Effect of manipulation of growth and fermentation patterns on nutrient availability and performance of feedlot cattle

A THESIS

SUBMITTED TO THE FACULTY OF

UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

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August 2019

ACKNOWLEDGEMENTS

My greatest passion in life has always been cattle, but that passion would not have grown into what it is today without the love and support of the people surrounding me. I am humbled and blessed to have the support and guidance of a wonderful group of friends, family, and mentors throughout the years.

First, I would like to thank my parents and sister for their continuous support. My dad, Steve, for his support of my passion for cattle and helping me to achieve all that I strive for in life. My mom, Michelle, for her support of my pursuit of higher education and her countless hours spent editing my papers even into my graduate school years. I cannot thank them enough as I would not be who I am today without them.

The sincerest of thanks is extended to my co-advisors Dr. Marshall Stern and Dr. Alfredo DiCostanzo. It is because of Dr. Stern that my passion for research began. I truly cherish his enthusiasm to bring me into his lab both as an undergraduate and Ph.D. student, and shape me to be the scientist I am today. Likewise, because of Dr. DiCostanzo's willingness to expose me to the feedlot industry, my knowledge has grown far beyond the science. I am truly thankful for the time he spent challenging me to look at the bigger picture as well as developing my passion for modeling and statistics.

I'd also like to thank my fellow lab mates, Alex Hohertz, Katie Nenn, Isaac Salfer, Allison VanDerWal, Josh Zeltwanger, and Trent Dado, for their support, guidance, and encouragement throughout my program. To my committee members, Dr. Grant Crawford, Dr. Brad Heins, and Dr. Doug Mashek, thank you for your patience and encouragement throughout this journey -- I will never forget your cookies again. And to

all the others who have worked to inspire, motivate, and support me throughout my graduate program and into my career, your presence in my life is invaluable and I am immensely grateful.

I would additionally like to acknowledge the Hueg-Harrison Fellowship, Land'O Lakes John Brandt Fellowship, Robert J Hartle Fellowship, Minnesota Corn Growers, Minnesota Soybean Growers, and AURI for funding my graduate program and research.

To Myron Larson,

Your daily sacrifices and dedication to the beef industry throughout your life continues to inspire me to reach for greater understanding

ABSTRACT

Alteration of ruminal environment by nutritional strategy presents opportunities for manipulating beef cattle growth. Changing fermentation patterns by altering feed type can impact both the composition and rate of gain in beef cattle. Three experiments were conducted to examine the effect of manipulation of growth and fermentation patterns on nutrient availability and performance of feedlot cattle. Experiment 1 studied the impact of ruminal environment on the passage of omega-3 fatty acids through the rumen of grass-fed verses grain-fed cattle. Omasal samples were collected from steers fed either fresh green chop, a high grain diet supplemented with flaxseed oil, or a high grain diet supplemented with corn oil. Regardless of diet, fatty acids with the greatest number of unsaturated bonds found in the highest abundance were transformed preferentially. Despite no differences ($P = 0.88$) in omega-3 transformation across treatments, grain-fed cattle with a flaxseed oil supplement demonstrated the greatest ($P < 0.01$) g of omega-3 fatty acid flow escaping biohydrogenation due to an increase in intake of dietary omega-3 fatty acids. Experiment 2 evaluated how utilization of bio-fuel byproducts impacts rumen environment and fatty acid profile at end point of fermentation. Omasal samples were collected from steers fed a steam flaked corn (SFC) basal diet with a portion of corn replaced by distillers grains (DGS) (40%), crude glycerin (GLY) (10%) or both. Total unsaturated fatty acids concentration in digesta were not different ($P = 0.43$) for the main effect of GLY, which indicates GLY is an effective alternative to corn when provided at the dietary concentrations evaluated in this study. The decrease ($P < 0.01$) in unsaturated fatty acids in digesta for main effect of DGS may be beneficial for shelf life stability of meat. Experiment 3 used a meta-analysis approach

to examine how nutritional strategy and performance during a post-weaning growing phase can be used to predict finishing performance and carcass characteristics. When examining feedlot and carcass performance attributes by growing strategy it factors, like $ADG_{growing}$, $DOF_{growing}$, and initial $BW_{finishing}$, interacted to create the most optimal compensatory gain response. Optimal combinations of $DOF_{growing}$ and initial $BW_{growing}$ differed between performance characteristics (Table 4.6) but as an average across dressing percent, LMA, Final $BW_{finishing}$, and HCW it was identified that maximized performance occurred when cattle began the growing phase around 240 kg.

