	0.05	0.30
Activity per gram protein, U/g protein	6.67	7.80	6.71	6.92	0.649	0.53	0.32	0.49
Total activity, U	31.6	47.5	32.6	46.6	6.40	0.99	0.03	0.88

[1]Steers were abomasally infused with 3.94 g raw corn starch/kg BW per day with either water or 10% sodium caseinate solution to provide 1.30 g casein/kg BW per day. Steers received subcutaneous injections of vehicle or 100 ug GLP-2/kg of BW per day.

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

Table 7.6 Effects of postruminal casein infusion and exogenous GLP-2 administration on jejunal mass, cellularity, and α-glucosidase activity in steers abomasally infused with corn starch.[1]

	Treatment							
	Water		Casein			*P*-value		
Item	BSA	GLP-2	BSA	GLP-2	SEM[1]	Casein	GLP-2	Casein × GLP-2
Jejunal mass								
Absolute mass, kg	1.81	2.25	2.01	2.84	0.139	0.01	<0.01	0.21
Absolute mucosal mass, kg	1.02	1.43	1.17	1.76	0.0838	0.01	<0.01	0.27
Mass relative to BW, g/kg of BW	7.32	9.11	7.92	11.3	0.589	0.03	<0.01	0.24
Mucosal mass relative to BW, g/kg of BW	4.12	5.80	4.57	7.02	0.359	0.03	<0.01	0.30
Mucosa, % of total mass	56.3	61.5	57.5	62.0	1.30	0.53	<0.01	0.80
Jejunal cellularity								
DNA concentration, mg/g jejunal mucosa	11.2	11.2	13.1	11.2	1.67	0.58	0.58	0.58
Total DNA content, g	11.5	15.8	15.2	19.6	2.14	0.10	0.06	0.97
Protein concentration, mg/g jejunal mucosa	73.0	74.0	81.5	78.0	3.41	0.09	0.72	0.52
Total protein content, g	74.8	105	96.5	137	8.73	<0.01	<0.01	0.56
Protein:DNA	7.59	5.06	6.57	4.24	1.05	0.39	0.04	0.93
Jejunal maltase activity								
Activity per gram mucosa, U/g	0.335	0.496	0.378	0.423	0.0664	0.82	0.14	0.40
Activity per gram protein, U/g protein	4.53	6.67	4.69	5.35	0.808	0.48	0.10	0.38

Table 7.6 (continued)

Total activity, kU	0.341	0.684	0.457	0.741	0.0916	0.36	<0.01	0.75
Jejunal isomaltase activity								
Activity per gram mucosa, U/g	0.194	0.236	0.217	0.224	0.0255	0.84	0.34	0.51
Activity per gram protein, U/g protein	2.65	3.21	2.67	2.85	0.302	0.59	0.24	0.53
Total activity, kU	0.200	0.329	0.261	0.394	0.0361	0.10	<0.01	0.94
Jejunal glucoamylase activity								
Activity per gram mucosa, U/g	0.319	0.394	0.337	0.331	0.0554	0.69	0.53	0.48
Activity per gram protein, U/g protein	4.32	5.30	4.27	4.22	0.726	0.45	0.53	0.49
Total activity, kU	0.326	0.536	0.404	0.585	0.0686	0.37	0.01	0.84
Jejunal α-glucosidase activity								
Activity per gram mucosa, U/g	0.848	1.13	0.931	0.979	0.135	0.82	0.25	0.41
Activity per gram protein, U/g protein	11.5	15.2	11.6	12.4	1.68	0.45	0.20	0.40
Total activity, kU	0.868	1.55	1.12	1.72	0.182	0.26	<0.01	0.83

[1]Steers were abomasally infused with 3.94 g raw corn starch/kg BW per day with either water or 10% sodium caseinate solution to provide 1.30 g casein/kg BW per day. Steers received subcutaneous injections of vehicle or 100 ug GLP-2/kg of BW per day.

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

Table 7.7 Effects of postruminal casein infusion and exogenous GLP-2 administration on ileal mass, cellularity, and α-glucosidase activity in steers abomasally infused with corn starch.[1]

	Treatment							
	Water		Casein			*P*-value		
Item	BSA	GLP-2	BSA	GLP-2	SEM[1]	Casein	GLP-2	Casein × GLP-2
Ileal mass								
Absolute mass, kg	2.60	2.94	2.60	3.23	0.108	0.18	<0.01	0.17
Absolute mucosal mass, kg	1.33	1.68	1.37	1.78	0.0729	0.37	<0.01	0.67
Mass relative to BW, g/kg of BW	10.5	11.9	10.3	12.8	0.479	0.49	<0.01	0.20
Mucosal mass relative to BW, g/kg of BW	5.41	6.77	5.42	7.03	0.285	0.64	<0.01	0.67
Mucosa, % of total mass	51.2	56.4	52.9	54.9	1.84	0.95	0.07	0.40
Ileal cellularity								
DNA concentration, mg/g ileal mucosa	11.3	12.0	12.1	11.8	1.70	0.87	0.92	0.78
Total DNA content, g	14.3	20.0	16.4	21.0	2.67	0.58	0.07	0.84
Protein concentration, mg/g ileal mucosa	67.4	72.7	67.0	69.3	2.38	0.44	0.13	0.55
Total protein content, g	89.1	123	92.1	124	7.98	0.81	<0.01	0.90
Protein:DNA	6.86	6.60	5.95	6.71	0.871	0.65	0.77	0.57
Ileal maltase activity								
Activity per gram mucosa, U/g	0.357[ab]	0.631[b]	0.378[ab]	0.338[a]	0.0705	0.07	0.12	0.04
Activity per gram protein, U/g protein	5.33[ab]	8.70[b]	5.63[ab]	4.71[a]	0.968	0.08	0.22	0.04

Table 7.7 (continued)

Total activity, kU	0.477[a]	1.08[b]	0.527[a]	0.608[ab]	0.120	0.10	0.01	0.05
Ileal isomaltase activity								
Activity per gram mucosa, U/g	0.189	0.291	0.323	0.305	0.0472	0.14	0.38	0.23
Activity per gram protein, U/g protein	2.80	3.91	4.78	4.46	0.712	0.10	0.59	0.33
Total activity, kU	0.240	0.510	0.449	0.544	0.0833	0.17	0.04	0.31
Ileal glucoamylase activity								
Activity per gram mucosa, U/g	0.305[a]	0.496[b]	0.330[ab]	0.265[a]	0.0416	0.03	0.15	<0.01
Activity per gram protein, U/g protein	4.56[a]	6.81[b]	4.91[ab]	3.80[a]	0.514	0.02	0.29	<0.01
Total activity, kU	0.410[a]	0.863[b]	0.457[a]	0.471[a]	0.0823	0.05	0.01	0.02
Ileal α-glucosidase activity								
Activity per gram mucosa, U/g	0.851[a]	1.42[b]	1.03[ab]	0.908[a]	0.121	0.19	0.09	0.01
Activity per gram protein, U/g protein	12.7[a]	19.4[b]	15.3[ab]	13.0[ab]	1.59	0.25	0.19	0.01
Total activity, kU	1.13[a]	2.46[b]	1.43[a]	1.62[ab]	0.234	0.28	<0.01	0.03

[1]Steers were abomasally infused with 3.94 g raw corn starch/kg BW per day with either water or 10% sodium caseinate solution to provide 1.30 g casein/kg BW per day. Steers received subcutaneous injections of vehicle or 100 ug GLP-2/kg of BW per day.

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

CHAPTER 8. INFLUENCE OF POSTRUMINAL CASEIN INFUSION AND EXOGENOUS GLUCAGON-LIKE PEPTIDE 2 ADMINISTRATION ON THE JEJUNAL MUCOSAL TRANSCRIPTOME IN CATTLE

8.1 Introduction

Decades of research evaluating the effects of exogenous GLP-2 administration in mammals has shown positive effects on small intestinal mucosal growth (Drucker et al., 1996; Brubaker et al., 1997; Tsai et al., 1997a; Tsai et al., 1997b; Burrin et al., 2000; Burrin et al., 2005; Taylor-Edwards et al., 2011), digestive enzyme activity (Brubaker et al., 1997; Petersen et al., 2002), nutrient absorption (Cheeseman and Tsang, 1996; Brubaker et al., 1997; Cheeseman, 1997; Cheeseman and O'Neill, 1998; Kato et al., 1999; Au et al., 2002), barrier function (Benjamin et al., 2000; Walker et al., 2015), and mesenteric artery and hepatic portal blood flow (Guan et al., 2003; Guan et al., 2006; Stephens et al., 2006; Taylor-Edwards et al., 2011; Taylor-Edwards et al., 2012). Cellular and molecular mechanisms describing GLP-2-mediated physiological responses have been investigated. However, Drucker (2019) noted that much of the early research investigating physiological effects of GLP-2 was narrow in focus, and thus, may not have completely described mechanisms associated with GLP-2-mediated physiological responses.

Similarly, decades of research evaluating the effects of postruminal casein infusion in ruminants has shown positive effects on feed intake (Khalili and Huhtanen, 2002; Martineau et al., 2016), ruminal digestion of low-quality forages (Bandyk et al., 2001; Wickersham et al., 2004), small intestinal starch digestion (Richards et al., 2002; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016), glucose and amino acid absorption (Guerino et al., 1991a; Taniguchi et al., 1995; Mabjeesh et al., 2003), muscle cell proliferation and turnover (Reecy et al., 1996), energy and nitrogen retention (Little and

Mitchell Jr, 1967; Derrig et al., 1974; Clark, 1975; Beermann et al., 1991; Swanson et al., 2004b; Ardalan et al., 2022; Acharya et al., 2023), and milk production and composition (Derrig et al., 1974; Clark, 1975; Cohick et al., 1986; Ardalan et al., 2022). Attempts have been made to identify groups of amino acids (essential, nonessential, brached-chain) or individual amino acids (glutamic acid, arginine) that might have mediated positive responses to postruminal casein infusion (Gow et al., 1979; Titgemeyer and Merchen, 1990; Mackle et al., 2000; Brake et al., 2014a). Attempts to identify relationships among blood hormone concentrations with positive responses to postruminal casein infusion have largely been inconclusive (Clark, 1975; Rodriguez et al., 1985; Cohick et al., 1986; Guerino et al., 1991b; Swanson et al., 2004a). Therefore, as others have previously concluded, the mechanisms associated with the positive effects of postruminal casein infusion are poorly understood (Reynolds et al., 1994).

