THE EFFECTS OF METABOLIZABLE PROTEIN INTAKE AND POST-RUMINAL FLOW OF AMINO ACIDS ON GROWTH PERFORMANCE AND PANCREATIC DIGESTIVE ENZYMES IN STEERS

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Title

The Effects Of Metabolizable Protein Intake And Post-Ruminal Flow Of Amino Acids On Growth Performance And Pancreatic Digestive Enzymes In Steers

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MASTER OF SCIENCE

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ABSTRACT

Excessive dietary protein may affect MP use because of energetic costs of excreting excess N. Amino acids also may influence post-ruminal digestion. Therefore, two experiments were designed to evaluate the effects of MP intake and post-ruminal flow of AA on growth performance and pancreatic digestive enzymes. In experiment 1, treatments supplied different amounts of MP intake to cattle and the effects on growth performance and feeding behavior were evaluated. In experiment 2, duodenal infusion of glutamate or casein was examined and the effects on pancreatic enzymes were measured. Experiment 1 suggests that feeding steers 906 g MP/d in finishing diets supplied enough MP for the greatest growth performance and carcass characteristics. Interestingly, MP intake caused different responses on feeding behavior with greater effects on steers fed 626 and 1444 g MP/d. In experiment 2, casein infusion increased α-amylase activity but not trypsin activity. Glutamate did not influence pancreatic digestive enzymes.

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DEDICATION

This thesis is dedicated to my father Jose Claudio Sitorski and my mother Eliege Fatima Gomes Sitorski, parents who gave me a life that has allowed me to take advantage of several opportunities and have taught me to strive for my personal happiness and success and to always respect the people around me. Every single accomplishment of mine is theirs as well.

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LIST OF ABBREVIATIONS

AA.....Amino Acid

ADG.....Average Daily Gain

ADF.....Acid Detergent Fiber

ATP.....Adenosine TriPhosphate

BCFA.....Branched-Chain Fatty Acid

BCNRM.....Beef Cattle Nutrient Requirement Model

BW.....Body Weight

C.....Celsius

CO_2.....Carbon Dioxide

CP.....Crude Protein

d.....Day

DDGS.....Dried Distillers Grains with Solubles

DM.....Dry Matter

DMI.....Dry Matter Intake

g.....Gram

G:F.....Gain to Feed Ratio

HCW.....Hot Carcass Weight

kg.....Kilogram

kg/d.....Kilogram per Day

KPH.....Kidney Pelvic Heart

L.....Liter

LM.....Loin Eye Muscle

MCP.....Microbial Crude Protein

ME.....Metabolizable Energy

mg .....Milligram

min .....Minute

mL .....Milliliter

m*M* .....Millimolar

MP .....Metabolizable Protein

mRNA .....Messenger Ribonucleic Acid

mTOR .....Mechanistic Target of Rapamycin

N .....Nitrogen

NaCl .....Sodium Chloride

NDF .....Neutral Detergent Fiber

NDSU .....North Dakota State University

NEm .....Net Energy for Maintenance

NH_3 .....Ammonia

NH_4^+ .....Ammonium Ion

NPN .....Non-Protein Nitrogen

NRC .....National Research Council

RDP .....Ruminally Degradable Protein

RNA .....Ribonucleic Acid

RUP .....Ruminally Undegradable Protein

USA .....United States of America

VFA .....Volatile Fatty Acid

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The feedlot industry in the USA has evolved since the 1850s when the first feed yards were developed by cottonseed oil-mill operators (NCBA, 2016). In 2016, it was estimated that a total of 30,000 feedlots were in operation in the country and the vast majority (93%) of this total was composed of feedlots with less than one thousand head capacity (NCBA, 2016). In addition, often small operations have limitations such as nutrients storage capacity, infrastructure, and cash flow when compared to large operations which makes the first more susceptible to market fluctuations. Therefore, the success of the feedlot industry in the USA relies on feeding cattle efficiently by improving nutrient absorption with the least dietary cost per unit of gain. Given the above, ruminant nutritionists have been adopting feeding strategies designed to increase starch fermentation (Vasconcelos and Galyean, 2007) and optimizing protein inclusion in finishing diets (Samuelson et al., 2016) to improve production efficiency. Digestibility of carbohydrates and proteins are important variables that contribute to production in ruminants and may affect feeding behavior of steers. However, the effects of protein on feeding behavior, and the effects of amino acids flowing to the small intestine on pancreatic digestive enzymes remains unclear. Therefore, two experiments were conducted to 1) determine the effects of metabolizable protein intake on growth performance, carcass traits, and feeding behavior of finishing steers, and 2) investigate the effects of post-ruminal flows of glutamic acid or casein on pancreatic amylase and trypsin activity in steers.

1.2. Literature Review

1.2.1. Characteristics of High Grain Diets

In the USA, feedlot facilities typically finish their cattle on high grain diets (> 70% grain in DM basis) aiming for maximum productivity (Vasconcelos and Galyean, 2007). Economical characteristics of the feed market have forced producers to exploit the abilities of ruminal microbes in fermenting and converting a variety of feed sources into energy and protein sources used by the animal. Furthermore, feedlot operations have been practicing the use of low levels of roughage inclusion in the diets to maximize operational efficiency (Contadini et al., 2017). The operational efficiency occurs due to reducing the labor needed to produce, store, and feed roughage sources; therefore, these factors contribute to increased profitability. Utilization of high grain diets became popular in feedlots because usually grains provide the least expensive cost per unit of energy when compared to roughage sources (Bevans, 2005; Buttrey et al., 2012). However, roughage is a component that is often necessary to minimize the incidence of digestive disturbances, affecting DMI and pH changes in the rumen (Owens et al., 1998; May et al., 2010). According to Brown et al. (2006), lower levels of roughage in finishing diets had a positive effect on feed efficiency, carcass yield, and feed costs.

High-grain diets are often introduced to cattle by slowly increasing the proportion of grain in the diet allowing efficiently and uniformly ruminal exposure to the feed. The idea behind the method is to minimize metabolic disorders, by giving time for the ruminal microflora and microfauna to effectively adapt to the use of fermentable carbohydrates that become available in the rumen (Owens et al., 1998). Metabolic disorders account for the second leading mortality cause in feedlot cattle, with ruminal acidosis being the number one digestive disorder (Nagaraja and Titgemeyer, 2007). One long-term effect in animals that survive a bout of acidosis is

decreased gain:feed as a result of damaged ruminal epithelium (Owens et al., 1998). According to Counette and Prins (1981), the animal is adapted to a high-grain diet when it consumes concentrate diets at a level of feed intake that would cause acidosis to a non-adapted animal, without having adverse effects.

In most of the USA, corn is the most common starch source included in high grain diets because of its high starch concentration and cost per unit energy. Processing methods applied to corn grain have been developed to increase ruminal starch digestibility. As a result of feeding the processed corn, metabolizable energy availability in the rumen increases which may improve animal performance in feedlots. Contrastingly, several results have shown that processing corn did not improve feedlot performance over whole grain corn in finishing diets (Owens et al., 1997). Moreover, processed corn has been shown to have greater starch digestibility than whole corn diets when fed to yearling animals; however, these trials have failed to prove advantages in animal performance for processing corn which may be caused due to the difference of chewing capacity between animals of different ages (Loerch and Gorocica, 2006). In addition, greater starch digestibility in processed corn may affect the RDP requirements because of the synchronization of N and energy release into the rumen. Therefore, animals fed whole grain corn may have lower RDP requirements than animals fed processed corn (Loerch and Gorocica, 2006). Furthermore, it is important to state that grain processing contributes to ration conditioning and uniform mixing of ingredients, which is another reasons why processed corn is commonly fed in North American feedlots.

Currently, inclusion of dry distillers grains with solubles (DDGS) as a protein and energy source in finishing diets is common because of the expansion of the ethanol industry. The industry expansion increased the availability of production byproducts (DDGS) resulting in

attractive prices to beef cattle producers including those in North Dakota. Although, corn grain is the primary feedstock used for ethanol production in North America; wheat, sorghum and other grains may also be used for ethanol and DDGS production. In this production process, starch from the grain is fermented to produce ethanol and the residue of this fermentation (DDGS) has approximately a 3-fold greater concentration of protein, fiber, and other components than the original grain (Klopfenstein et al., 2008). However, the ethanol production process uses sulfuric acid which leads to greater S accumulation in its byproduct (Felix and Loerch, 2011). Dry distillers grains with solubles can be used as a protein source for finishing animals; however, care should be taken with the amount of DDGS inclusion due to S concentration and the risk for toxicity. Increasing dietary inclusion of DDGS up to 60% (DM basis) has been shown to reduce growth performance and carcass quality in finishing cattle which is likely partly due to S concentration (Klopfenstein et al., 2008; Felix et al., 2015).

1.2.2. Nitrogen Metabolism in Ruminants

1.2.2.1. Fate of Dietary Protein

Ruminal fermentation of carbohydrates and proteins make it challenging to predict what nutrients are absorbed. Available carbohydrates, such as starches, are largely digested by the ruminal microbes while dietary protein may be more or less degraded depending upon protein source and microbial efficiency. The lack of dietary amino acid flow to the intestine tends to be offset by microbial crude protein (MCP) synthesis. In the rumen, excessive amounts of fermentable protein in finishing diets results in greater ammonia (NH_3) release, which is absorbed, converted to urea primarily in the liver, and can be excreted leading to environmental implications (Amaral et al., 2018). Contrastingly, when ruminants are fed diets deficient in ruminally degradable protein (RDP), MCP synthesis is at least partially maintained by using

endogenous recycled urea. Dietary protein can be subjectively divided into RDP and ruminally undegradable protein (RUP). The RDP is broken down to peptides, amino acids, and NH_3, which are incorporated into MCP. Synthesis of MCP is dependent on the ruminal environment, ruminal bacteria efficiency, passage rate, dietary protein, and non-protein nitrogen (NPN) as well as nitrogen (N) recycled to the rumen. Microbial crude protein represents 50% to 80% of the total amount of amino acids absorbed by a ruminant (Clark et al., 1992). However, RUP is used to make up for deficient amino acids delivered to the small intestine. These amino acids are derived from RUP, microbial crude protein, and from endogenous protein (Miranda et al., 2012). Previous studies have shown that intestinal digestibility of amino acids from RUP vary widely according to different feed sources (Mjoun et al., 2010). In order to meet maintenance and production requirements, ruminants need to have a proper balance between MCP production from RDP as well as RUP as sources of amino acids (Lascano et al., 2016).