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INTRODUCTION

Dietary fatty acid composition varies immensely depending upon forages and concentrates fed. Most fatty acids that contribute to the dietary lipid fraction, regardless of diet, are comprised of 16- or 18-carbon. These fatty acids, in particular omega-3 and omega-6 18-carbon chains, have been in the spotlight by the modern consumer. Consumers increasingly demand information on the fatty acid composition of ruminant food products, particularly those with polyunsaturated fatty acids (PUFA) that fall into the 18-carbon chain category. Various studies have proven that omega-3 and omega-6 fatty acid concentrations in beef and milk products are directly impacted by the diet of the animal (Wood et al., 2004).

Greater omega-3 concentrations of meat and milk have led some consumer groups to begin classifying omega-3 enriched meat and milk products as “functional foods”. Although omega-3 content of milk or meat products may increase, the amount of omega-3 fatty acids consumed from that product is not enough to be considered a significant intake source for omega-3 fatty acids. Instead, an increase in omega-3 content balances the omega-6 to omega-3 ratio of the consumers’ diet. Human dietitians recommend a ratio of 4:1 omega-6 to omega-3 (Daley et al., 2010). A typical American diet has an omega-6 to omega-3 ratio of around 15 to 20:1 (Simopoulos, 2006) mainly due to American’s high consumption of grain-based products. The increase in demand by

consumers for omega-3 rich products has led to a trend in cattle feeding research to understand the most effective method to incorporate omega-3 fatty acids from the diet into meat or milk.

A study conducted by Cherfaoui et al. (2011) determined that the long-chain PUFA associated with human health benefits, such as eicosapentaenoic acid (EPA; 25:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), can be synthesized in bovine liver, adipose, and muscle tissues if supplied with post-ruminal alpha linolenic acid (ALA; 18:3). This observation led to the conclusion that ALA leaving the rumen unaltered beneficially impacts omega-3 concentrations in meat and milk products. Increasing ALA content in the diet through grass feeding can shift this ratio in meat products from 6-7:1 to 2:1 (Nuernberg et al., 2005).

Impact of differences in dietary structure of polyunsaturated fatty acids

Alpha linolenic acid (ALA) is the most common dietary form of omega-3 consumed by cattle. In fresh grasses, approximately 50% to 75% of total fatty acids (FA) are linolenic acid (Hawke, 1973). Unlike grasses, cereal-based crops, which make up a majority of conventional Midwestern feedlot and dairy diets, are high in linoleic acid, an omega-6 fatty acid.

Based on dietary source, structure of fat varies, in turn altering efficiency of its rate of breakdown. Most cereal grains or concentrate sources store lipids in the form of triglycerides while lipids found in forage sources are galactolipids, sulpholipids, and phospholipids predominantly found within the chloroplast membrane . These lipids differ from triglycerides because they bind 2 fatty acid chains rather than 3 in

triglycerides. Thus, energy content per fat unit is higher for cereals (i.e.- triglycerides) than forages (i.e.-galactolipids, sulpholipids).

RUMINAL LIPID TRANSFORMATIONS

Triglycerides and forage lipids in the rumen are hydrolyzed by enzymes of rumen microorganisms (Garton et al., 1958; Dawson et al., 1977). Breakdown of fats by rumen microbes varies based on the form in which it is stored. This transformation occurs via two major processes in the rumen, lipolysis and biohydrogenation.

Microbial roles in lipid breakdown processes are differentiated by species-specific enzyme production and dietary fat structure. *Butyrivibrio fibrisolvens* is a known producer of phospholipase enzymes, establishing its role in lipolysis of forage-derived fat sources (galactolipids and sulpholids; Hazelwood and Dawson, 1975). While *Anaerovibrio lipolytica* plays a role in lipolysis of triglycerides (cereal lipids) but not phospholipids or galactolipids (Hobson and Mann, 1961; Prins et al., 1975). During lipolysis, microbial lipases work to breakdown lipids by hydrolyzing ester linkages within lipid structures to release fatty acids and expose a free carboxyl group (Garton et al., 1961; Dawson et al., 1977). The exposure of a free carboxyl group on a fatty acid is imperative for the next transformation process, biohydrogenation. The carboxyl group serves as an electronegative region for the lipase enzyme to bind hydrogen. When hydrogen is bound, there is a shift of electrons allowing isomerization to occur and saturation of double bonds to proceed (Harfoot and Hazelwood, 1988). Thus, transformation from the unsaturated to saturated form is complete.