Targeted approaches to describe cellular and molecular mechanisms are hypothesis-driven and useful when it is known which mechanisms may be associated with *in vivo* responses. To our knowledge, there are no studies that have investigated the jejunal transcriptomic response to intestinal casein supply or exogenous GLP-2 administration using an untargeted approach in any mammalian species. Therefore, the objective of this experiment was to evaluate the effects of postruminal casein infusion and exogenous GLP-2 administration on transcriptomic pathways and functions of the jejunal mucosa using next-generation RNA sequencing. It was hypothesized that RNA sequencing analyses would lead to the discovery of novel pathways affected by postruminal casein infusion and exogenous GLP-2 administration that have not been previously reported and the discovery

of novel pathways which could be associated with already established functions of postruminal casein infusion and exogenous GLP-2 administration.

8.2 Materials and methods

All surgical, animal care, and experimental protocols were approved by the University of Kentucky Animal Care and Use Committee (2020-3479).

8.2.1 *Animals, diet, and experimental design*

The experimental design was previously described in Chapter 7. Briefly, 24 Holstein steers (initial shrunk BW = 240 ± 22.2 kg) were initially housed in individual pens (3 m × 3 m) in the Intensive Research Building of the University of Kentucky C. Oran Little Agricultural Research Center in Versailles, KY. Steers were surgically fitted with infusion catheters in the abomasum that were constructed of Tygon tubing (6.35-mm internal diameter; Saint-Gobain North America, Malvern, PA) (McLeod et al., 2007). Steers were limit-fed 5.65 ± 0.468 kg of an alfalfa hay cube-based diet (82.9% alfalfa hay cubes, 16.2% finely ground corn, 0.45% trace mineral premix, 0.45% vitamin premix; 40.7% neutral detergent fiber, 33.3% acid detergent fiber, 15.4% crude protein; 13.8% total starch, 1.32 Mcal/kg net energy for maintenance) on a dry-matter (DM) basis. The diet was formulated to supply 1.33 times the net energy required for maintenance and exceed requirements for vitamins, minerals, ruminally degradable protein, and metabolizable protein for a steer gaining 0.46 kg/d. Rations were provided twice daily at 0800 and 2000 h in equal portions. Steers were adapted to the basal diet for at least 7 d before the start of the treatment period.

Steers were transferred to metabolism stalls (1.2 m × 2.4 m) for the treatment period (7 d). Due to limited stall space and infusion apparatus, steers were stratified by BW into 6 replicate groups (blocks). Steers received subcutaneous injections in two equal portions daily of either: 5 g/L bovine serum albumin (BSA) diluted in 9 g/L NaCl as a vehicle or 100 µg GLP-2 in vehicle · kg BW^{-1} · d^{-1}. All steers were abomasally infused with 3.94 ± 0.245 g raw corn starch · kg BW^{-1} · d^{-1}. Along with raw corn starch, steers were abomasally infused with either water or 10% wt:wt sodium caseinate solution to provide 0 or 1.30 ± 0.299 g sodium caseinate · kg BW^{-1} · d^{-1}. This resulted in four treatments: 1) abomasal water + vehicle injection (control), 2) abomasal water + GLP-2 injection (GLP-2), 3) abomasal 10% sodium caseinate solution + vehicle injection (casein), and 4) abomasal 10% sodium caseinate solution + GLP-2 injection (casein + GLP-2).

8.2.2 *Tissue collection*

Upon completion of the 7-d infusion period, the infusion tubing was disconnected from abomasal infusion catheters and steers were transported to the University of Kentucky Meats Laboratory within 1 h. Steers were humanely stunned via captive bolt and exsanguinated. After slaughter, the viscera were rapidly removed and 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 2.43-m board (Kreikemeier et al., 1990b). The small intestine was separated into the duodenum (0.1 to 1.1 m caudal to the pyloric sphincter), jejunum (proximal half of non-duodenal

small intestine), and ileum (distal half of non-duodenal small intestine) (Liao et al., 2008). Each intestinal section (duodenum, jejunum, ileum) was weighed and sampled (1 m) from the midpoint. Each 1-m segment was cut into three equidistant subsamples, everted, and rinsed in ice-cold saline. The first subsample was cut laterally, scraped with a glass microscope slide, and 200 mg of mucosa was weighed and preserved with RNAlater stabilization solution (Thermo Scientific Inc., Waltham, MA) in RNase-free tubes and stored at -80°C. The second subsample was scraped as described previously and the mucosa was flash-frozen in liquid nitrogen in an aluminum pouch and stored at -80°C for cellularity and enzymatic analyses. The third subsample was weighed, mucosa scraped, and then the isolated mucosa was weighed.

8.2.3 *RNA isolation, library preparation, and sequencing*

Approximately 200 mg of jejunal mucosal tissue was shipped for RNA extraction, library preparation, and RNA sequencing by Novogene Corporation Inc. (Sacramento, CA). Total RNA was extracted from jejunal mucosal tissue using TRIzol reagent (Thermo Fisher Scientific Inc., Waltham, MA). RNA concentration (1003 ± 863 ng/µL) was measured at 260 nm using a microvolume UV-VIS spectrophotometer (NanoDrop; Thermo Fisher Scientific Inc., Waltham, MA) and purity was assessed using the 260/280 nm ratio for protein contamination and 260/230 nm ratio for nucleic acid contamination. Samples were assessed for RNA integrity (6.6 ± 1.30) using the RNA 6000 Nano Kit (Agilent Technologies Inc., Santa Clara, CA) and automated electrophoresis (2100 Bioanalyzer Instrument; Agilent Technologies Inc., Santa Clara, CA). One sample in the control treatment was not prepared for RNA sequencing because of low RNA integrity.

Strand-specific cDNA library preparation was conducted by Novogene Corporation Inc. (Sacramento, CA). Messenger RNA was isolated from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was facilitated using divalent cations under elevated temperature in First Strand Synthesis Reaction Buffer (5X). First-strand cDNA was synthesized using random hexamer primer and Moloney murine leukemia virus reverse transcriptase and second-strand cDNA was synthesized using DNA polymerase I and RNAse H. Remaining overhangs were converted to blunt ends using exonuclease and polymerase activities. Three-prime ends of DNA fragments were adenylated and adaptors with hairpin loop structures were ligated for hybridization preparation. Library fragments were purified with AMPure XP System (Beckman Coulter Inc., Brea, CA) and 370-420 bp cDNA fragments were selected for PCR amplification. Polymerase chain reaction products were purified and assessed with the 2100 Bioanalyzer Instrument (Agilent Technologies Inc.). Strand-specific RNA sequencing was conducted NovaSeq 6000 Sequencing System (Illumina Inc., San Diego, CA) to generate 150-bp paired-end reads at an average depth of 27,833,203 reads in each direction.

8.2.4 *Bioinformatic and statistical analyses*

Raw reads were filtered to remove sequencing adaptors, low-complexity reads, and reads containing low-quality bases. Quality control scores (Q20 = 96.8 ± 0.370%; Q30 = 91.41 ± 0.831 %) and GC content (47.9 ± 1.25%) were calculated. Paired-end clean reads were aligned to the *Bos taurus* reference genome (ARS-UCD1.2) using HISAT 2 (version 2.0.5) (Kim et al., 2019). Mapped reads of each sample were assembled using StringTie (version 1.3.3b) (Pertea et al., 2015) in a reference-based approach. There was an average of 20,322,229 uniquely mapped reads in each direction per sample. Reads per gene were

counted using featureCounts (version 1.5.0-p3) (Liao et al., 2014). Quantification of each gene was calculated as the number of fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM). Visualization of box plots, violin plots, and FPKM density plots confirmed that gene expression was distributed equally across each steer and that the FPKM density conformed to a negative binomial distribution. Differential gene expression (DEG) analysis was quantified using the DESeq2 R-package (version 1.20) (Love et al., 2014). The DEG analysis used the negative binomial generalized linear model to fit gene expression level as a negative binomial distribution and Wald statistics to perform hypothesis testing. Multiple testing adjustment of the *P*-values was performed using the Benjamini-Hochberg procedure for controlling the false discovery rate (FDR). Genes were identified as differentially expressed if the FDR-adjusted *P*-value was ≤ 0.05. Gene ontology and KEGG pathway enrichment analyses of DEGs were conducted using the clusterProfiler R-package (Yu et al., 2012). Enrichment analyses were considered significant if the FDR-adjusted *P*-value was ≤ 0.05. Changes in gene expression were classified as upregulated or downregulated based on the sign of the log2 fold change. Because postruminal casein infusion did not generate DEG that collectively affected KEGG pathway or gene ontology enrichment, all subsequent data analyses were conducted comparing only the main effect of exogenous GLP-2 administration (n = 11 for BSA, n = 12 for GLP-2).

8.3 Results

8.3.1 *Summary of results*

A summary of the effects of exogenous GLP-2 administration on differentially expressed genes, KEGG pathways, and gene ontologies is presented in Table 8.1. Exogenous GLP-2 administration upregulated (*padj* < 0.05) 667 DEGs, 26 KEGG pathways, 198 biological processes, 56 cellular components, and 60 molecular functions. Exogenous GLP-2 downregulated (*padj* < 0.05) 1101 DEGs, 14 KEGG pathways, 270 biological processes, 105 cellular components, and 46 molecular functions. Postruminal casein infusion did not result in any enriched KEGG pathways or gene ontologies of the jejunal mucosa.