Ruminal rate of proteolysis is an important factor that contributes to the efficiency of protein digestion and it is related to dietary protein solubility. For the ruminant, increased solubility generally results in the N being more available for microbial breakdown. In the rumen, soluble compounds are rapidly exposed and digested more completely because of differences in microbial access than insoluble compounds (Hedqvist and Udén, 2005). In most protein sources, only a small fraction is soluble. Specific structural and chemical composition of proteins probably determine solubility and degradation rate in the rumen. However, considering a variety of diets, protein solubility alone is a poor predictor for ruminal degradation rate because different soluble compounds are digested at different rates (Owens and Zinn, 1998). Additionally, previous studies have shown that ruminal degradation of insoluble proteins in ruminal buffers range from 35 to 50% (Owens and Zinn, 1998). Protein solubility may be a useful predictor of

amino acid out-flow from the rumen. Ruminal out-flow of amino acids varies between soluble and insoluble fractions (Hogan and Weston, 1970). An alternative to reduce protein solubility of any plant source is applying heat treatment which results in denaturation of cytoplasmic proteins (Van Soest, 1982).

As previously mentioned the quantity and quality of protein that reaches the small intestine depends on the combined effects of RDP degradation and MCP synthesis (Owens, 1982). Biological value (which is the proportion of absorbed N incorporated into the organism) of proteins in the small intestine is largely dependent on the microbes present in the rumen. If the dietary protein has a low biological value, the biological value of RUP that reaches the small intestine may increase with enzymatic actions to the dietary protein (Owens and Zinn, 1998). On the other hand, if dietary protein has a high biological value the ruminal microbial degradation may decrease its biological value before escaping the rumen.

1.2.2.2. Ruminal Ammonia and N Recycling

The literature refers to input and output of N in the rumen as being the net balance between absorption of NH_3 from the rumen and recycling of N to the rumen. According to Owens and Zinn (1998), when CP inclusion in cattle diets is below 13% (DM basis), the protein output from the rumen to the small intestine generally exceeds input from the diet, and the opposite is true when dietary crude protein is above 13% (DM basis). Recycled N enters the rumen through saliva or by diffusion or transport from the blood stream through the ruminal wall. Thereby, ruminant animals are capable to survive in a N-limited environment without essential amino acids in the diet because microbes synthesize essential amino acids through MCP. However, MCP does not provide sufficient amino acids to meet the needs for rapid growth and high production which is dependent on dietary supply. In the rumen, most microbes (bacteria

are majority present) use NH_3 as a source of N. However, previous research has shown that ruminal bacteria use amino acids or peptides as a major source of N, being able to survive in critical conditions with NH_3 as the sole N source (Karsli and Russel, 2002). The concentration of ruminal NH_3 increases with increased degradation of dietary protein and NPN, hydrolysis of urea that is recycled to the rumen, and intestinal digestion of MCP. On the other hand, NH_3 decreases in the rumen with increased N uptake from the microbes, increased NH_3 passage to the abomasum, and increased absorption through the ruminal wall. Ruminal NH_3 concentration changes with time after feeding, achieving earlier concentration peaks depending upon the N source in the diet (Pichard and Van Soest, 1977). As ruminal NH_3 concentration increases, NH_3 absorption from the rumen increases as well. The NH_3 present in the blood stream is detoxified primarily by the liver via the urea cycle and can be reutilized by the organism or excreted via urine. Importantly, low concentrations of NH_3 in the rumen may also decrease bacterial efficiency due to NH_3 starvation, negatively affecting digestion rate and feed intake of the animal (Owens and Zinn, 1998). Therefore, diets with low protein inclusion affect growth performance in cattle.

The continuous N recycling from the blood stream to the rumen is affected by ruminal NH_3 concentration. Two possible routes for urea entering the rumen are through saliva secretion and ruminal wall diffusion. Urea that passes through diffusion from the blood stream is converted to NH_3 and CO_2 by bacterial urease enzymes present in the ruminal epithelium. The lower ruminal pH induces urea flow from blood to the rumen and converts it to ammonium ion (NH_4^+), therefore capture of NH_4^+ increases as the ruminal pH decreases. Inside the rumen, the NH_4^+ is less readily absorbed through the ruminal wall to the bloodstream than NH_3 because of the lower lipophilic nature of NH_4^+ across cell membranes. Therefore, reduction in N recycling

rates occurs with high ruminal NH_3 concentration due to inhibition of urease in the ruminal wall or by decreasing the diffusion gradient for ruminal NH_3. Contrastingly, feeding urea generally increases ruminal pH due to stimulation of VFA absorption and increases NH_3 concentration in the rumen, resulting in NH_3 absorption through the rumen wall due to the diffusion gradient. In addition, no animal tissue produces urease enzyme but 10-15% of the bacteria present in the rumen produce it (Owens and Zinn, 1998). Therefore, shifts in the rumen microbial species may affect urea hydrolysis rate. The amount of N that will be recycled through saliva depends upon blood urea concentration and saliva production. Salivary secretion is increased by promoting chewing activity with high fiber diets in dairy cows (Beauchemin, 2018). Therefore, salivary N recycling is stimulated by inclusion of fibrous dietary sources in the diet.

Clinical indicators of NH_3 toxicity in ruminants include ruminal NH_3 concentration above 1000 mg/L, ruminal pH above 8, and blood plasma NH_3 above 20 mg/L (Owens and Zinn, 1998). Feeding excessive levels of urea (e.g., NPN) to cattle increases the production and absorption of NH_3 to the bloodstream resulting in toxicity. Ammonia concentration is increased in the rumen by microbial fermentation of urea. Ruminal pH increases due to greater VFA absorption, changing the epithelial concentration gradient which allows greater NH_3 levels to reach the liver through the bloodstream. In the liver, starvation of ATP supply stops the urea cycle which is responsible for making NH_3 into urea. Destabilization of the nervous stimulus on neurons affects synthesis and release of neurotransmitters resulting in depression of the central nervous system and possible future death (Antonelli et al. 2009). However, adequate use of supplemented urea has shown to increase microbial digestibility and growth performance in ruminants (Zanetti et al. 2000; Khattab et al., 2013).

Supplementation of dietary urea to ruminants is a feeding strategy that often results in reduced costs associated with protein supplementation while maintaining production efficiency. Feed grade urea has an average of 46% N = 287% CP (DM basis) and is the most commonly used source of NPN because it has the cheapest cost per unit of N. The use of dietary urea provides NH_3 to ruminal bacteria which can be incorporated into MCP synthesis. Usually, cattle fed with urea-based diets have shorter but more frequent meals (Owens and Zinn, 1998). In addition, feed intake and growth performance of cattle generally is slightly reduced for about the first month when animals are introduced to a diet containing urea, with a later improvement in growth performance (Owens and Zinn, 1998). It is hypothesized that this effect on growth performance may be caused because of a shortage in the supply of dietary amino acids from the protein source that reaches the small intestine until the microbes adapt to the urea and MCP is efficiently synthesized (Owens and Zinn, 1998). However, the reason for the microbial adaptation to the dietary urea remains unclear due to the knowledge that urea is continuously recycled to the rumen even without dietary supplementation. Therefore, initial feed intake may be affected when cattle are fed urea-based diets, and the fluctuation on growth performance remains unclear.

Microbial growth in the rumen is dependent on the balance between NH_3 concentration and energy availability. Consequently, MCP synthesis is dependent of how fast or slow ruminal NH_3 is released. However, previous studies have shown that NH_3 production and utilization in the rumen are not coordinated in time and that its concentration has a large fluctuation especially the first five hours after feeding (Bergen and Owens, 1985). During NH_3 concentration peaks, N recycling to the rumen apparently compensates for rapid NH_3 release, providing non-toxic concentrations. Furthermore, past research in cattle has failed to prove N utilization

improvements by slowing NH_3 release rates to be closer to where energy becomes available for bacterial growth (Owens and Zinn, 1998). Therefore, slowed NH_3 released rates may not improve N utilization and animal growth performance; however, it certainly helps to avoid NH_3 toxicity in cattle.

1.2.2.3. Nutrients Synchronization and Microbial Crude Protein

Optimum balance between protein and energy in ruminant diets is important to support high performance and production in cattle. Efficient ruminal microbial growth and optimum MCP synthesis is dependent on the ruminal synchronization between N and energy availability from dietary protein and carbohydrates (Kebreab et al., 2002; Neto et al., 2007). Previous studies have shown enhancement in microbial efficiency by capturing N and utilizing ATP for microbial growth with the synchronization of N and energy in the rumen (Herrera-Saldana, 1989). Additionally, Caton et al. (1993) reported that MCP synthesis is influenced by energy and nitrogen source, as well as microbial species present in the rumen. Furthermore, factors such as N and carbohydrate sources, ratio of forage and concentrate in the diet, dry matter intake of the animal, and synchronization of N and simultaneous release of energy affected MCP synthesis (Hoover and Stokes, 1991). Polan (1988) reported that insufficient N availability affects ruminal microbial growth resulting in uncoupled protein fermentation, therefore ATP is not efficiently used and energy is lost as heat. Additionally, previous studies have shown that ewes fed diets with 7% CP (DM basis) increased MCP synthesis due to infusion of 22 g/d of NPN into the rumen (Obtisu et al., 1992). In order to have optimum MCP synthesis, the N requirement of ruminal bacteria needs to be met (Karsli and Russel, 2002) because generally dietary protein is more rapidly fermented than dietary carbohydrate (Suhada et al., 2016). The carbohydrate profile of diets is also important to microbial efficiency. Voigt et al. (1993) fed different starch sources

to cattle and reported differences in ruminal microbial growth and amino acid composition flowing to the duodenum. According to Tamminga et al., (2007), the optimal N and energy ratio to achieve maximal microbial growth and minimum N loss in dairy cattle is 32 g of N/kg of starch. Furthermore, ruminant diets containing a mixture of structural and non-structural carbohydrates may affect MCP synthesis due to microbial diversity in the ruminal environment. In addition to NPN available in the rumen, a synchrony of amino acids and peptides and structural and non-structural carbohydrates sources are necessary for optimal MCP synthesis and amino acids supply to the small intestine.