Saturation of each unsaturated bond within a PUFA makes up a different phase of the biohydrogenation pathway. Dietary sources of linoleic acid and linolenic acid are transformed by rumen bacteria from an unsaturated structure to the saturated fatty acid stearic acid (18:0) via a pathway involving various isoforms (Figure A1.11; Wilde and Dawson, 1966; Kramer et al., 2004). Each phase of fatty acid transformation involves two distinct groups of bacteria, Group A and B, as classified by Kemp and Lander (1984). Group A hydrogenates polyunsaturated fatty acids to trans 18:1 isoforms, while Group B hydrogenates the trans 18:1 isoform to stearic acid (18:0) (Kemp and Lander, 1984; Harfoot and Hazelwood, 1997).

Role of Biohydrogenation in Rumen

Three primary theories on biohydrogenation have evolved to explain the purpose of lipid transformation in the rumen. One theory stemmed from observations by Hazelwood and Dawson (1979) about the two primary groups of bacteria involved in fatty acid transformations (Group A and Group B). Group A bacteria demonstrated the ability to incorporate trans-isoforms of linolenic or linoleic acids into membrane lipids. Therefore, suggesting that the biohydrogenation pathway served a role in the utilization of dietary fatty acids to synthesize membranes in certain bacterial species. Because these species make up such a small fraction of the total microbial population, it is unrealistic to think that supporting this small group is the reason behind such a significant ruminal process (Harfoot and Hazelwood, 1988). A more debated theory suggested by Lennarz (1966), is that biohydrogenation serves as a hydrogen disposal for bacteria requiring a reduced environment. Unsaturated bonds serve as a sink for free hydrogen in the rumen; therefore, this theory does hold merit. However, Harfoot and Hazelwood (1988) discredit

this theory, citing methanogenesis as a much more efficient process for disposal of excess reducing power (i.e.- hydrogen). The third and perhaps most widely known theory for the purpose of biohydrogenation is as a role in the detoxification of fatty acids (Kemp and Lander, 1984). Maia et al. (2010) supported this theory by showing a negative impact of linoleic and linolenic acid on cell growth and integrity of rumen microbes. By hydrogenating unsaturated fatty acids, PUFA are converted to a form useable by the rumen microbes and the inhibitory effects associated with unsaturated forms decrease. Within this third theory of biohydrogenation, there are three schools of thought from which the idea of toxicity of unsaturated fats stems:

1. Double bonds alter the shape of molecule and disrupt microbial lipid bilayer structure altering cell integrity
2. Chemiosmotic destruction of membrane potential causing ion leakage and /or decoupling intramembrane pathways
3. Metabolic pathway disruption

Knowledge that 0.5% of total cell protein of *Butyrivibrio fibrisolvens*, an active contributor to both lipolysis and biohydrogenation, is devoted to the reductase that converts conjugated linoleic acid (cis-9, trans-11 C18:2) to vaccenic acid (trans-11 C18:2) indicates a significant dedication of cellular resources to this process (Maia el al., 2010). This would imply a vital function of the biohydrogenation process. *Butyrivibrio fibrisolvens* cell wall structure has been shown to have an extremely thin cellular envelope (Cheng and Costerton, 1977). This evidence also supports toxicity theory one and two, knowing toxic effects would be greater for bacteria with less protection from surrounding environment. However, strong evidence exists in favor of theory three as

well. Paillard et al. (2007) determined that, in the presence of overwhelming amounts of unsaturated fatty (specifically linoleic acid) acids, there was a shift in utilization of ruminal CoA metabolic pools used to synthesize butyrate. Within the rumen, butyrate is synthesized via two separate enzymes; butyrate kinase and butyryl-CoA CoA-transferase (Figure A1.12). Strains of *B. fibrisolvens* were identified to carry the gene for either butyrate kinase or butyryl-CoA CoA-transferase. Strains that carry the gene for butyrate kinase were shown to have an increase in vulnerability to toxic effects of linoleic acid (Paillard et al., 2007). Thus, it was concluded that toxicity to unsaturated fatty acids is strain specific. However, to this point, no evidence has been provided to distinctly conclude if toxicity is caused from disruption of cell integrity, chemiosmotic changes, or metabolic pathway interruptions. Regardless, from this work it seems logical to conclude that the toxic effect of PUFA on rumen microbes is a primary driver behind need for biohydrogenation in the rumen.