8.3.2 *Differentially expressed genes*

Exogenous GLP-2 administration resulted in 1768 DEGs (*padj* < 0.05) with 667 upregulated genes and 1101 downregulated genes (Fig. 8.1). Postruminal casein infusion resulted in 7 DEGs (*padj* < 0.05) with 0 upregulated genes and 7 downregulated genes in the jejunal mucosa.

8.3.3 *Gene ontologies*

The top 10 biological processes upregulated (*padj* < 0.05) with exogenous GLP-2 administration were the alcohol metabolic process, sphingolipid metabolic process, organic hydroxy compound metabolic process, anion transport, cellular lipid catabolic process, monocarboxylic acid metabolic process, carbohydrate metabolic process, transition metal ion transport, small molecule catabolic process, and lipid catabolic process (Fig. 3). The top 10 cellular components upregulated (*padj* < 0.05) with exogenous GLP-2

administration were apical junction complex, apical part of the cell, brush border, Golgi subcompartment, Golgi apparatus part, apical plasma membrane, the cluster of actin-based cell projections, cell-cell junction, occluding junction, and Golgi membrane. The top 10 molecular functions upregulated (*padj* < 0.05) with exogenous GLP-2 administration were hydrolase activity (hydrolyzing *O*-glycosyl compounds), hydrolase activity (hydrolyzing glycosyl bonds), anion transmembrane transporter activity, coenzyme binding, transition metal ion transmembrane transporter activity, lipid transporter activity, organic anion transmembrane transporter activity, active transmembrane transporter activity, UDP-glycosyltransferase activity, and cofactor binding.

The top 10 biological processes downregulated (*padj* < 0.05) with exogenous GLP-2 administration were ribonucleoprotein complex biogenesis, ribosome biogenesis, rRNA metabolic process, rRNA processing, ncRNA processing, ribosomal large subunit biogenesis, ribonucleoprotein complex assembly, ribosomal small subunit biogenesis, and ribonucleoprotein complex subunit organization. The top 10 cellular components downregulated (*padj* < 0.05) with exogenous GLP-2 administration were ribosomal subunit, cytosolic ribosome, ribosome, cytosolic part, cytosolic large ribosomal subunit, preribosome, large ribosomal subunit, cytosolic small ribosomal subunit, and nucleolar part. The top 10 molecular functions downregulated (*padj* < 0.05) with exogenous GLP-2 administration were the structural constituent of ribosome, rRNA binding, translation factor activity (RNA binding), catalytic activity (acting on RNA), translation initiation factor activity, catalytic activity (acting on DNA), chromatin binding, snoRNA binding, histone binding, and helicase activity.

8.3.4 *KEGG pathways*

The KEGG pathways that were upregulated (*padj* < 0.05) with exogenous GLP-2 administration were sphingolipid metabolism, adherens junction, bile secretion, galactose metabolism, vitamin digestion and absorption, mineral absorption, peroxisome, carbon metabolism, tight junction, other glycan degradation, starch and sucrose metabolism, retinol metabolism, lysosome, insulin resistance, ascorbate and aldarate metabolism, *N*-glycan biosynthesis, valine leucine and isoleucine degradation, glycerolipid metabolism, PPAR signaling pathway, and lysine degradation, protein digestion and absorption, AMPK signaling pathway, fat digestion and absorption, propanoate metabolism, gastric cancer, and various types of *N*-glycan biosynthesis (Fig. 5).

Downregulated KEGG pathways (*padj* < 0.05) include ribosome, RNA transport, DNA replication, ribosome biogenesis in eukaryotes, spliceosome, cell cycle, base excision repair, mismatch repair, nucleotide excision repair, primary immunodeficiency, RNA polymerase, Fanconi anemia pathway, B cell receptor signaling pathway, and leishmaniasis.

8.4 Discussion

8.4.1 *Influence of postruminal casein infusion on the jejunal transcriptome*

To our knowledge, this is the first study that has evaluated the effects of postruminal casein infusion on the jejunal mucosal transcriptome in ruminants. Several plausible explanations exist as to why postruminal casein infusion for 7 d did not affect the jejunal transcriptome in the current study. The basal diet was formulated to exceed net energy and metabolizable protein requirements, whereas effects of postruminal casein infusion might

have been detected if dietary energy or protein was limiting. When postruminal casein was added to a protein-free diet, small intestinal starch disappearance increased by 37.6 percentage units (from 54.9% to 92.5%) (Taniguchi et al., 1993). By comparison, postruminal casein infusion only increased small intestinal starch disappearance by a maximum of 10.2 percentage units when dietary metabolizable protein requirements were met (Brake et al., 2014b). Increased small intestinal starch disappearance is one of the most consistent effects observed with postruminal casein infusion (Taniguchi et al., 1993; Richards et al., 2002; Mendoza and Britton, 2003; Brake et al., 2014a; Brake et al., 2014b; Blom et al., 2016; Acharya et al., 2023). Supporting this concept, a recent review summarized the effects of postruminal casein infusion from 51 studies and concluded that casein increased DM intake when metabolizable protein supply was deficient and that casein had no effect or decreased DM intake when metabolizable protein supply was in excess (Martineau et al., 2016). Therefore, it is possible that the lack of transcriptomic response to postruminal casein infusion was due to the positive energy and metabolizable protein balance of steers in the current study.

The method of nutrient administration, time to collect jejunal samples after infusion termination, and approach used to measure gene expression could have influenced the outcome of the current experiment. In the current study, casein was delivered continuously via postruminal infusion for 7 d. It is possible that the continuous postruminal infusion of casein masked effects that would be observed with a single pulse dose or multiple pulse doses per day. Previous research demonstrated that the timing of sampling in relation to luminal nutrient flow is important for detecting DEGs in the intestinal mucosa of cattle (Li et al., 2019). Li et al. (2019) found that the number of DEGs in duodenal mucosa decreased

from 1490 DEGs to 482 DEGs 24 h after terminating the postruminal infusion of partially hydolyzed starch. Small intestinal transit time is approximately 3 h in cattle (Brake et al., 2014b) and all steers in the current study were slaughtered within a maximum of 3 to 4 h after termination of the 7-d postruminal infusion. Therefore, it is possible that the final portion of luminal casein had passed the jejunum by the time samples were collected for analysis. Because the intestinal mucosa is a heterogeneous tissue, approaches using single-cell RNA sequencing technology could potentially detect DEGs of lowly expressed cell populations, such as enteroendocrine cells, that were likely underrepresented in the current study (Haber et al., 2017).

A common finding between the current study and Li et al. (2019) is that 7-d postruminal infusion of partially hydrolyzed starch or raw corn starch with casein does not influence many functions of the intestinal epithelium. Although previous research detected 1490 DEGs, the enrichment analyses showed that DEGs found after 7-d of postruminal partially hydrolyzed starch infusion only affected a few, generic gene ontologies of the duodenal mucosa (such as biological process, cellular process, molecular function, binding) (Li et al., 2019). Likewise, postruminal infusion of casein did not result in any enriched KEGG pathways or gene ontologies in the jejunal mucosa in the current study. These findings collectively suggest that continuous postruminal infusion of starch or starch with casein for 7 d does not influence the transcriptome of the intestinal mucosa in cattle.

If the FDR-corrected *padj* is ignored, then KEGG pathways such as pancreatic secretion (8/99), GABAergic synapse (8/74), morphine addiction (7/76) are among the top pathways identified in the jejunal mucosa to be affected by postruminal casein infusion. A large body of research has demonstrated that postruminal casein infusion affects pancreatic

exocrine function in ruminants (Wang and Taniguchi, 1998; Swanson et al., 2002a; Swanson et al., 2002b; Richards et al., 2003; Swanson et al., 2003; Swanson et al., 2004a; Trotta et al., 2020c). Casein contains relatively high proportions of glutamate and glutamine (Lapierre et al., 2012) and a few casein hydrolysate peptides have been shown to interact with GABA receptors associated with sleep promotion in mice (Miclo et al., 2001; dela Pena et al., 2016; Qian et al., 2021). Casein contains opiate-like peptides (ß-casomorphins) which are thought to slow gastrointestinal transit and reticular motility in cattle through interactions with opiate receptors (Brantl et al., 1979; Daniel et al., 1990; Kil and Froetschel, 1994). Because postruminal casein infusion did not significantly affect any KEGG pathways or gene ontologies in the current study, the remainder of the discussion will focus on effects of exogenous GLP-2.

8.4.2 *Effects of exogenous GLP-2 on jejunal mucosal growth*

It is well-established that exogeneous GLP-2 administration increases the growth of the small intestinal mucosa, but net tissue accretion can be due to a combination of increasing cellular proliferation and survival and decreasing cellular apoptosis and proteolysis. Previous research has shown that increased mucosal mass with exogenous GLP-2 administration could occur via both mechanisms of increasing tissue accretion, but activation of these pathways could vary based on physiological state, the presence of luminal nutrient supply, and with mucosal inflammation/injury (Burrin et al., 2000; Burrin et al., 2005; Burrin et al., 2007). We previously demonstrated that exogenous GLP-2 administration for 7 d increased jejunal mucosal mass, mucosal DNA content, and mucosal protein content. Because increased mucosal mass can occur via increasing proliferation/decreasing apoptosis, there can be multiple mediators of GLP-2-induced

mucosal growth (Dubé and Brubaker, 2007). After GLP-2 binds to its receptor, it is generally thought that independent actions of insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF) are critical downstream mediators of increased cellular proliferation (Dubé et al., 2006; Dubé and Brubaker, 2007; Yusta et al., 2009; Fesler et al., 2020). These mediators are thought to lead to activation of Wnt/β-catenin and PI3K/Akt signaling pathways to increased cellular proliferation and differentiation (Dubé and Brubaker, 2007). Currently, there are no annotations of GLP-2 signaling pathways in the KEGG pathway or gene ontology databases. Nevertheless, several transcripts associated with EGF receptor signaling (*ERBB2*, *ERBB3*), insulin signaling (*INSR*, *IRS2*), Wnt/β-catenin signaling (*WNT1*, *WNT8B*, *WNT11*, *FZD3*, *FZD5*, *DVL1*, *GSK3B*, *CTNNB1*, *TCF7L2*) and PI3K/Akt signaling (*PDPK1*) pathways were upregulated with exogenous GLP-2 administration. These data suggest that part of the increase in jejunal mucosal growth with exogenous GLP-2 administration might have occurred through the activation of GLP-2 signaling pathways.