A characteristic of mammalian tissues is the inability to synthesize essential amino acids, relying on exogenous amino acids sources such as MCP synthesis plus RUP supply. Protein requirements in ruminants can be difficult to assess because of ruminal fermentation of dietary amino acids, and differences in absorption of amino acids from the alimentary tract. Generally, the approach used to assess essential amino acids requirements in cattle is post-ruminal infusion of the most limiting amino acid to determine the optimum level based on N balance, plasma amino acids, urea concentration and excretion, feed intake and milk production (Owens and Zinn, 1998). By estimation of the difference between the quantity of amino acid supplemented and the quantity flowing through the large intestine the estimated total amount required is obtained. However, it should be taken into account that essential amino acid requirements are influenced by growth rates of the animals (NASEM, 2016).

Synthesis of MCP can be limited by truly fermentable organic matter. Some specific peptides and amino acids released during protein digestion serve as branched-chain volatile fatty acids (BCVFA) which are growth factors for cellulolytic bacteria. Therefore, fiber digestion is dependent on BCVFA supply either from the diet or other microbes in the rumen. Ruminal

deficiency of BCVFA, NH_3 and other nutrients may decrease ATP availability due to decreased fiber digestion. Some ruminal bacteria can grow without a carbohydrate source for energy. These bacteria use carbon structures from amino acids. Previous studies have shown that bacteria which use amino acids as an energy source often have a two-phase growth pattern whom provided low levels of free amino acids plus NH_3 (Owens and Zinn, 1998). Rapid growth occurs as long as amino acids remain available, but growth rate slows as availability of amino acids decrease and the bacteria use N from NH_3. Bacteria can also shift from MCP synthesis to intracellular polysaccharide synthesis which is also an ATP demanding process when ruminal N is small. In N-limiting conditions, over 33% of the potential anaerobic ATP yield from glucose is used to store the glucose as a polysaccharide (Owens and Zinn, 1998).

1.2.2.4. Post-ruminal Digestion and Metabolizable Protein (MP)

Pancreatic secretion of enzymes such as proteases (e.g. trypsin and chymotrypsin) can increase as the flow of protein increases to the small intestine. Previous studies in ruminants have shown that the small intestine has a high digestive and absorptive capacity for protein because neither digestion nor absorption of protein has been exceeded when infused at very high levels of protein (Owens and Zinn, 1998). Apparent small intestinal digestion of N compounds in ruminants has been estimated to be between 65-75% of duodenal N flow (Santos et al., 1984). Supply and digestibility rates of materials in the small intestine must be considered separately from each other (Owens and Zinn, 1998). The more a material is fermented in the rumen, the less of that material reaches the small intestine. Past research has hypothesized that increasing potential digestion rate in the rumen by feed processing, may increase potential digestion rate of material that reaches the small intestine (Owens and Zinn, 1998). Contrastingly, Huang et al. (2015) reported that feeding pellets to dairy cattle increased CP degradation in the rumen, shifted

protein digestion site from the small intestine to the rumen, and affected MP supply to the small intestine because of reduced RUP availability and altered ruminal N to energy synchronization. Likely, these results were observed because after the pelleting process (heat, moisture, and pressure) feed particles are larger and may be more digestible to the ruminal microbes. Therefore, processing feed strategically to decrease protein solubility (e.g. heat treatment) and reducing ruminal microbial attack may increase proportional amount of protein digested in the small intestine. Interestingly, a unique characteristic of ruminants is the greater volume of pancreatic ribonuclease enzyme secretion which results in about 80% of small intestinal digestion of microbial nucleic acids (Owens and Zinn, 1998). This enzymatic post-ruminal RNA degradation contributes to N recycling and MP supply.

Metabolizable protein is the true protein (amino acids and peptides) absorbed from the small intestine, supplied by MCP, RUP, and epithelial sloughed cells (Das et al., 2014); which is available to the animal for maintenance, growth, and production. Differently from metabolizable energy and N balance which are calculated by difference, MP values are estimated either by summing all protein outputs from the rumen that are absorbed (Van Soest, 1982) or by differences between protein flowing from the duodenum to the ileum. Several feeding systems have been developed to use current knowledge of N metabolism in ruminants to formulate diets (Tedeschi et al., 2015). However, predicting MP requirements in ruminants is still not fully understood and more research is needed to improve current feeding systems.

1.2.2.5. Starch and Amino Acid (AA) Effects on Pancreatic Enzymes

Digestion of starch may be limited in the small intestine of cattle. Post-ruminal digestion of nutrients that flows to the small intestine begins with the secretion of pancreatic enzymes. As reported by Harmon et al. (2004), the starch assimilation process begins in the duodenum with

the secretion and action of the pancreatic α-amylase enzyme. According to Owens et al. (1986), starch digested in the small intestine provides 42% more energy than starch fermented in the rumen. Therefore, improving post-ruminal starch digestion is an important goal to future research aiming for productivity improvements. However, it is hypothesized that greater inclusion of starch in finishing diets may limit starch digestion due to greater starch flow to the small intestine, resulting in inadequate pancreatic α-amylase secretion (Harmon, 1992). Interestingly, greater post-ruminal flows of starch in dairy cattle decreased efficiency of starch digestion in the small intestine (Nocek and Tamminga, 1991). Apparently, greater flow of starch to the small intestine of calves affects pancreatic secretions (α-amylase) which are responsible for starch hydrolysis (Kreikemeier et al., 1990; Swanson et al., 2002). However, small intestinal infusion of casein has been shown to increase α-amylase secretion in the presence of starch in ruminants (Wang and Taniguchi, 1998). Swanson et al. (2002) reported that greater small intestinal protein flow enhanced pancreatic weight as well as α-amylase and trypsin activity in calves. Interestingly, previous reports in ruminants have shown or suggested effects of protein (i.e., casein) as a regulator of pancreatic secretory processes (Richards et al., 2003; Swanson et al., 2004; Brake et al., 2014). Different sources of protein that reaches the small intestine may influence pancreatic secretions. Guilloteau et al. (2011) reported that twice as much trypsin was required after feeding milk formula based on soybean concentrate when compared to skim milk powder to obtain maximal intestinal nutrient digestibility in milk-fed calves. Gastrointestinal hormones were thought to influence enzymatic secretion by the pancreas due to complex interactions with absorbed nutrients; however, changes in hormone concentration have been shown to not be correlated with pancreatic enzyme secretion (Swanson et al., 2004). Therefore,

further research is needed to better define the mechanisms regulating exocrine pancreatic function in ruminants.

Stimulating the pancreas to produce greater quantity of digestive enzymes may be an effective alternative to improve production in cattle by improving nutrient utilization. Furthermore, Gressley et al., (2011) reported that undigested starch that passes through the ileal-cecal junction may be fermented in the large intestine resulting in energy waste and potentially large intestinal acidosis.

Differently from non-ruminants, which have large fluctuations in digesta flow to the small intestine, ruminants have a more continuous flow of carbohydrates and MP supply to the small intestine which is thought to minimize daily fluctuations in pancreatic juice secretion (Merchen 1988). Ruminants have small concentrations of pancreatic enzymes at birth, but enzyme concentrations and exocrine secretions increase as the animal matures (McCormick and Stewart 1966; Siddons 1968). Pancreatic exocrine cells (acinar cells) are responsible for the production of enzymes such as amylase, lipase, and proteases. These enzymes are important for the post-gastric digestion in the gastrointestinal tract of ruminants and non-ruminants. Several previous studies have reported potential regulation of synthesis and secretion of pancreatic enzymes by amino acid flow (e.g. leucine, isoleucine, phenylalanine, and glutamate) to the small intestine of cattle (Blom et al., 2016; Guo et al., 2018; Guo et al., 2018b).

Appearance of high-quality protein in the small intestine of ruminants has been shown to influence post-ruminal starch digestion and α-amylase secretion by the pancreas. Indeed, Brake and Swanson (2018) reported that several authors speculate that asynchrony between AA and carbohydrate flow through the gastrointestinal tract may limit small intestinal starch digestion. Interestingly, individual flow of AA such as glutamic acid (glutamate) to the small intestine has

increased small intestinal starch digestion in ruminants (Brake et al., 2014; Blom et al., 2016; Brake and Swanson, 2018). Additionally, Windmueller and Spaeth (1975) have reported that apparently all absorbed dietary glutamate and glutamine are metabolized by the small intestine of rats as metabolic fuels for the small intestinal epithelium; however, the mechanism of action of AA on pancreatic secretion is not fully understood in cattle.

It is hypothesized that the mechanism of α-amylase synthesis in calves and other species is under transcriptional (mRNA) control which is regulated via signaling pathways (e.g. mTOR) (Swanson et al., 2002; Guo et al., 2018b). These signaling pathways are responsible for controlling protein synthesis into cells, including pancreatic cells (Wu, 2009). Therefore, greater amino acid flow to the small intestine is hypothesized to affect mTOR signaling in pancreatic cells, increasing synthesis of protein thereby stimulating pancreatic enzymes production.