Greater need for biohydrogenation exists in the rumen of grass-fed animals because *B. fibrisolvens*, particularly the cellulolytic-butyrate-producing strains, is present in greater concentrations in this environment. Greater rates of biohydrogenation are necessary in the rumen of grass-fed cattle because linolenic acid, the primary fatty acid found in grasses, has greater toxicity to rumen-butyrate producers than linoleic acid (Maia et al., 2010). It is estimated that approximately 85 to 100% of dietary ALA is biohydrogenated in the rumen, with 0 to 15% passing through unchanged (Doreau and Ferlay, 1994). In contrast, 70 to95% of dietary linoleic acid is biohydrogenated in the rumen. The increase in *B. fibrisolvens* population with fiber-based diets resulting from

greater dietary concentration of linolenic acid could explain why greater amounts of linolenic not linoleic acid are hydrogenated.

Within modern grain-based cattle feeding systems, greater supply of dietary linoleic acid concentration became a topic of interest as feeding ethanol coproducts such as distillers grains and corn oil increased. Ethanol is produced via fermentation of starch found within corn grain. By-products from this process consisting of the remaining fractions of corn grain are commonly utilized as feed sources for livestock. As the starch fraction is removed from the grain, the concentration of fat within the remaining by-products increases. Unsaturated fatty acids make up over 80% of the fat composition of corn, with over 50% of unsaturated fatty acids being linoleic acid. Hendersen (1973) demonstrated in pure culture that unsaturated fatty acids exhibited greater negative impacts on growth of cellulolytic bacteria than saturated fats. Various researchers observed a shift in rumen fermentation with an increase in supplementation of concentrations of fat in the diet (Czerkawski 1966a; Clapperton et al, 1969; Dinius et al, 1974; Zinn 1988). A decrease in ruminal acetate production with an increase in ruminal propionate and a decrease in methane production are consistent responses to addition of dietary fat (Clapperton et al., 1969). A decrease in growth of cellulolytic bacteria in response to supplementation of dietary fat explains the decrease in ruminal acetate concentration and lowered methane production (Rasmussen and Harrison, 2011). An increase in ruminal propionate concentration can be explained by increased lipolysis of triglycerides freeing glycerol backbones which ferment to propionate in the rumen (Wang et al., 2009). A decrease in enteric ruminal methane production with dietary PUFA supplementation is hypothesized to be a response to the double bonds within the fatty

acid serving as direct competition to other hydrogen utilizing mechanisms, such as methane production (Rasmussen and Harrison, 2011).

A compelling counter explanation for the impact of fat on rumen fermentation is that the fat source can physically coat the feed particles and retard feed exposure to microbial enzymatic attack. This coating effect is especially pertinent for fatty acids with a melting point that is near or exceeds the temperature of rumen (MacLeod and Buchanan-Smith, 1972; Chalupa et al., 1984). Zinn et al (2000) demonstrated that high concentrations of dietary fat (> 8% of diet DM) negatively impacted microbial nitrogen flow out of the rumen; an effect proposed to have resulted directly from a decrease in ruminal digestion of organic matter (OM).

To avoid detrimental impacts of high concentrations of dietary fatty acids on rumen fermentation yet still maintain an energy dense feed ingredient, investigations into alternative by-products, such as glycerin, have been conducted. Glycerin is a byproduct of the biodiesel industry and is comprised of the glycerol backbone of a triglyceride fat. Glycerol has been suggested to ferment directly to propionate (Wang et al., 2009). However dietary glycerin, similar to unsaturated fatty acids, has negative effects on certain strains of fiber-digesting bacteria (*Butyvibrio fibrosolvens* and *Selenomonas ruminantium*; AbuGhazaleh et al., 2011). Thus, a decrease in acetate and increase in propionate would be expected when feeding glycerin or unsaturated fats.

Impact of ruminal pH on PUFA

As fermentation patterns change (in both amount and profile) from dietary additions of fat as well as differing ratios of concentrates:forages within a ration, the

rumen microbial population will shift production of fermentation end products. This shift in fermentation products, such as VFA, can lead to differences in ruminal pH due to varying pKa associated with differing VFA profiles, as well as changes in total acid production. Associations between decreases in ruminal pH and decreases in rates of lipolysis and biohydrogenation of PUFA were observed (Nevel and Demeyer, 1996; Loor et al., 2003). As shown in Table A1.11, there is a definite impact of ruminal pH on both lipid transformation processes once ruminal pH drops to a borderline acidotic level.