In the current study, many KEGG pathways associated with genetic information processing were downregulated with exogenous GLP-2 administration including ribosome, RNA transport, DNA replication, ribosome biogenesis in eukaryotes, spliceosome, cell cycle, base excision repair, mismatch repair, nucleotide excision repair, and RNA polymerase. In addition, several transcripts associated with ubiquitin-mediated proteolysis (30/166) were downregulated with exogenous GLP-2 administration, which might suggest that exogenous GLP-2 increased jejunal mucosal growth in the current study through increased cellular proliferation and decreased proteolysis. Steers received subcutaneous GLP-2 injections twice daily for 7 d in the current study, and tissues were collected on day

8. Therefore, jejunal mucosa was collected approximately 14-15 h after the last GLP-2 injection was given on day 7. Glucagon-like peptide 2 has a short half-life (7 min) in plasma in humans (Hartmann et al., 2000) but, subcutaneous GLP-2 administration can sustain elevated pharmacological plasma GLP-2 concentrations for more than 7 h in cattle (Taylor-Edwards et al., 2011). It is possible that changes in the mRNA expression of genes in genetic information processing pathways are transient and that desensitization of the GLP-2 receptor could have occurred with chronic GLP-2 treatment for 7 d (Taylor-Edwards et al., 2011). Therefore, it remains unclear if the effects of exogenous GLP-2 for 7 d on genetic information processing pathways was coordinated with decreased cellular apopotosis and proteolysis, chronic exposure causing GLP-2 receptor desentisization, timing of sampling, or because the pharmacological dose given could potentially differ from responses that would occur under normal physiological conditions.

8.4.3 *Effects of exogenous GLP-2 on nutrient digestion, absorption, and metabolism*

Previous research has demonstrated that GLP-2 can increase the mRNA expression and enzyme activity of brush-border carbohydrases (Brubaker et al., 1997; Kitchen et al., 2000; Petersen et al., 2001; Petersen et al., 2002); mRNA expression, protein abundance, and activity of intestinal glucose transporters (Cheeseman and Tsang, 1996; Cheeseman, 1997; Cheeseman and O'Neill, 1998; Au et al., 2002); and net portal glucose flux (Guan et al., 2003). In the current study, exogenous GLP-2 upregulated mRNA expression of genes involved with starch and sucrose metabolism and galactose metabolism KEGG pathways. In addition, exogenous GLP-2 upregulated several gene ontologies related to carbohydrate digestion, absorption, and metabolism including carbohydrate metabolic process, hydrolase activity, glucosidase activity, monosaccharide metabolic process, and hexose

metabolic process. Genes associated with brush-border carbohydrate digestion (*MGAM*, *LCT*, *TREH*), intestinal glucose transport (*SLC5A1, SLC2A2*), glycolysis/gluconeogenesis (*HKDC1*, *HK2*, *PGM1*, *PCK2*, *PDHA1*) and the tricarboxylic acid cycle (*ACO1*, *IDH1*, *SDHA*, *PC*, *DLD*, *SUCLG1*, *SUCLG2*) were upregulated with exogenous GLP-2 administration, suggesting that the capacity for small intestinal carbohydrate assimilation might have increased with exogenous GLP-2 administration.

In the current study, exogenous GLP-2 upregulated mRNA expression of genes involved with protein digestion and absorption KEGG pathway and exopeptidase gene ontology. Exogenous GLP-2 increased mRNA expression of genes associated with luminal protease/peptidase activity (*LOC618826, CPA3*) and membrane-bound peptidase activity (*DPP4*, *XPNPEP2*, *ACE2*, *MEP1A*, *MEP1B*, *MME*). Exogenous GLP-2 increased mRNA expression of genes associated with apical amino acid transport (*SLC1A1*, *SLC6A19*, *SLC36A1*, *SLC3A1*, *SLC7A9*) and basolateral amino acid transport (*SLC38A2*, *SLC3A2*, *SLC7A8*, *SLC7A7*). Previous research demonstrated that exogenous GLP-2 administration decreased net portal-drained visceral release of several amino acids including leucine, isoleucine, and lysine (Taylor-Edwards et al., 2012). In the current study, exogeneous GLP-2 administration upregulated valine, leucine and isoleucine degradation and lysine degradation KEGG pathways. These data suggest that these amino acids were used by cells of the jejunal mucosa to support increased energy/amino acid requirements of GLP-2-induced growth. Several gene ontologies related to protein digestion, absorption, and metabolism were upregulated with exogenous GLP-2 including cellular response to nitrogen compound, alpha-amino acid metabolic process, peptide catabolic process, amino acid transport, and neutral amino acid transport.

Although past research primarily focused on determining the effects of GLP-2 on intestinal glucose and amino acid utilization, more recent research has also observed that GLP-2 is involved with lipid digestion, absorption, and metabolism in the small intestine (Newberry and Davidson, 2009). In the current study, exogenous GLP-2 upregulated mRNA expression of genes involved with bile secretion and fat digestion and absorption KEGG pathways. There is a link between bile acid secretion and endogenous GLP-2 secretion because the enteral infusion of chenodeoxycholic acid resulted in increased small intestinal mucosal mass, villus:crypt, and plasma GLP-2 concentration (Jain et al., 2012). Bile emulsifies lipids in the aqueous environment of the small intestinal lumen to aid in micelle formation to facilitate lipid digestion and absorption. Glucagon-like peptide 2 has been shown to increase chylomicron production and lipid absorption through increased lymph flow, apical fatty acid absorption, and chylomicron formation and secretion (Hsieh et al., 2009; Hsieh et al., 2015; Grande et al., 2022; Syed-Abdul et al., 2022). In the current study, genes associated with apical fatty acid and cholesterol transport (*CD36*, *SCARB1*, *NPC1L1*, *ABCG5*, *ABCG8*), intracellular fatty acid transport (*FBP1*, *SLC27A4*, *MTTP*), and intracellular glycerolipid metabolism (*PLPP1*, *PLPP2*, *MOGAT2*, *DGAT1*) were upregulated with exogenous GLP-2 administration. Numerous gene ontology terms related to lipid digestion, absorption, and metabolism were upregulated with exogenous GLP-2 including cellular lipid catabolic process, lipid localization, lipid transport, fatty acid transport, fatty acid metabolic process, lipid homeostasis, cholesterol metabolic process, triglyceride metabolic process, etc.

Exogenous GLP-2 administration upregulated vitamin digestion and absorption and mineral absorption KEGG pathways in the current study. Expression of mRNA associated

with apical membrane vitamin absorption that were upregulated with exogenous GLP-2 include cubilin (*CUBN*; vitamin B_{12}-intrinsic factor complex transporter), solute carrier family 19 member 2 (*SLC19A2*; thiamine transporter 1), solute carrier family 52 member 3 (*SLC52A3*; riboflavin transporter), solute carrier family 19 member 1 (*SLC19A1*; folate transporter 1), and scavenger receptor class B member 1 (*SCARB1*; vitamin A and vitamin E transporter). Expression of LMBR1 domain containing 1 (*LMBR1*) and transcobalamin 2 (*TCN2*) which aid in the lysosomal export of vitamin B_{12}-intrinsic factor complex and basolateral transport of vitamin B_{12} were increased with exogenous GLP-2 administration. Increased retinol binding protein 2 (*RBP2*) and lecithin retinol acetyltransferase (*LRAT*) mRNA expression might aid in the absorption, intracellular metabolism, and esterification of vitamin A. Additionally, 19 transcripts of retinol metabolism and 10 transcripts of ascorbate and aldarate metabolism KEGG pathways were upregulated with exogenous GLP-2. Genes associated with apical mineral absorption (*TRPM6*, *TRPM7*, *SLC11A2*, *SLC5A1*, *SLC6A19*, *SLC31A1*, *SLC9A3*, *SLC39A4*), basolateral mineral absorption (*SLC30A1*, *SLC40A1*, *CLCN2*), and intracellular iron oxidation and transport (*HEPH*). Gene ontology terms related to mineral absorption including transition metal ion transport and homeostasis; zinc, copper, and iron ion transport; zinc, iron, and anion transmembrane transport were upregulated with exogenous GLP-2. Results from RNA-sequencing analyses expand on previous findings to show that GLP-2 may have increased digestion, absorption, and metabolism of all nutrients in the jejunal mucosa in the current study.