1.2.2.6. Mechanisms Regulating Feed Intake and Feeding Behavior of Cattle

Mechanisms regulating feed intake in ruminants are complex and not well defined. However, previous studies have shown that ruminal digesta disappearance rate, ruminal capacity, and chemostatic mechanisms may be major factors regulating feed intake (Jones, 1972). A general negative relationship between feed intake and energy concentration of the diet was proposed by Montgomery and Baumgardt (1965). This hypothesis suggests that rumen distention (physical capacity) is related to low nutritive dietary values and chemical mechanisms are related to high nutritive dietary values (Figure 1). Therefore, feed intake of ruminants peaks at a point where dietary energy plays a larger role in regulating DMI than distension, decreasing thereafter; even though increased dietary energy concentration of the diet results in energy intake remaining constant with less feed intake.

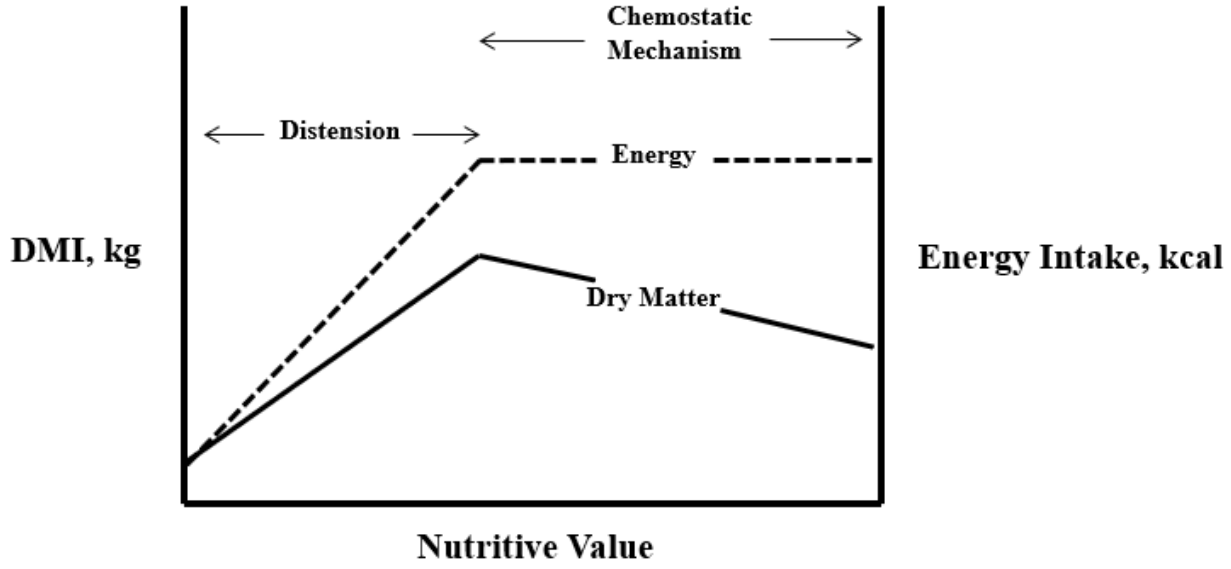

Figure 1. Effects of dietary nutritive value on feed intake of ruminants. Adapted from Montgomery and Baumgardt (1965).

Receptors which are present in several tissues and in the gastrointestinal tract of ruminants are responsible for numerous feedback signals such as rumen distention, VFA and free fatty acids concentration, etc. (Baumgardt, 1970). The hypothalamus receives these signals and to maintain energy balance, regulates feed intake of the animal. Additionally, Grant and Albright (1995) have reported that external factors such as palatability, social interactions, individual learning behavior of the animal, cattle management, and environment modulate feed intake and feeding behavior. Therefore, producers should have high quality facility and feeding management programs to maximize intake in finishing cattle. Ruminal fill and chemostatic mechanisms are a function of body size, age, and physiological state of the animals (Grant and Albright, 1995). Gaining a better understanding of how nutrition and management programs influence feeding behavior and how these changes in feeding behavior influence production efficiency is needed so that new approaches for improving production efficiency can be developed.

1.3. Literature Summary

Oftentimes feed intake and feeding behavior of cattle are determinative to the success of feedlot industry. Digestibility of starch and protein are important variables that contribute to production efficiency in ruminants and may affect feeding behavior of steers. Greater productivity may be reached with improvements in supply of metabolizable energy and metabolizable protein to cattle. However, there is a lack of research about how protein affects feeding behavior, and how amino acid flow to the small intestine impacts pancreatic secretion which may affect post-ruminal starch digestion. Therefore, the objectives of these studies were to 1) determine the effect of metabolizable protein intake on growth performance, carcass traits, and feeding behavior of finishing steers (Chapter 2), and to 2) investigate the effects of post-ruminal flows of glutamic acid or casein on pancreatic amylase and trypsin activity in steers (Chapter 3).

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2. THE EFFECT OF METABOLIZABLE PROTEIN INTAKE IN FINISHING STEERS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, AND FEEDING BEHAVIOR

2.1. Abstract

One-hundred thirty-two steers (316 ± 3.1 kg of BW) were used to study the effect of metabolizable protein intake in finishing steers on growth performance, carcass characteristics, and feeding behavior. Steers were stratified by initial BW across five pens and randomly assigned to one of four dietary treatments (626, 906, 1209 and 1444 g MP/d; n = 33 per treatment). Average daily gain responded quadratically ($P < 0.01$) with ADG increasing in steers fed 906 g MP/d and plateauing thereafter. Dry-matter intake (kg) responded quadratically ($P = 0.009$) with DMI increasing with MP intake up to 1209 g/d MP and decreasing thereafter. Gain:feed increased linearly ($P = 0.04$) and tended ($P = 0.10$) to respond quadratically, as G:F increased up to 906 g MP/d. A quadratic response ($P = 0.04$ and $P = 0.02$, respectively) was observed for marbling score and 12th rib subcutaneous fat thickness with steers fed 1209 g MP/d having the greatest marbling score and back fat thickness. For feeding behavior, a visit was defined as each time the Insentec system detected a steer at the feed bunk. A meal was defined as eating periods by intervals no longer than 7 min. A quadratic effect for visits and meals per day was observed ($P < 0.01$) with steers fed 1209 g MP/d treatments having the least visits and meals per day. Additionally, time eating per visit responded quadratically ($P = 0.05$) with time increasing from 626 to 906 g MP/d. There was a linear increase ($P \leq 0.02$) in time eating per meal and per day with increasing MP intake. A quadratic effect ($P < 0.03$) was observed for DMI per visit, meal, and minute with steers fed 1209 g MP/d having the greatest DMI. In summary, steers fed 626 g MP/d had increased visits and meals per day. However, DMI per visit, meal, and

minute was greater in steers fed 1209 g MP/d. These data indicate that MP supply (from deficient to excess) influences growth performance, carcass characteristics, and feeding behavior of finishing steers.

2.2. Introduction

Feed costs represent the most expensive component in a feedlot production system (Lanna et al., 1999). Dietary protein is often the nutrient that has greater cost per unit compared to dietary starch, being responsible for a large contribution to the ration cost. Protein supplementation to ruminants often increases growth performance, which makes protein a limiting nutrient for production (Medeiros and Marino, 2015). However, excessive use of protein in finishing diets leads to economic losses and environmental implications due to the excess of nitrogen (N) excreted by the animals (Amaral et al., 2018). In addition, N excretion in ruminants is not only related to N intake, but also microbial efficiency which influences metabolizable protein (MP) supply (Niu et al., 2016). Metabolizable protein is the true protein absorbed from the small intestine, supplied by microbial true protein, ruminal undegradable protein (RUP), and epithelial sloughed cells; which is available to the animal for maintenance, growth, and production (Das et al., 2014).

In the USA, the typical percentage of crude protein (CP) in growing and finishing cattle diets ranges from 13 to 17% DM (Samuelson et al., 2016). These concentrations usually exceed the metabolizable protein requirements for growing and finishing cattle according to the Nutrient Requirements of Beef Cattle (NASEM, 2016). Furthermore, previous studies have shown that exceeding 13% CP of DM in finishing diets might not improve growth performance (Gleghorn et al., 2004). In addition, increasing inclusion of CP in finishing cattle diets from 13 to 20% CP of

DM decreased energetic efficiency, which may also have a potential negative impact on growth performance due to decreased production efficiency of steers (Hales et al., 2013).

Under typical feeding conditions, decreasing CP concentrations in finishing diets could potentially cause adverse effects on DMI and animal health (Galyean, 1996). Recent data have shown that increasing inclusion of CP in growing or finishing diets from 10 to 14% CP (DM basis) did not affect DMI (Amaral et al., 2018). However, Cole et al. (2003) reported that steers fed finishing diets containing 14% CP (DM basis) during the first 56 d had greater DMI than steers fed diets containing 12% CP (DM basis) during the same period. It is known that feeding behavior can be influenced in cattle fed finishing diets; however, there is a lack of research about how specific nutrients, such as protein influence feeding behavior in finishing cattle. The objectives of this project were to determine the effect of metabolizable protein intake on growth performance, carcass traits, and feeding behavior of finishing steers.

2.3. Materials and Methods

2.3.1. Animals, Experimental Design, and Dietary Treatments

All procedures with animals were approved by the North Dakota State University Animal Care and Use Committee. One-hundred thirty-two steers (316 ± 3.1 kg of BW) predominantly of Angus, Simmental and Shorthorn breeding were stratified by initial BW across five pens (n = 26 to 32 steers/pen). Each pen contained 8 automated feeders (Insentec; Hokofarm B.V. Repelweg 10, 8316 PV Marknesse, The Netherlands) and each diet was delivered to 2 troughs per pen. Steers were randomly assigned to 1 of 4 dietary treatments (n = 33 steers/treatment; actual MP intake): 1) 626 g MP/d intake, 2) 906 g MP/d intake, 3) 1209 g MP/d intake, and 4) 1444 g MP/d intake (Table 1). Diets were formulated using the Beef Cattle Nutrient Requirements Model (BCNRM) software (NASEM, 2016). Individual predicted MP requirement was 880 g MP/d

intake (NASEM, 2016). Treatment 2 was designed to meet the RDP requirement. The supply of predicted metabolizable protein intake was formulated to differ by a similar amount between adjacent dietary treatments. After completion of the experiment, actual MP intake was calculated using nutrient analysis and DM intake data (NASEM, 2016). Diets were offered for ad libitum intake, and the steers had free access to water. Steers were adapted to experimental diets by transitioning from 60% to 90% concentrate diets over 28 d. Steers were implanted with 4 mg of estradiol and 20 mg of trenbolone acetate (Revalor-XS; Merck Animal Health, Whitehouse Station, N.J.) at d 0 of the experiment.