8.4.4 *Effects of exogenous GLP-2 on intestinal barrier function*

In addition to nutrient digestion and absorption, a major role of the gastrointestinal tract is to act as a barrier by limiting permeability to potentially toxic or pathogenic

organisms and compounds. Epithelial permeability of the intestinal mucosa can occur through transcellular transport associated with solute or water transport through a cell or paracellular transport in between cell-cell junctions (Groschwitz and Hogan, 2009). Exogenous GLP-2 administration has been shown to decrease jejunal conductance and *ex vivo* fluxes of Na^+, CrEDTA, and horseradish peroxidase in mice (Benjamin et al., 2000). Cameron and Perdue (2005) found that exogenous GLP-2 administration decreased bacteria adhering to and penetrating the intestinal mucosa in stress-induced mice. Others have found that exogenous GLP-2 or GLP-2 receptor agonist administration increases mRNA expression of tight junction proteins (Cani et al., 2009; Moran et al., 2012; Walker et al., 2015; Reiner et al., 2022). In the current study, exogenous GLP-2 administration upregulated adherens junction and tight junction KEGG pathways. In these KEGG pathways, 36% and 25% of the total number of transcripts in each KEGG pathway term were upregulated, respectively. Several gene ontologies including transcripts encoding tight junction proteins were upregulated with exogenous GLP-2 administration including apical junction complex, apical, basolateral, and lateral plasma membrane; bicellular tight, cell-cell, occluding junction; cell, cell-cell junction organization; epithelial cell differentiation and morphogenesis; apical junction, cell, cell-cell, and bicellular tight junction assembly; and anion and chloride transport. Also, connections of adherens and tight junction proteins to the actin cytoskeleton through adaptor proteins may have been improved, as indicated by an upregulated cluster of actin-based cell projections, anchoring junction, actin-based cell projections, actin cytoskeleton, and desmosome gene ontologies. Upregulation of gene ontologies related to phospholipid and sphingolipid metabolism and terms related to apical membrane suggest that exogenous GLP-2 increased the physical

barrier (plasma membrane) of individual cells of the jejunal mucosa. These data suggest that exogenous GLP-2 administration enhanced the physical barrier function of the jejunal mucosa by decreasing transcellular (plasma membrane) and paracellular (tight and adherens junction) permeability.

In addition to the physical barrier of the small intestine, intestinal permeability can be affected by exogenous factors, epithelial cell turnover, cytokines, and immune cells (Groschwitz and Hogan, 2009). Notably, intestinal barrier function dysfunction is associated with increased intestinal inflammation and initiation of immunoregulatory processes (Turner, 2009). In the current study, exogenous GLP-2 administration downregulated primary immunodeficiency and B cell receptor signaling KEGG pathways. Gene ontologies related to immune function that were downregulated with exogenous GLP-2 administration include B cell activation, regulation of B cell activation, immunoglobulin-mediated immune response, B cell-mediated immunity, immunoglobulin production, regulation of lymphocyte activation, adaptive immune response, and others. These data suggest that the adaptive immune function of the jejunal mucosa was improved with exogenous GLP-2 administration. Collectively, next-generation RNA sequencing findings of the current study suggest that exogenous GLP-2 administration improved intestinal barrier function, which was supported by changes in KEGG pathways and gene ontologies associated with decreased intestinal permeability, inflammation, and immune system activation.

8.4.5 *Implications*

To our knowledge, this is the first report that has investigated the effects of exogenous GLP-2 on jejunal mucosal function using an untargeted RNA-sequencing

approach in mammals. This dataset could be useful for identifying novel functions of exogenous GLP-2 and lead to future investigations detailing mechanisms of GLP-2-mediated responses in the small intestinal mucosa. Comparing the findings of the current study with other RNA-sequencing datasets shows that DEGs and KEGG pathways affected by exogenous GLP-2 are similar to DEGs and KEGG pathways identified in cattle with divergent average daily gain (Lindholm-Perry et al., 2016; Foote et al., 2017). Notably, the mRNA expression of *APOB*, *CLCA4*, *CUBN*, *CYP2B6*, and *SLC9A3* were differentially expressed in the jejunal mucosa in all 3 studies. Furthermore, upregulation of vitamin digestion and absorption, galactose metabolism, mineral absorption, bile secretion, retinol metabolism, starch and sucrose metabolism, protein digestion and absorption, PPAR signaling pathway, and insulin resistance KEGG pathways are similar to findings between Foote et al. (2017) with divergent average daily gain cattle and the current study with exogenous GLP-2 administration. Fitzsimons et al. (2017) hypothesized that beef cattle with greater gain:feed had a greater capacity for nutrient absorption from the small intestine. Supporting this hypothesis, others have found that jejunal mucosal density is positively correlated with gain:feed (Meyer et al., 2014). Also, the number of cells in the duodenal and ileal mucosa is positively correlated with improvements in feed efficiency (Montanholi et al., 2013). Those authors suggested that the benefits of increased metabolic activity in the small intestinal mucosa due to increased cellularity would be greater than the increased energetic cost of maintaining the tissue, leading to improved feed efficiency (Montanholi et al., 2013). Therefore, some of the metabolic pathways and molecular functions of the jejunal mucosa affected by exogenous GLP-2 administration may also underlie physiological differences in feed efficiency in beef cattle. These observations

warrant future research to investigate if potential changes to small intestinal function with exogenous GLP-2 administration could lead to improved growth, health, or feed efficiency of feedlot cattle.

8.5 Conclusions

Postruminal casein infusion for 7 d did not affect the jejunal mucosal transcriptome in cattle. This is the first report that has investigated the effects of exogenous GLP-2 administration on jejunal mucosal function using an untargeted RNA sequencing approach in mammals. Results from the current study can be used to discover novel pathways affected by exogenous GLP-2 administration that have not been previously reported and to discover novel pathways which could be associated with already established functions of exogenous GLP-2 administration. Next-generation RNA sequencing generated novel targets for future research to elucidate mechanisms of GLP-2-mediated responses in the jejunal mucosa.

Table 8.1 Summary of the effects of exogenous GLP-2 administration on differentially expressed genes, KEGG pathways, and gene ontologies of the jejunal mucosa.

Analysis	Upregulated	Downregulated
Differentially expressed genes	667	1101
KEGG pathways	26	14
Gene ontologies		
Biological processes	198	270
Cellular components	56	105
Molecular function	60	46

Table 8.2 Enriched KEGG pathways of the jejunal mucosa that were upregulated with exogenous GLP-2 administration.[1]

KEGG ID	Description	GeneRatio	BgRatio	*pvalue*	*padj*
bta00600	Sphingolipid metabolism	25/1021	48/7748	<0.0001	<0.0001
bta04520	Adherens junction	27/1021	75/7748	<0.0001	<0.0001
bta04976	Bile secretion	30/1021	96/7748	<0.0001	0.0003
bta00052	Galactose metabolism	14/1021	30/7748	<0.0001	0.0006
bta04977	Vitamin digestion and absorption	13/1021	27/7748	<0.0001	0.0006
bta04978	Mineral absorption	21/1021	60/7748	<0.0001	0.0006
bta4146	Peroxisome	27/1021	89/7748	<0.0001	0.0007
bta01200	Carbon metabolism	34/1021	126/7748	<0.0001	0.0008
bta04530	Tight junction	41/1021	166/7748	<0.0001	0.0011
bta00511	Other glycan degradation	10/1021	19/7748	<0.0001	0.0012
bta00500	Starch and sucrose metabolism	13/1021	30/7748	<0.0001	0.0012
bta00830	Retinol metabolism	19/1021	57/7748	<0.0001	0.0017
bta4142	Lysosome	34/1021	133/7748	<0.0001	0.0017
bta04931	Insulin resistance	29/1021	107/7748	<0.0001	0.0017
bta00053	Ascorbate and aldarate metabolism	10/1021	23/7748	0.0003	0.0068
bta00510	N-Glycan biosynthesis	16/1021	50/7748	0.0004	0.0083
bta00280	Valine, leucine and isoleucine degradation	16/1021	51/7748	0.0005	0.0099
bta00561	Glycerolipid metabolism	18/1021	61/7748	0.0006	0.0099
bta03320	PPAR signaling pathway	21/1021	79/7748	0.0010	0.0159
bta00310	Lysine degradation	18/1021	65/7748	0.0013	0.0204
bta04974	Protein digestion and absorption	23/1021	92/7748	0.0014	0.0204
bta04152	AMPK signaling pathway	28/1021	120/7748	0.0014	0.0204

Table 8.2 (continued)

bta04975	Fat digestion and absorption	14/1021	46/7748	0.0017	0.0225
bta00640	Propanoate metabolism	11/1021	34/7748	0.0030	0.0391
bta05226	Gastric cancer	30/1021	140/7748	0.0040	0.0479
bta00513	Various types of N-glycan biosynthesis	12/1021	40/7748	0.0040	0.0479

[1]GeneRatio = ratio of upregulated DEGs to all genes in a KEGG term; BgRatio = background ratio, ratio of all genes concerning this KEGG term to all genes in the background KEGG database; *pvalue* = probability value for hypergeometric test; *padj* = probability value for hypergeometric test adjusted for Benjamini-Hochberg FDR correction.

Table 8.3 Enriched KEGG pathways of the jejunal mucosa that were downregulated with exogenous GLP-2 administration.[1]

KEGG ID	Description	GeneRatio	BgRatio	*pvalue*	*padj*
bta03010	Ribosome	121/1121	296/7788	<0.0001	<0.0001
bta03013	RNA transport	72/1121	187/7788	<0.0001	<0.0001
bta03030	DNA replication	26/1121	38/7788	<0.0001	<0.0001
bta03008	Ribosome biogenesis in eukaryotes	35/1121	76/7788	<0.0001	<0.0001
bta03040	Spliceosome	56/1121	170/7788	<0.0001	<0.0001
bta04110	Cell cycle	47/1121	143/7788	<0.0001	<0.0001
bta03410	Base excision repair	18/1121	33/7788	<0.0001	<0.0001
bta03430	Mismatch repair	15/1121	24/7788	<0.0001	<0.0001
bta03420	Nucleotide excision repair	19/1121	47/7788	<0.0001	0.0004
bta05340	Primary immunodeficiency	20/1121	60/7788	0.0002	0.0051
bta03020	RNA polymerase	13/1121	32/7788	0.0003	0.0068
bta03460	Fanconi anemia pathway	18/1121	53/7788	0.0003	0.0068
bta04662	B cell receptor signaling pathway	29/1121	109/7788	0.0006	0.0133
bta05140	Leishmaniasis	28/1121	106/7788	0.0008	0.0173

[1]GeneRatio = ratio of downregulated DEGs to all genes in a KEGG term; BgRatio = background ratio, ratio of all genes concerning this KEGG term to all genes in the background KEGG database; *pvalue* = probability value for hypergeometric test; *padj* = probability value for hypergeometric test adjusted for Benjamini-Hochberg FDR correction.

Figure 8.1 Volcano plot of jejunal mucosal genes influenced by exogenous GLP-2 administration. Data points that are highlighted in red were differentially expressed (*padj* < 0.05).

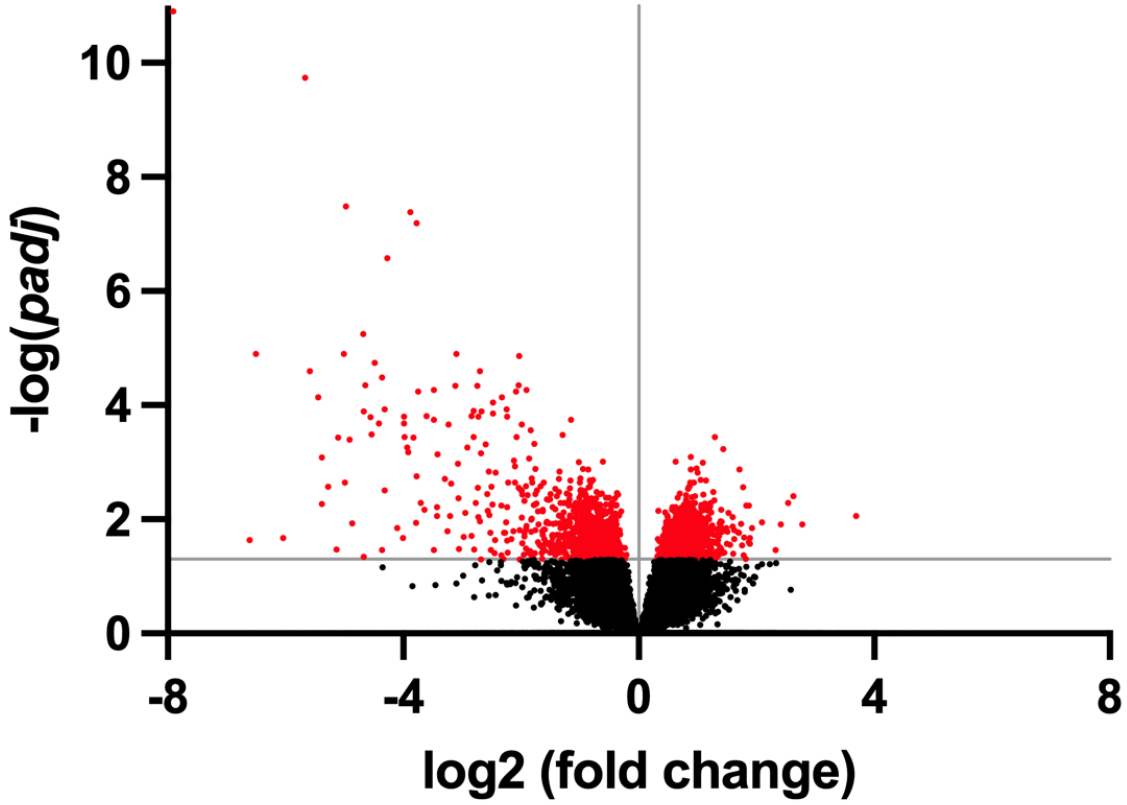


Figure 8.2 Top enriched gene ontology terms of the jejunal mucosa that were upregulated with exogenous GLP-2 administration.

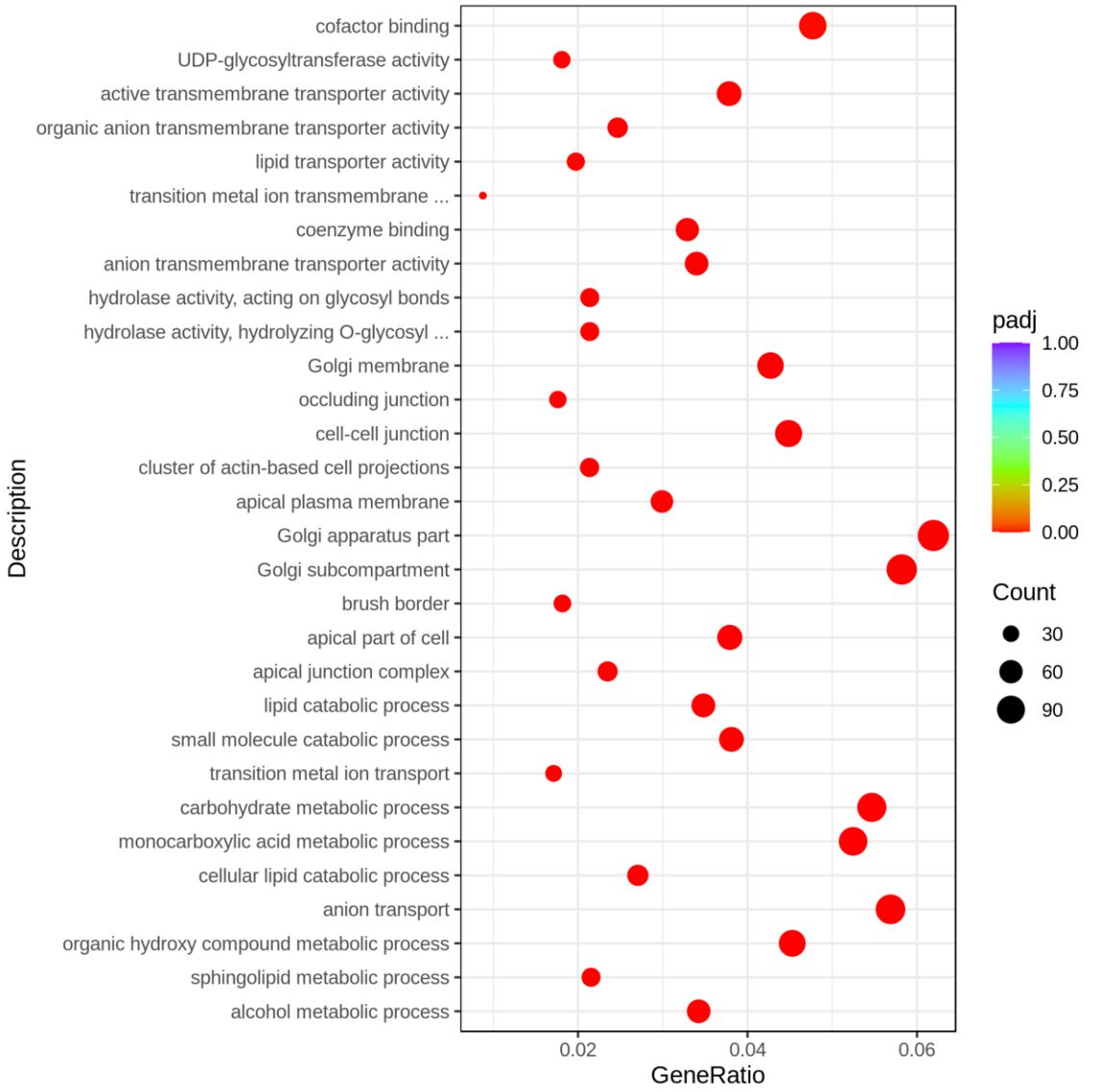
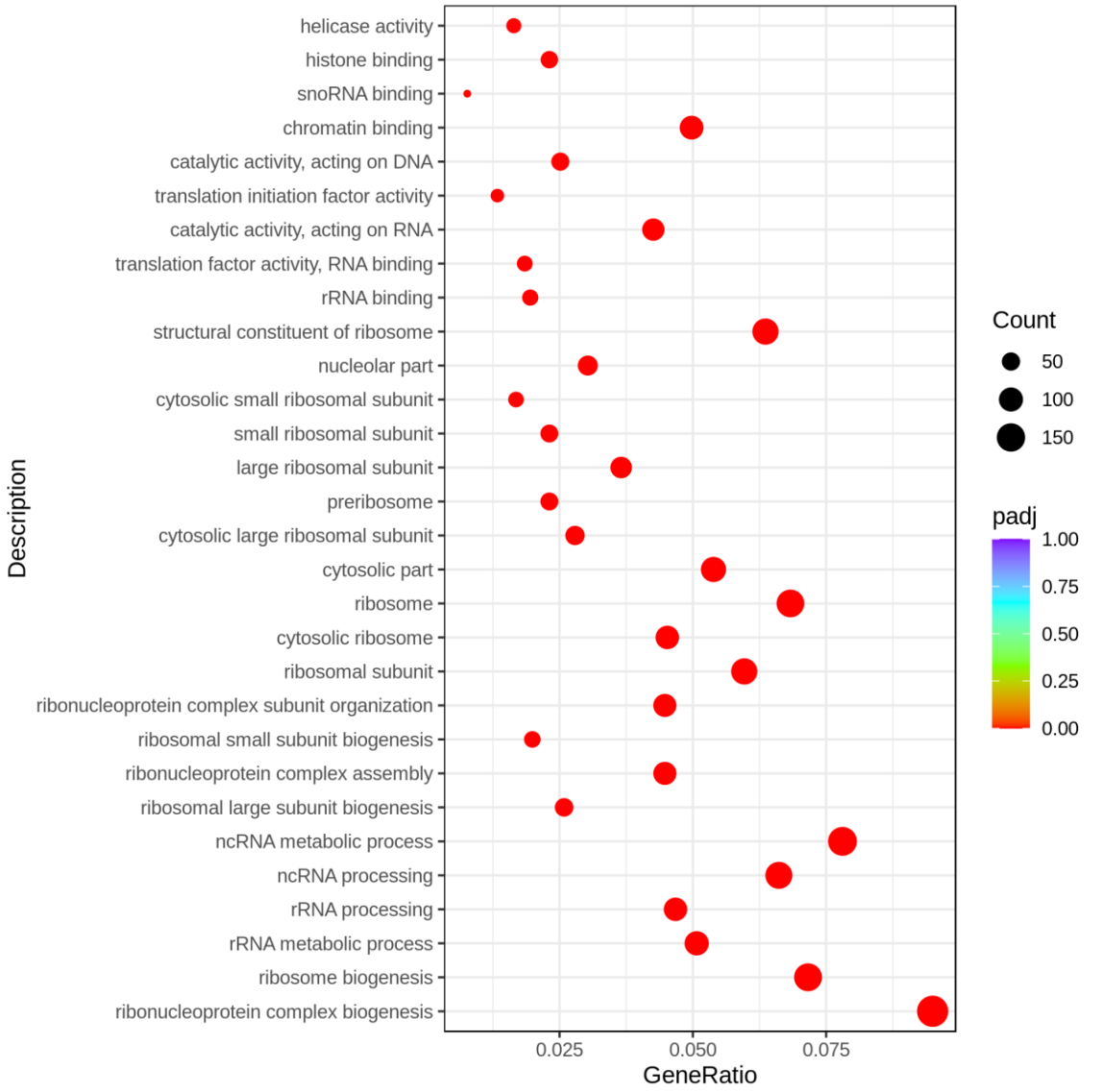