Table 1. Diet and nutrient composition of the dietary treatments

Item	Treatment, g/d of MP intake			
	626	906	1209	1444
Ingredient, % of DM				
Corn	77.7	77.7	57.1	36.0
Corn silage	10.0	10.0	10.0	10.0
Wheat straw	5.00	5.00	5.00	5.00
DDGS	-	-	21.8	44.0
Corn oil	2.30	2.30	1.10	-
Urea	-	1.50	1.50	1.50
Limestone	1.80	1.80	1.80	1.80
Salt	0.24	0.24	0.24	0.24
Fine ground corn	2.87	1.37	1.37	1.37
Vitamin premix[1]	0.01	0.01	0.01	0.01
Trace mineral premix[2]	0.05	0.05	0.05	0.05
Monensin premix[3]	0.02	0.02	0.02	0.02
Tylosin premix[4]	0.01	0.01	0.01	0.01
Nutrient analyses[5]				
Dry matter (DM), %	73.3	73.8	74.2	74.1
Organic matter, % of DM	95.2	95.2	94.2	93.5
Metabolizable protein[6], g/d	626	906	1209	1444
Crude protein, % of DM	7.84	11.7	17.2	20.9
Neutral detergent fiber, % of DM	25.9	24.9	31.0	34.7
Acid detergent fiber, % of DM	10.7	10.3	12.6	13.8
Fat (ether extract), % of DM	4.89	4.80	5.02	4.57
Calcium, % of DM	0.54	0.56	0.57	0.56
Phosphorus, % of DM	0.27	0.26	0.42	0.53
Sulfur[6], % of DM	0.10	0.10	0.22	0.34

[1]Contained 48,510 kIU/kg vitamin A and 4,630 kIU/kg vitamin D.

[2]Contained 3.62% calcium (Ca), 2.56% copper (Cu), 16% zinc (Zn), 6.5% iron (Fe), 4% manganese (Mn), 1,050 mg/kg iodine (I) and 250 mg/kg cobalt (Co).

[3]Contained 176.4 g of monensin/kg premix.

[4]Contained 88.2 g of tylosin/kg premix.

[5]Average of weekly samples.

[6]Calculated using tabular values reported in National Academies of Sciences, Engineering, and Medicine (2016).

2.3.2. Body Weight and Feed Intake Measurements

Body weight measurements were taken on two consecutive days prior to the beginning of the experiment and every 28 days throughout the experiment. Final BW was estimated using linear regression (days on feed × predicted ADG as predicted by the slope of the BW by day regression; average $r^2 = 0.98$).

A radio frequency identification tag was placed in the right ear of each steer before the beginning of the experiment to allow for use of the Insentec automated feeding system (Hokofarm B.V. Repelweg 10, 8316 PV Marknesse, The Netherlands). As previously described by Montanholi et al. (2010) and Wood et al. (2011), the Insentec automated feeding systems allows to offer specific dietary treatments and to monitor individual feed intake and feeding behavior characteristics of the animals. Feeding behavior measurements were quantified as described by Montanholi et al. (2010) as follows: events (number of daily visits and meals to the feed bunk), time eating in minutes (per visit, per meal, and per day), and feed intake in grams (per visit, per meal, and per minute). Feeding behavior data were summarized as the average of each individual steer over the entire experiment including the dietary adaptation period (28 d). A visit was defined as each time the Insentec system detected a steer at the feed bunk. A meal was defined as eating periods that might include short breaks separated by intervals no longer than 7 min (Forbes, 1995; Montanholi et al., 2010).

2.3.3. Feed Analysis

Diet samples were collected weekly throughout the experiment. Weekly samples were dried in a 55°C oven for at least 48 h and ground to pass a 1-mm screen. Weekly samples were analyzed for DM, ash, N (Kjehldahl method), Ca, and P by standard procedures (AOAC, 1990). In addition, weekly samples also were analyzed for NDF (assayed with heat stable amylase and

sodium sulfite and expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) concentration by the method of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology Corp., Fairport, NY). Percent CP was calculated by multiplying N concentration × 6.25. Samples also were analyzed for ether extract (AOAC, 1990). After weekly sample analyses from the entire experiment, average dietary composition results were updated into the feed composition table in the BCNRM software (NASEM, 2016). Updated feed composition and feed intake values provided the calculated metabolizable protein intake.

2.3.4. Carcass Characteristics

Steers were fed until they achieved an average BW of 599 ± 48 kg and marketed in 5 slaughter groups. The first group was fed for 172 d (n = 15), the second group for 179 days (n = 40), the third group for 186 days (n = 44), the fourth group for 195 days (n = 9), and the fifth group for 200 days (n = 24). After the fourth group was sent to slaughter, the remaining cattle were combined into 1 pen for the remainder of the experiment. Carcass characteristics were provided by the commercial slaughter facility; hot carcass weight data were measured right after slaughter of the animal, whereas marbling score, subcutaneous fat thickness at the 12th rib (back fat), longissimus area, and kidney, pelvic and heart fat percentage (KPH) were measured after carcass chilling.

2.3.5. Blood Collection and Plasma Glucose and Urea-N Analysis

Blood samples were collected by jugular venipuncture into Vacutainer tubes containing sodium heparin (Becton Dickinson, Rutherford, NJ) on d 0, 86, and 172 before feeding. Plasma was isolated by centrifugation at 3,000 × g for 20 min at 4°C and stored at –20°C until analysis. Plasma glucose analysis was performed using the hexokinase/glucose-6-phosphate dehydrogenase method (Farrance, 1987) with a kit from Thermo Scientific. Plasma urea-N was

determined using the urease/Berthelot procedure (Fawcett and Scott, 1960; Chaney and Marbach, 1962).

2.3.6. Statistical Analysis

Data were analyzed as a completely randomized block (slaughter group) design using the General Linear Model (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC) for growth performance, carcass traits, and feeding behavior data. Linear and quadratic effects of MP intake were tested using orthogonal contrast statements. Contrast coefficients were determined using the IML procedure of SAS. For plasma glucose and urea-N, data were analyzed as a randomized block (slaughter group) design with repeated measures and tested for the effects of treatment, day, and treatment × day using the Mixed procedure of SAS. Appropriate (minimize information criterion) covariance structures were utilized (Wang and Goonewardene, 2004). Data were considered statistically significant when $P \leq 0.05$ and trends were discussed at $0.05 < P < 0.10$.

2.4. Results

Average daily gain responded quadratically ($P < 0.01$) with ADG increasing in steers fed 906 g MP/d and plateauing thereafter (Table 2). Dry-matter intake (% of BW/day) was not different ($P > 0.10$) among treatments. However, dry-matter intake (kg/day) responded quadratically ($P = 0.009$) with DMI increasing with MP intake up to 1209 g MP/d and decreasing thereafter. Gain:feed increased linearly ($P = 0.04$) and tended ($P = 0.10$) to respond quadratically, as G:F increased up to 906 g MP/d. Longissimus muscle area was not affected ($P > 0.10$) by MP intake. Hot carcass weight responded quadratically ($P = 0.02$) as HCW increased to the greatest extent when MP intake increased from 626 g MP/d to 906 g MP/d with smaller increases thereafter. A quadratic effect ($P = 0.04$) was observed for marbling score with steers fed 1209 g MP/d having the greatest marbling score. In addition, there was a quadratic effect (*P*

= 0.02) for back fat thickness with steers fed 1209 g MP/d having the greatest back fat thickness and plateauing thereafter. Kidney, pelvic and heart fat percentage also responded quadratically (P = 0.01) as KPH increased from 626 g MP/d to 906 g MP/d intake and plateaued thereafter.

Table 2. Effect of metabolizable protein intake on growth performance and carcass characteristics of finishing steers

	Treatments, g/d of MP intake					Contrast *P*-value	
Item	626	906	1209	1444	SEM[a]	Linear	Quad.[b]
Average daily gain, kg/d	1.46	1.67	1.63	1.63	0.027	<0.001	<0.001
Dry-matter intake, % BW/d	3.05	2.98	3.03	2.98	0.045	0.34	0.81
Dry-matter intake, kg/d	9.19	9.79	9.92	9.64	0.170	0.03	0.009
Gain:feed, g/kg	0.15	0.17	0.16	0.17	0.003	0.04	0.10
Hot carcass weight, kg	341	362	366	370	3.668	<0.001	0.02
Marbling score[c]	431	481	500	479	17.25	0.02	0.04
Back fat, cm	0.91	1.22	1.34	1.32	0.068	<0.001	0.02
Longissimus area, cm^2	83.9	84.6	85.4	86.4	1.437	0.17	0.89
KPH fat[d], %	1.81	1.92	1.98	1.92	0.033	0.004	0.01

[a]Standard error of the mean where n = 33/treatment.
[b]Quadratic effects.
[c]For marbling score 400 = slight, 500 = small, 600 = moderate.
[d]Kidney, pelvic and heart fat.

A quadratic effect for visits (P = 0.002) and meals (P = 0.005) per day was observed with steers fed 1209 g MP/d having the least visits and meals per day (Table 3). Time eating per visit responded quadratically (P = 0.05) with time increasing from 626 to 906 g MP/d and plateauing thereafter. There was a linear increase in time eating per meal (P < 0.001) and per day (P = 0.02) with increasing MP intake. Dry-matter intake (g) responded quadratically per visit (P < 0.001), per meal (P < 0.001), and per minute (P = 0.03) with DMI being greatest when MP intake was 1209 g MP/d.