Figure 8.3 Top enriched gene ontology terms of the jejunal mucosa that were downregulated with exogenous GLP-2 administration.



CHAPTER 9. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

Research generated in this dissertation can be used to develop and refine strategies to improve flake quality, optimize starch digestion, and reduce methane emissions of feedlot cattle. To improve our understanding of small intestinal starch assimilation, more research is needed to address specific knowledge gaps. Current datasets evaluating small intestinal starch disappearance were generated with data from experiments in which cattle were fed whole or rolled forms of processed grain. There are limited data describing the effects of extensive grain processing (steam-flaking, high-moisture processing) on small intestinal starch disappearance. Most studies where information is available about extensive grain processing are limited in their application because: 1) postruminal starch disappearance was measured instead of small intestinal starch disappearance, 2) diets were fed below production levels of intake, and 3) dietary models are difficult to separate effects of ruminal digestion on small intestinal starch digestion. Once starch reaches the small intestine, it is still unclear which carbohydrases are most-limiting. In particular, more research is needed to investigate the physiological explanation for the absence of sucrase activity in cattle and if it is related to quantitative limitations of small intestinal starch digestion. There needs to be an experiment that specifically measures the enzymatic digestion of starch in the small intestine of cattle using tracers, as it remains unknown how much is enzymatically digested and authors have speculated that excessive microbial fermentation and/or visceral metabolism is responsible for the large portion of carbon that cannot be accounted for by lack of glucose appearance in portal blood. Evaluating the apical GLUT2 hypothesis (Kellett and Helliwell, 2000) under physiological conditions in cattle is warranted to increase knowledge of intestinal glucose transport mechanisms in ruminants.

There needs to be more research on starch retrogradation of steam-flaked corn. At present, it seems like retrogradation may be a practical strategy to shift the site of starch digestion from the rumen to the small intestine. Research would be needed to evaluate if starch retrogradation affects ruminal passage rate, small intestinal starch digestibility, and total-tract starch digestibility to confirm that predictions of increased energetic efficiency are achievable. If this concept remains true, then additional research would be needed to assess the impact of shifting the site of starch digestion to the small intestine on enteric methane emissions, ruminal microbial protein synthesis, and nitrogen balance. These studies would need to be conducted with varying ratios of retrograded:non-retrograded steam-flaked corn and with varying inclusions of steam-flaked corn in finishing diets, as these criteria may influence conclusions about feeding retrograded steam-flaked corn in finishing diets.

As noted previously, 25% of feedlot producers do not use steam-flaking as their primary or secondary method of grain processing, indicating a large market to find suitable alternatives to improve efficiency for those producers. Consistently, complete replacement of rolled corn with fine-ground corn in finishing diets has shown depressions in growth and gain:feed, and increased incidence of ruminal acidosis. However, no research has evaluated including fine-ground corn at low inclusion levels with rolled corn. Based on ruminal solubilities, it appears that fine-ground corn would be quite similar to 335 g/L steam-flaked corn.

The inclusion of *Y. schidigera* extract or *Q. saponaria* extract in finishing diets to reduce methane emissions was not successful. Several factors may have influenced the outcome of this experiment. However, research to replace feed additives classified as

antibiotics in finishing diets is needed. The same concepts should be applied when evaluating other potential non-antibiotic feed additives. To be competitive with ionophores, non-antibiotic feed additives will need to contain anti-methanogenic and anti-coccidial activity, improve average daily gain and gain:feed, and decrease the incidence of liver abscesses.

The use of exogenous GLP-2 and postruminal casein infusion to selectively target endogenous pancreatic and small intestinal carbohydrase activities was successful. This overcomes a major limitation of coordinating luminal nutrient flows with carbohydrase activity – as there are no previously known methods to selectively increase carbohydrase activities with only luminal nutrient flows. It is expected that this model will be useful for determining physiological mechanisms associated with increased carbohydrase activity. One possible refinement of the model would be to find an alternative pancreatic α-amylase stimulant instead of postruminal casein infusion. Postruminal casein infusion increases energy and nitrogen intake in the current model. Use of a hormone, such as cholecystokinin, would avoid this complication; yet, it is unclear if exogenous cholecystokinin administration would increase pancreatic α-amylase activity in ruminants and there are additional limitations from using exogenous hormones in this model, as there are other neural, hormonal, and feedback signals that influence pancreatic α-amylase activity.

Exogenous GLP-2 administration affected the expression of genes involved in many metabolic pathways and molecular functions of the jejunal mucosa. This information will be useful to discover novel functions of exogenous GLP-2 and discover novel pathways that are associated with already-known functions of exogenous GLP-2. The effects of

exogenous GLP-2 on jejunal mucosal KEGG pathways are similar to cattle that have greater average daily gain. Thus, it may be warranted to further investigate if the changes in the jejunal mucosa induced by exogenous GLP-2 would improve average daily gain and/or gain:feed of finishing beef cattle. Exogenous GLP-2 or other GLP-2 receptor agonists might be suitable candidates to be administered through ear implants, as previous research has shown that exogenous GLP-2 can mediate intestinotrophic effects through intravenous, subcutaneous, intramuscular, or intraperitoneal administration.

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VITA

EDUCATION

Master of Science in Animal Science (Ruminant Nutritional Physiology) Dec. 2019

North Dakota State University

Thesis: Biological abnormalities in the ruminant small intestine and its relationship to carbohydrate assimilation

Major Advisor: Kendall C. Swanson

Bachelor of Science in Animal Sciences Dec. 2017

University of Kentucky

Minor in Biological Sciences

PROFESSIONAL EXPERIENCE

Graduate Research Assistant Jan. 2020 – Present

Department of Animal & Food Sciences

University of Kentucky, Lexington, KY

Supervisor: David L. Harmon

Graduate Research Assistant Jan. 2018 – Dec. 2019

Department of Animal Sciences

North Dakota State University, Fargo, ND

Supervisor: Kendall C. Swanson

Undergraduate Research Assistant May 2017 – Dec. 2017

Forage-Animal Production Research Unit

United States Department of Agriculture – Agricultural Research Service, Lexington, KY

Supervisor: James L. Klotz

Animal and Food Sciences Student Worker Nov. 2014 – Dec. 2017

Department of Animal & Food Sciences

University of Kentucky, Lexington, KY

Supervisor: David L. Harmon

SCHOLASTIC AND PROFESSIONAL HONORS

ASAS Wettemann Graduate Scholar in Physiology Award	Jul. 2023
ASAS National Young Scholar Award	Jul. 2022
ASAS Wilson G. Pond International Travel Award	Jul. 2022
UK Animal and Food Science Outstanding PhD Student Award	Jun. 2022
UK Animal and Food Science Graduate Poster Symposium – 2nd Place	Jun. 2022
Plains Nutrition Conference Graduate Poster Competition – Runner-Up	Apr. 2022
Certified Angus Beef Graduate Colvin Scholarship	Aug. 2021
Plains Nutrition Conference Graduate Poster Competition – Runner-Up	Apr. 2021
UK CAFE Richards Graduate Student Research Activity Award	Oct. 2020
Plains Nutrition Conference Graduate Poster Competition – 2nd Place	Apr. 2020
Featured Cover Article for The Journal of Nutrition Volume 150, Issue 4	Apr. 2020
North Dakota Soybean Council Graduate Scholarship	Aug. 2019
North Dakota Livestock Endowment Foundation Scholarship	Aug. 2019
Alltech Young Scientist North American Undergraduate Award	May 2018
Commencement Honors: Cum Laude	Dec. 2017
University of Kentucky Dean's List	Dec. 2017
Central States Meat Association Scholarship	Aug. 2017
University of Kentucky Dean's List	May 2017

University of Kentucky Dean's List	Dec. 2016
Central States Meat Association Scholarship	Aug. 2016
Dairy Farmers of America Scholarship	Aug. 2016
Agriculture Phone-a-thon Scholarship	Aug. 2015
Stephanie Weiser Green Scholarship	Aug. 2014

PROFESSIONAL PUBLICATIONS

Published Journal Articles

1. **Trotta, R. J.,** D. L. Harmon, H. Ji, and J. L. Klotz. 2023. Duration of ergovaline exposure influences serotonin-mediated vasoactivity of bovine mesenteric vasculature. J. Anim. Sci. doi:10.1093/jas/skad100

2. Schiff, A. P., **R. J. Trotta**, V. Holder, K. K. Kreikemeier, and D. L. Harmon. 2023. In vitro gas production kinetics are influenced by grain processing, flake density, starch retrogradation, and *Aspergillus oryzae* fermentation extract containing α-amylase activity. J. Anim. Sci. doi:10.1093/jas/skad031

3. Webb, K. L., **R. J. Trotta**, Y. Jia, P. J. Bridges, and J. C. Matthews. 2022. Influence of form of selenium supplementation and tall fescue endophyte toxicity on growth performance, serum parameters, and tissue masses of grazing beef steers. Transl. Anim. Sci. 6:txac122. doi:10.1093/tas/txac122

4. **Trotta, R. J.**, K. K. Kreikemeier, R. F. Royle, T. Milton, and D. L. Harmon. 2022. Corn processing, flake density, and starch retrogradation influence ruminal solubility of starch, fiber, protein, and minerals. J. Anim. Sci. 100:skac149. doi:10.1093/skac149

5. **Trotta, R. J.,** D. L. Harmon, J. C. Matthews, and K. C. Swanson. 2022. Nutritional and physiological constraints contributing to limitations in small intestinal starch digestion and glucose absorption in ruminants. Ruminants. 2:1-26. doi:10.3390/ruminants2010001

6. **Trotta, R. J.**, K. K. Kreikemeier, R. F. Royle, T. Milton, and D. L. Harmon. 2021. Flake density and starch retrogradation influence *in situ* ruminal degradability

characteristics of steam-flaked corn and predicted starch digestibility and energetic efficiency. J. Anim. Sci. 99:skab298. doi:10.1093/jas/skab298

7. **Trotta, R. J.**, K. K. Kreikemeier, R. F. Royle, T. Milton, and D. L. Harmon. 2021. Influence of air equilibration time, sampling techniques, and storage temperature on enzymatic starch availability of steam-flaked corn. J. Anim. Sci. 99:skab162. doi:10.1093/jas/skab162

8. **Trotta, R. J.**, and K. C. Swanson. 2021. Prenatal and postnatal nutrition influence pancreatic and intestinal carbohydrase activity of ruminants. Animals. 11:171. doi:10.3390/ani11010171

9. **Trotta, R. J.**, K. R. Maddock-Carlin, and K. C. Swanson. 2021. Influence of ractopamine hydrochloride supplementation on pancreatic digestive enzyme activity in finishing steers. Can. J. Anim. Sci. 101:191-195. doi:10.1139/CJAS-2020-0092

10. **Trotta, R. J.**, C. O. Lemley, K. A. Vonnahme, and K. C. Swanson. 2021. Effects of nutrient restriction and melatonin supplementation from mid- to late-gestation on maternal and fetal small intestinal carbohydrase activities in sheep. Domest. Anim. Endocrinol. 74:106555. doi:10.1016/j.domaniend.2020.106555.

11. **Trotta, R. J.**, F. E. Keomanivong, J. L. Peine, J. S. Caton, and K. C. Swanson. 2020. Effects of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activities of lamb offspring. Livest. Sci. 241:104246. doi:10.1016/j.livsci.2020.104246

12. **Trotta, R. J.**, A. K. Ward, and K. C. Swanson. 2020. Influence of dietary fructose supplementation on visceral organ mass, carbohydrase activity, and mRNA expression of genes involved in small intestinal carbohydrate assimilation in neonatal calves. J. Dairy Sci. 103:10060-10073. doi:10.3168/jds.2020-18145

13. **Trotta, R. J.**, M. A. Vasquez-Hidalgo, K. A. Vonnahme, and K. C. Swanson. 2020. Effects of nutrient restriction during midgestation to late gestation on maternal and fetal postruminal carbohydrase activities in sheep. J. Anim. Sci. 98:skz393. doi:10.1093/jas/skz393

14. **Trotta, R. J.,** L. G. Sitorski, S. Acharya, D. W. Brake, and K. C. Swanson. 2020. Duodenal infusions of starch with casein or glutamic acid influence pancreatic and

small intestinal carbohydrase activities in cattle. J. Nutr. 150:784-791. doi:10.1093/jn/nxz319

15. **Trotta, R. J.**, and K. C. Swanson. 2020. Effects of dietary supplement sources on the rate and extent of in vitro ruminal degradation of alfalfa-based diets for cattle. Can. J. Anim. Sci. 100:242-252. doi:10.1139/CJAS-2019-0105

16. **Trotta, R. J.**, K. R. Maddock-Carlin, and K. C. Swanson. 2019. Effects of ractopamine hydrochloride supplementation on feeding behavior, growth performance, and carcass characteristics of finishing steers. Transl. Anim. Sci. 3:1143-1152. doi:10.1093/tas/txz114

17. **Trotta, R. J.**, D. L. Harmon, and J. L. Klotz. 2018. Interaction of ergovaline with serotonin receptor 5-HT_{2A} in bovine ruminal and mesenteric vasculature. J. Anim. Sci. 96:4912-4922. doi:10.1093/jas/sky346

18. **Trotta, R. J.**, J. L. Klotz, and D. L. Harmon. 2018. Effects of source and level of dietary energy supplementation on in vitro digestibility and methane production from tall fescue-based diets. Anim. Feed. Sci. Technol. 242:41-47. doi:10.1016/j.anifeedsci.2018.05.010

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2. **Trotta, R. J.**, K. K. Kreikemeier, R. S. Foote, K. R. McLeod, and D. L. Harmon. 2022. O104 Influence of decoquinate and Yucca schidigera extract supplementation on whole-body methane production and ruminal fermentation in steers fed a high-concentrate diet. In: Proceedings of the 7th EAAP International Symposium on Energy and Protein Metabolism and Nutrition (ISEP 2022). Animal – Science proceedings, Elsevier. Granada, Spain. p. 371-373.

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4. **Trotta, R. J.,** K. K. Kreikemeier, R. F. Royle, C. T. Milton, and D. L. Harmon. 2021. Flake density and starch retrogradation influence effective ruminal degradability of steam-flaked corn. In: Proceedings of the 2021 Plains Nutrition Council Spring Conference. San Antonio, TX. p. 95.

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Abstracts

1. **Trotta, R. J.** 2022. 388 Young Scholar Award Talk: Increasing efficiency of finishing beef production: Optimizing starch digestion, improving flaking quality, and reducing methane emissions. J. Anim. Sci. 100(suppl_3):193. doi:10.1093/jas/skac247.355

2. **Trotta, R. J.,** H. C. Freetly, R. A. Cushman, W. T. Oliver, A. K. Lindholm-Perry, J. S. Caton, A. K. Ward, and M. S. Crouse. 2022. PSV-A-5 One-carbon metabolite supplementation influences pancreatic α-amylase activity and protein concentration in a dose-dependent manner in beef heifers. J. Anim. Sci. 100(suppl_3):259. doi:10.1093/jas/skac247.469

3. **Trotta, R. J.,** K. K. Kreikemeier, R. F. Royle, T. Milton, and D. L. Harmon. 2021. PSIV-9 Effective ruminal degradability of steam-flaked corn is influenced by flake density and starch retrogradation. J. Anim. Sci. 99(suppl_3):296-297. doi:10.1093/jas/skab235.545

4. **Trotta, R. J.,** M. A. Vasquez-Hidalgo, B. I. Smith, S. A. Reed, K. E. Govoni, K. A. Vonnahme, and K. C. Swanson. 2021. 248 Maternal nutrient restriction during mid-

gestation decreases uteroplacental release and fetal uptake of essential amino acids in sheep. J. Anim. Sci. 99(suppl_3):130-131. doi:10.1093/jas/skab235.238

5. Webb, K., **R. J. Trotta**, P. Bridges, and J. Matthews. 2021. 177 Ad libitum consumption of a 1:1 blend of inorganic:organic Se by steers grazing endophyte-infected tall fescue increases serum prolactin and alkaline phosphatase activity, but not average daily gain. J. Anim. Sci. 99(suppl_3):94-95. doi:10.1093/jas/skab235.169

6. **Trotta, R. J.**, J. L. Klotz, J. C. Matthews, and K. C. Swanson. 2020. PSIX-7 Grazing toxic endophyte-infected tall fescue does not influence pancreatic or small intestinal digestive enzyme activities in steers. J. Anim. Sci. 98(suppl_4):410-411. doi:10.1093/jas/skaa278.718

7. **Trotta, R. J.**, F. E. Keomanivong, J. L. Peine, J. S. Caton, and K. C. Swanson. 2020. 219 Effects of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activities of lamb offspring. J. Anim. Sci. 98(suppl_3):45. doi:10.1093/jas/skaa054.081

8. **Trotta, R. J.**, and K. C. Swanson. 2019. The potential for different supplement sources to reduce frothy legume bloat based on in vitro degradation characteristics. J. Anim. Sci. 97(suppl_3):390-391. doi:10.1093/jas/skz258.778

9. **Trotta, R. J.**, K. A. Vonnahme, and K. C. Swanson. 2019. PSIV-1 Effects of nutrient restriction and realimentation of gestating ewes on fetal carbohydrase activities in the small intestine. J. Anim. Sci. 97(suppl_3):221. doi:10.1093/jas/skz258.452

10. **Trotta, R. J.,** T. M. Czech, M. A. Vasquez-Hidalgo, K. A. Vonnahme, and K. C. Swanson. 2019. 340 Effects of nutrient restriction during mid- to late-gestation on maternal and fetal pancreatic exocrine function in sheep. J. Anim. Sci. 97(suppl_3):95. doi:10.1093/jas/skz258.197

11. **Trotta, R. J.**, D. L. Harmon, and J. L. Klotz. 2018. PSV-14 Ergovaline acts as both an agonist and antagonist on serotonin receptor 5-HT_{2A} in bovine ruminal and mesenteric vasculature. J. Anim. Sci. 96(suppl_3):46-47. doi:10.1093/jas/sky404.104