Table 3. Effect of metabolizable protein intake on feeding behavior of finishing steers

	Treatments, g/d of MP intake					Contrast *P*-value	
Item	626	906	1209	1444	SEM[1]	Linear	Quad.[2]
Events, per d							
Visits	36.1	29.5	24.4	27.0	1.45	<0.001	0.002
Meals	10.36	9.54	8.92	9.38	0.223	<0.001	0.005
Time eating, min							
Per visit	2.84	3.80	4.63	4.57	0.243	<0.001	0.05
Per meal	9.87	10.95	11.79	12.10	0.423	<0.001	0.43
Per day	101	103	104	112	3.2	0.02	0.27
Dry-matter intake, g							
Per visit	257	362	447	400	20.8	<0.001	<0.001
Per meal	906	1053	1140	1053	30.4	<0.001	<0.001
Per min.	94.7	97.8	98.7	88.7	3.12	0.23	0.03

[1]Standard error of the mean where n = 33/treatment.
[2]Quadratic effects.

A significant day × treatment interaction ($P < 0.001$) was observed for plasma urea-N concentration, and there was a day effect ($P < 0.001$) for plasma urea-N and glucose concentration (Table 4). No day × treatment interaction for plasma glucose concentration was observed. A day × treatment interaction ($P < 0.001$) was observed for plasma urea N as concentrations increased to a greater extent over time in the higher MP treatments than in the lower MP treatments (Figure 1). Interestingly, at d 86 plasma urea-N of steers fed 626 g MP/d and 906 g MP/d decreased to levels lower than initial concentrations, followed by an increase thereafter.

Table 4. Effect of metabolizable protein intake on blood metabolites concentration of finishing steers

	Treatments, g/d of MP intake					*P*-value		
Item	626	906	1209	1444	SEM[1]	Treat.[2]	Day	Treat. × Day
Glucose, m*M*	90.4	92.8	91.4	96.0	2.36	0.35	<0.001	0.74
Urea-N, m*M*	10.7	13.2	17.2	19.9	0.34	<0.001	<0.001	<0.001

[1]Standard error of the mean where n = 33/treatment.
[2]Treatments.

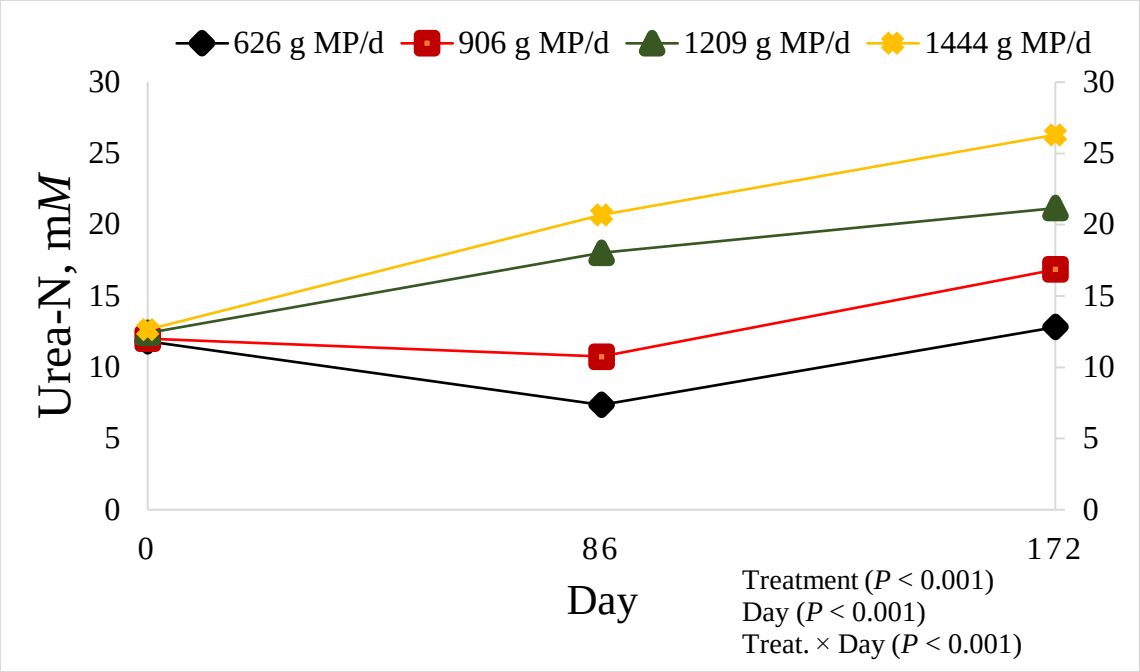

Figure 2. Effect of metabolizable protein intake on plasma urea-N of finishing steers.

2.5. Discussion

Optimization of metabolizable protein intake in finishing cattle diets is important for maximizing production and economic efficiency as well as minimizing environmental implications. The quadratic effect on ADG and the tendency for a quadratic effect on G:F likely occurred with steers fed 626 g MP/d having the lowest ADG and G:F because the MP supply was below the predicted requirements for finishing cattle gaining 2 kg/d (NASEM, 2016). Efficient ruminal microbial growth and optimum microbial protein synthesis may be impacted by ruminal synchronization between nitrogen and energy availability from dietary protein and carbohydrates (Kebreab et al., 2002; Neto et al., 2007). Limited dietary protein in the 626 g MP/d diet likely resulted in inadequate dietary nitrogen availability in the rumen, which may have also resulted in reduced energy utilization. Additionally, the low intake of RDP likely reduced microbial protein synthesis and amino acid supply (metabolizable protein) to the small intestine. The greatest ADG was observed in steers fed 906 g MP/d suggesting that the diet

provided adequate RDP as well as MP. Furthermore, ADG of steers that were fed 1209 and 1444 g MP/d diets did not decrease suggesting that the added energy required to excrete the excess nitrogen (Reed et al., 2017) must not have been great enough to negatively affect ADG of steers fed 1444 g MP/d.

Previous research has generally suggested increasing crude protein inclusion in finishing diets did not affect DMI (kg/day) in finishing cattle (Menezes et al., 2016; Amaral et al., 2018). However, our data showed that increasing MP intake in finishing diets quadratically influenced DMI (kg/day) with the steers fed 626 g/d MP having the least DMI. This may have occurred because these animals had lower ADG throughout the experiment and, therefore, weighed less. In addition, previous research suggested low supply of dietary N limits ruminal fermentation as well as passage rate of the digesta, which results in decreased feed intake (Campling, 1970). Additionally, past investigations have shown decreased DMI with increasing DDGS inclusion in finishing diets with at least part of the depression attributed to the high sulfur (S) content in DDGS-based diets (Klopfenstein et al., 2008; Felix et al., 2015). It is difficult to predict if the depression in intake at the greater MP intakes occurred because of increases in protein supply or other influences of the DDGS, such as dietary S concentration. However, corn oil was added to supply similar amounts of oil between treatments and, therefore, the observed effects were likely not the result of differences in oil concentration of the diets. In addition, Bach et al. (2005) have reported that there is a negative relationship between ruminal pH and bacterial N flow because of the increased supply of energy in the rumen from highly fermentable diets. Therefore, DMI of steers fed 1444 g/d MP might have been influenced by ruminal pH depression with greater DDGS concentration in the diet (Felix and Loerch, 2014) which may have decreased microbial

efficiency. Ruminal pH was not measured in this study, but no signs of ruminal acidosis or S toxicity were visually observed.

The increase in HCW in steers fed 906 g MP/d or greater was likely because of the observed greater ADG. These results are similar to past research in finishing bulls (Amaral et al., 2018) and in finishing lambs Ebrahimi et al. (2007). Similarly, quadratic effects were observed for marbling score, back fat thickness, and KPH in steers fed different MP intake. Protein and fat deposition in the carcasses of steers fed 626 g MP/d resulted in the lowest dressing percentage among treatments. It is likely that steers fed 626 g MP/d had insufficient RDP supply resulting in decreased microbial protein synthesis and VFA production in the rumen resulting in decreased protein and energy supply to the animal. An increase in plasma urea-N concentration of steers with increasing MP intake was observed. In ruminants it is estimated that up to 5% of energy lost as heat from the body can be attributed to urea synthesis in the liver (Huntington and Archibeque, 1999). Therefore, increasing MP supply may result in increased ureagenesis potentially influencing metabolizable energy (ME) use by ruminants. Interestingly, steers fed 1444 g MP/d had a decreased marbling score, back fat thickness, and KPH compared to steers fed 1209 g MP/d. This could suggest that steers fed 1444 g MP/d had greater ME expenditure towards urea synthesis and nitrogen excretion, having a negative effect on energy availability towards fat deposition.

Less is known about how metabolizable protein influences feeding behavior of steers. Quadratic effects for visits and meals per day were observed with steers fed 626 g MP/d having the greatest number of these events among the treatments. However, DMI per visit, per meal, and per minute was greater in steers fed 1209 g MP/d. The lower ruminal supply of N to the microbes in steers fed 626 g MP/d likely affected microbial efficiency, decreasing ruminal

digestibility and passage rate. Therefore, steers fed 626 g MP/d had more visits and number of meals possibly in an attempt to compensate for the RDP deficiency; however, DMI was lesser per visit and meal. Although steers fed 1209 g MP/d had greater DMI and less visits and meals per day, ADG was greater in steers fed 906 g MP/d. These results suggest that the depression in growth performance of steers fed 1209 g MP/d compared to steers fed 906 g MP/d could at least be in part the result of increased urea synthesis and nitrogen excretion influencing ME expenditure of the animal (Huntington and Archibeque, 1999). Ruminal pH is thought to be associated with feeding behavior (Gonzalez et al., 2012) and feeding behavior is influenced by feeding diets with different particle size (Swanson et al., 2014). In the current experiment, increasing MP intake quadratically influenced time eating per visit, and resulted in linear increases in time eating per meal and per day. Concomitantly with increasing total time eating, increasing MP intake of steers quadratically affected DMI (g/d). Interestingly, steers fed 1444 g MP/d consumed 10 g less per min when compared to steers fed 1209 g MP/d. This could be partially attributed to palatability and physical characteristics of the 1444 g MP/d diet, since the inclusion of 44% DDGS (DM basis) likely resulted in a diet having a smaller particle size (Swanson et al., 2014) when compared to the other diets. Alternatively, dietary protein could influence feed intake through chemostatic mechanisms regulated through the central nervous system (Hackmann and Spain, 2010; Allen, 2014). The transport of fuels such as VFA, glucose, lactate, and AA to the liver triggers brain feeding centers which is thought to affect feeding behavior in ruminants (Allen, 2014). Our results have shown that there were no effects on plasma glucose concentration among different MP intake treatments suggesting that glucose was not responsible for the observed differences in feeding behavior.

In conclusion, increasing from insufficient to high levels of MP intake of steers in finishing diets quadratically influenced growth performance, carcass characteristics, and feeding behavior. Steers fed 906 g MP/d had the greatest ADG while steers fed 1209 g MP/d had the greatest DMI. Hot carcass weight was greatest in steers fed 1444 g MP/d; however, the greatest marbling score, back fat thickness, and KPH was greatest in steers fed 1209 g MP/d. Steers that were fed 1209 g MP/d had the least visits and meals per day; however, had larger meals than the other steers. Additionally, steers that were fed 1444 g MP/d spent more time eating per day and had the slowest eating rate per minute. Therefore, data from the present study suggest that feeding steers 906 g MP/d in finishing diets supplied a sufficient amount of MP for the greatest growth performance and carcass characteristics. Interestingly, MP intake caused different responses on feeding behavior with the greatest effects observed in steers fed 626 and 1444 g MP/d. Additional research is needed to better understand how feeding behavior is affected by protein intake in finishing cattle which potentially could contribute to development of new feeding strategies.

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3. EFFECTS OF POST-RUMINAL FLOWS OF AMINO ACIDS ON SMALL INTESTINE STARCH DIGESTION IN BEEF STEERS

3.1. Abstract

Seventeen crossbred steers (188 ± 14 kg of BW), surgically fitted with duodenal infusion cannulas, were used to measure the effects of post-ruminal flows of glutamic acid or casein on pancreatic amylase and trypsin activity in steers. Steers were stratified in three replicates and randomly assigned to treatments. Steers were limit-fed a soybean hull-based diet at 1.5×NEm requirement for 0.48 kg/d gain of BW and exceeding MP requirements. Duodenal infusion treatments (14-L of aqueous solution containing 1,500 g/d cornstarch plus treatment) were water (control), glutamic acid (122 g/d), and casein (400 g/d); n = 5 to 6 per treatment. Steers were given a period of 35 d for surgical recovery and adaptation to the diet. After adaptation, steers were continuously infused with treatment for 58 d before tissue collection. At slaughter, the pancreas was weighed and a 10% subsample was collected and frozen for later analysis of total protein and enzymatic activities. Steers infused with casein had the greatest final BW (kg; P = 0.007) among all treatments. Concentration of α-amylase activity per gram of pancreas tended (P = 0.09) to be greater in steers duodenally-infused with casein. Furthermore, concentration of α-amylase activity per gram of protein was greatest (P = 0.04) in steers infused with casein. Content (kU/pancreas) of α-amylase activity tended (P = 0.07) to be greater in steers infused with casein. Additionally, α-amylase activity relative to BW (U/kg of BW) was greatest (P = 0.03) in steers infused with casein. When casein was infused, the ratio of α-amylase:trypsin activity was greater (P = 0.002) compared to the other treatments. In summary, steers infused with casein had greater α-amylase activity and a heavier final BW. These data indicate that post-ruminal casein infusion in beef steers increases α-amylase activity with no effect on trypsin

activity, whereas post-ruminal glutamate infusion did not influence pancreatic digestive enzymes.

3.2. Introduction

The feedlot industry relies heavily on grain inclusion in finishing diets (e.g., corn, barley, etc.) which contains a great amount of starch resulting in a high net energy availability for maintenance and production. Despite the majority of dietary starch being fermented in the rumen, when animals are fed high grain diets, significant amounts of starch reaches the small intestine (Owens et al., 1986; Harmon, 2009). Pancreatic α-amylase is responsible for the initial breakdown of starch to glucose in the small intestine (Wright, 1993). Additionally, trypsin is also released by the pancreas into the small intestinal lumen to break down protein to smaller peptides (Moran Jr., 2016). However, several researchers have suggested possible limitations in α-amylase secretion with the increase of starch flow through the small intestine of ruminants (Chittenden et al., 1984; Kreikemeier et al., 1990; Walker and Harmon, 1995; Swanson et al., 1998). Interestingly, greater amounts of pancreatic α-amylase and trypsin secretions were reported when proteins (e.g., casein) or amino acids were infused concomitantly with starch in the small intestine of cattle (Wang and Taniguchi, 1998; Swanson et al., 2002b; Yu et al., 2014). In addition, Brake et al. (2014b) reported that infusing casein increased small intestinal starch digestion but also increased flow of small intestinal α-glycosides. Interestingly, glutamic acid infusion increased post-ruminal starch digestion without increasing ileal flows of α-glycosides. Therefore, improvements in small intestinal starch digestion by duodenal casein and glutamic acid supply is thought to occur through different mechanisms (Brake et al., 2014b).

Clearly, there is a need for research about how protein and starch interact to affect pancreatic exocrine function and starch digestion in ruminants. Therefore, the objective of the

present experiment was to examine the effects of post-ruminal flows of glutamic acid or casein on pancreatic α-amylase and trypsin activity in steers.

3.3. Materials and Methods

3.3.1. Animals, Diet, Experimental Design, and Infusion Treatments

All procedures with animals were approved by the South Dakota State University Institutional Animal Care and Use Committee. Seventeen crossbred steers (188 ± 14 kg of BW) predominantly of British and Continental influenced breeds were surgically fitted with double L-shaped duodenal and ileal cannulas (Streeter et al., 1991; Brake et al., 2014). Steers were given a period of 35 d for surgical recovery and adaptation to the diet. From the adaptation period throughout the experiment steers were limit-fed a soybean hull-based diet (Table 1) formulated to supply 1.5 × NEm requirement for a steer gaining 0.48 kg/d, to exceed RDP and MP requirements (NRC, 2000), and steers had free access to water. Animals were housed and tethered individually in tie-stalls (1.7 × 1.2 m) in a controlled temperature (21°C) and light (16h light: 8h dark) environment.

Table 5. Composition of soybean hull-based diet[1]

Ingredients	% of DM
Soybean hulls	72.4
Brome hay	20.0
Corn steep liquor	6.0
Limestone	1.0
Salt	0.5
Mineral and vitamin premix[2]	0.1

[1]Diet formulated to supply 1.5 × NE_m requirement and to exceed RDP and MP requirements (NRC, 2000).
[2]Provided to diet (per kg diet DM): 50 mg of Mn, 50 mg of Zn, 10 mg of Cu, 0.5 mg of I, 0.2 mg of Se, 2,200 IU of vitamin A, 275 IU of vitamin D, and 25 IU of vitamin E.

Steers were stratified in three replicates and randomly assigned to 1 of 3 continuous duodenal infusion treatments (n = 5 per treatment; 14L of aqueous solution containing 1,500 g/d

cornstarch with either treatment): 1) water (control), 2) glutamic acid (122 g/d), and 3) casein (400 g/d). Treatments were continuously infused through Tygon tubing (2.38 mm i.d.; Saint-Gobain North America, Valley Forge, PA) with 14 L of aqueous solution using a peristaltic pump (model CP-78002-10; Cole-Parmer, Vernon Hills, IL). Two containers of treatment suspensions in aqueous solution were prepared daily immediately before infusion for use over 12 h intervals. Suspensions were maintained with continuous stirring by an electric mixer (Arrow 1750; Arrow Engineering Company, Hillside, NJ) and delivered at an infusion rate of 525 mL/h. Composition of cornstarch suspensions included 884 g of raw cornstarch (Common Starch 106; ADM Corn Processing, Clinton, IA), and deionized H_2O. The pH of the glutamic acid suspension was adjusted to near 7 with addition of 42.8 g of 40% (wt/wt) NaOH. The infusates were prepared daily by weight and the amount infused was determined by the weight of residual infusate after each 12-h period. To prevent accumulation of residual infusate, 100 ml of water was flushed through the tubing every 12 h.

3.3.2. Tissue Collection

At the conclusion of the infusion period (d 59), steers were weighed, and slaughtered after stunning via captive bolt for tissue collection. The total pancreas was removed, weighed and a sample of the body portion was collected (Swanson et al., 2002). Samples were frozen in liquid nitrogen.

3.3.3. Pancreatic α-Amylase and Trypsin Activity

After collection, pancreatic tissue that was in liquid nitrogen was stored at -80°C until analysis. Pancreas samples (0.25 g) were homogenized in 0.9% NaCl (2.25mL) using a polytron (Brinkmann Instruments Inc, Westbury, NY, USA). Protein concentration was determined using the bicinchoninic acid procedure with bovine serum albumin (BSA) used as the standard (Smith

et al., 1985). Using a commercial kit Teco Diagnostics (Anaheim, CA, USA) pancreatic α-amylase activity was determined through the procedure of Wallenfels et al. (1978). The method similar to previously reported by Geiger and Fritz (1986) was used for analysis of trypsin activity after activation with 100 U/L enterokinase (Swanson et al., 2008). Analyses were adapted for use on a microplate spectrophotometer (Synergy H1, Hybrid Multi-Mode Reader, Winooski, VT, USA). One unit (U) of enzyme activity equals to 1 μmol product produced per min. Enzyme activity data are expressed as U/g pancreas, U/g protein, kU/pancreas, and U/kg BW.

3.3.4. Statistical Analysis

Data were analyzed as a completely randomized block design using the General Linear Model (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC) for final body weight, total protein, and pancreatic enzymes activity data. The model contained treatment and replicates, and steer served as a random effect. The LSMEANS statement was applied to calculate treatment means. Data were considered significant different when $P \leq 0.05$ and trends were discussed at $0.05 < P < 0.10$.

3.4. Results

Final BW (kg) was greatest ($P = 0.007$) in steers infused with casein (Table 2) when compared to the control or glutamic acid infusates. Pancreatic mass (g and % of BW) were not different ($P > 0.05$) among treatments. There were no differences ($P > 0.05$) in total protein concentration and content (mg/g pancreas; g/pancreas; or mg/kg BW) among infusion treatments. However, the concentration of α-amylase activity per gram of pancreas (U/g pancreas) tended ($P = 0.09$) to be greater in steers infused with casein (Table 3). Additionally, concentration of α-amylase activity per gram of protein (U/g protein) was greatest ($P = 0.04$) in steers that received the casein treatment. When expressed as kU/pancreas, content of α-amylase

activity tended ($P = 0.07$) to be greater in animals that received casein infusion. Content of α-amylase activity relative to BW (U/kg BW) was greatest ($P = 0.03$) in steers infused with casein. Post-ruminal infusion treatment did not influence trypsin activity. The α-amylase:trypsin activity ratio was greater ($P = 0.002$) in steers that received the casein infusion treatment.

Table 6. Influence of duodenal infusion of casein and glutamic acid on BW, pancreatic mass and total protein in steers

Item	Treatment			SEM[2]	*P*
	Con.	Glut. acid[1]	Casein		
Final BW, kg	211.9[a]	211.9[a]	247.1[b]	7.30	0.007
Pancreas mass					
g	217.9	217.1	223.3	16.71	0.95
% of BW	0.103	0.103	0.093	0.008	0.68
Protein					
mg/g pancreas	111.0	108.4	87.81	10.90	0.29
g/pancreas	23.80	24.03	19.18	2.64	0.36
g/kg BW	1,137	1,145	7,932	1,493	0.19

[1]Glutamic acid.
[2]Standard error of the mean where n = 5.
[a,b]Data are presented as least square means per treatment ± SEM. Different letters in the same row signify means are different ($P < 0.05$).

Table 7. Effect of duodenal infusion of casein or glutamic acid on pancreatic α-amylase and trypsin activity in steers receiving 1.5 kg/d of duodenal infusion of raw cornstarch

	Treatment				
Activity	Con.	Glut. acid[1]	Casein	SEM[2]	*P*
α-Amylase					
U/g pancreas	94.2[a]	81.9[a]	228.3[b]	48.94	0.09
U/g protein	795.3[a]	820.2[a]	2553.2[b]	501.2	0.04
kU/pancreas	19.7[a]	17.2[a]	51.0[b]	10.77	0.07
U/kg BW	95.1[a]	81.2[a]	230[b]	39.70	0.03
Trypsin					
U/g pancreas	61.85	57.20	70.40	12.90	0.72
U/g protein	541.48	544.5	831.0	125.1	0.19
kU/pancreas	13.23	12.27	15.65	3.01	0.68
U/kg BW	62.77	57.88	71.87	13.05	0.69
α-Amylase:trypsin	1.36[a]	1.50[a]	4.29[b]	0.525	0.002

[1]Glutamic acid.
[2]Standard error of the mean where n = 5.
[a,b]Data are presented as least square means per treatment ± SEM. Different letters in the same row signify means are different ($P < 0.05$).

3.5. Discussion

Starch is often the major dietary energy source in the dairy or beef industry. Even though ruminants have limited ability to digest starch post-ruminally, measures of post-ruminal starch digestion are variable (35-60%) in comparison to the total amount of starch which enters the small intestine (Harmon, 2009). As previously reported by Owens et al. (1986), starch that is assimilated in the small intestine may offer 42% greater energetic efficiency to the animal than starch digested into the rumen. Improvements in pancreatic enzyme secretions responsible for small intestinal starch digestion might provide great benefits to beef and dairy production systems (Brake et al., 2014b; Yu et al., 2014). Therefore, this experiment was conducted to determine the effects of post-ruminal flows of casein and glutamic acid on pancreatic enzyme activity.

Final BW increased with post-ruminal casein infusion when compared to other treatments. Previous research have reported that protein infusion increased α-amylase activity and small intestinal starch digestion in cattle (Richards et al., 1997; Brake et al., 2014b). Even though visceral fat was not analyzed in the current study, a possible explanation is that the effect on final BW may be driven by greater metabolizable energy availability resulting in greater visceral fat deposition in steers. Several authors have reported that enhancing pancreatic protein concentration may increase small intestinal hydrolysis of nutrients (Corring, 1980; Swanson et al., 2000; Keomanivong et al., 2017). Additionally, Swanson et al. (2002) reported that post-ruminal casein flow enhanced pancreatic weight and enzymatic activities in calves. In the current study, final BW was greater in steers post-ruminally infused with casein; however, pancreatic mass (% of BW) and pancreatic protein did not differ among treatments. This result suggests that casein infusion likely improved digestion and utilization of nutrients in the small intestinal lumen without affecting pancreatic tissue growth.

Greater concentration of pancreatic α-amylase activity was observed in steers infused with casein which agrees with previous literature reported by Swanson et al. (2002). However, in the present study trypsin activity was not affected by post-ruminal infusion of casein. As expected due to α-amylase and trypsin activity results, the ratio of the concentration of pancreatic α-amylase:trypsin activity was greater in steers that received the casein infusion. This suggests that pancreatic α-amylase and trypsin may have different regulatory production mechanisms which are not fully understood yet and that post-ruminal protein may have a greater effect on α-amylase than trypsin activity.

Results obtained from the current experiment have demonstrated that casein infusion affects α-amylase activity in the pancreas without altering pancreatic mass in steers.

Additionally, casein flowing to the small intestine increased final BW likely due to greater post-ruminal starch digestion. Neither casein nor glutamic acid infusions affected trypsin activity suggesting that pancreatic enzymes have different regulatory mechanisms. Our results may support the theory suggested by Brake et al. (2014b) where casein and glutamic acid improved small intestinal starch digestion potentially by different mechanisms, where casein may facilitate greater hydrolytic capacity of α-amylase and glutamic acid may increase brush border α-glycosidase activity. A better understanding of the regulatory mechanisms of pancreatic enzymes may contribute to development of feeding strategies to improve starch digestion and metabolizable energy utilization in cattle.

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4. SUMMARY AND CONCLUSIONS

My first study indicates that increasing from insufficient to high levels of MP intake of steers in finishing diets quadratically influenced growth performance, carcass characteristics, and feeding behavior. The greatest ADG was observed in steers fed 906 g MP/d suggesting that this diet treatment provided adequate RDP as well as MP. Furthermore, ADG of steers fed 1209 and 1444 g MP/d diets did not decrease suggesting that if added energy was required to excrete the excess nitrogen it must not have been great enough to negatively affect ADG. Dry matter intake (kg/day) was quadratically influenced by increasing MP intake in finishing diets with the steers fed 626 g MP/d having the least DMI. This effect may have occurred because these animals had lower ADG throughout the experiment and therefore weighed less. Hot carcass weight increased in steers fed 906 g MP/d or greater likely because of the observed greater ADG. Steers fed 626 g MP/d resulted in the lowest dressing percentage among treatments. This effect on dressing percentage is likely because steers fed 626 g MP/d had insufficient RDP supply resulting in decreased microbial protein synthesis and VFA production in the rumen resulting in decreased protein and energy supply to the animal.

Regarding feeding behavior of the steers in the first experiment, DMI per visit, per meal, and per minute was greatest (quadratic effect) in steers fed 1209 g MP/d. The lower ruminal supply of N to the microbes in steers fed 626 g MP/d likely affected microbial efficiency, decreasing ruminal digestibility and passage rate. This may have been why steers fed 626 g MP/d had more visits and number of meals per day. Although steers fed 1209 g MP/d had greater DMI and less visits and meals per day, ADG was greater in steers fed 906 g MP/d. These results suggest that the depression in growth performance of steers fed 1209 g MP/d compared to steers fed 906 g MP/d could at least be in part the result of increased urea synthesis and nitrogen

excretion influencing ME expenditure of the animal. Interestingly, steers fed 1444 g MP/d consumed 10 g of DM less per min when compared to steers fed 1209 g MP/d. This could be partially attributed to palatability and physical characteristics of the 1444 g MP/d diet, since the inclusion of 44% DDGS (DM basis) likely resulted in a diet having a smaller particle size.

In the second experiment, results obtained demonstrated that post-ruminal casein infusion increases α-amylase activity in the pancreas without altering pancreatic mass in steers. Final BW increased with post-ruminal casein infusion when compared to other treatments. Even though visceral fat was not analyzed in the second study, it is speculated that the effect on final BW may be partially driven by greater small intestinal starch digestion and metabolizable energy availability resulting in greater visceral fat deposition. Interestingly, pancreatic mass (% of BW) and pancreatic protein did not differ among treatments. This result suggests that casein infusion likely improved digestion and utilization of nutrients in the small intestinal lumen without affecting pancreatic tissue growth. Greater concentration of pancreatic α-amylase activity was observed in steers infused with casein but there was no effect on trypsin activity, whereas glutamic acid infusion did not influence pancreatic digestive enzymes. This suggests that pancreatic α-amylase and trypsin may have different regulatory production mechanisms which are not fully understood.

Our results show that different metabolizable protein intakes play interesting roles on growth performance and feeding behavior in finishing cattle. These data leave room for future research to understand which mechanisms are involved in regulating feeding behavior. Development of feeding strategies based on predicted feeding behavior may minimize metabolic disorders and environmental implications, improve production efficiency, and decrease operational cost of finishing cattle. Furthermore, this research increases our knowledge on the

effects of amino acid flow to the small intestine on digestive enzymes. Little is known about how pancreatic enzyme production is regulated, especially since the potential to improve the efficiency of starch digestibility in ruminants. There is a vast research area available for future findings to better understand and manipulate exocrine enzyme secretion that could contribute to the development of more efficient feeding strategies.