Two major theories have emerged to explain changes in biohydrogenation rate associated with lowered pH. One theory suggests that pH-sensitivity of lipolytic bacteria is a primary cause for the depression of hydrogenated fatty acid products in the rumen. Lipolytic bacterial species, *Anaerovibrio lipolytica*, has a decrease in growth at pH 5.7 and completely inhibited lipolytic activity at pH 5.3 (Hobson, 1965). In vitro, *Butyrivibrio fibrisolvens* exhibited a 25% decrease in yield at pH 5.75 and activity was completely inhibited at pH 5.5 (Russell and Dombrowski, 1980). By inhibiting enzymatic activity of these species, lipid breakdown to free fatty acids (FFA) will not occur as readily. Chalupa et al. (1984) observed that fat in the form of triglycerides did not inhibit ruminal digestion to the same extent as free fatty acids. Although a decrease in pH negatively impacts bacterial activity involved ruminal lipolysis and biohydrogenation, a lower presence of free fatty acids would lower the need for ruminal biohydrogenation.

Mackie et al. (1978) determined that *A. lipolytica* was not eliminated from the microbial population when donor cattle were adapted from a low- to a high-concentrate diet. These results cast doubt as to whether or not viable counts of lipolytic bacteria

could be a primary indicator of lipase activity. Results from earlier studies demonstrated that activity of lipase enzymes was highest at pH 7.4 but it was reduced 50% if pH dropped to 6.6 (Henderson, 1971). Because a decrease in ruminal pH is routinely associated with an increase in concentrates in a diet, the decrease in lipase activity became associated with the changes in microflora based on diet composition (Latham et al., 1972; Gerson et al.,1985). This reduction in activity has been theorized as an inhibition of the rumen microflora. Results from in vitro studies, demonstrated a secondary theory that the reduction in lipase activity associated with changes in dietary composition was due to a shift in the metabolic processes of microbes. High concentrations of carbohydrates, particularly glucose, reduced bacterial production of lipase under aerobic conditions (Papon and Talon, 1988; Jaeger et al., 1994).

The impact of ruminal pH on biohydrogenation rate is of great interest when observing the wide spectrum of diets consumed by cattle. It is accepted that average ruminal pH of grass-fed cattle is higher than that of grain-fed cattle due to an increase in ensalivation, an increase in rumen volume, less severe pKa of acetate compared with propionate, and natural buffering capacity of forages (McBurney et al., 1983; Moreira el al., 2013). Data from Van Nevel and Demeyer (1996; Table A1.11), indicates that average ruminal pH for a 24 h-period should have no effects on lipolysis or biohydrogenation. However, in that study pH did not reach a value below the decreased activity threshold of pH 5.7. It is assumed that the ruminal pH of grain-fed cattle spends a quantifiable amount of time below pH 5.7, which leads to the hypothesis that the total amount of biohydrogenated PUFA in digesta would be lower for grain- compared with grass-fed rumen. Depression in ruminal pH of grain-fed animals would lead to lower

biohydrogenation rates and greater concentration of dietary PUFA present in digesta entering the small intestine.

Effect of ionophores and antimicrobials on biohydrogenation

In addition to differences in ruminal pH, it must also be considered that many grain-based diets are supplemented with ionophores. Ionophores and other antimicrobials are thought to alter the amount of PUFA deposited in meat and milk products due to an interaction with the microbes involved in lipolysis and biohydrogenation (Marmer et al., 1985; Van Nevel and Demeyer, 1995; Fellner et al., 1997). Marmer et al. (1985) determined that inclusion of monensin in the diet did not change lipid and fatty acid content in tissue of steers, but it did demonstrate a decrease in saturated fatty acid content and an increase in unsaturated fatty acid content. Within adipose deposits of monensin-supplemented cattle, significant increases in trans-octadecenoic acids, a product of the first steps of biohydrogenation, were observed. This led to the hypothesis that monensin reduces biohydrogenation. Zinn (1988) determined that dietary inclusion of monensin increased *trans*-C18:1 by 34% and decreased C18:0 by 11% in the duodenal chyme of steers fed highly digestible finishing diets. Fellner et al. (1997) observed a decrease in complete hydrogenation of C18:2 to C18:0 but flows of *trans*-C18:1 to the small intestine were increased when ionophores were included in the diet. These results suggest that the first step in biohydrogenation is not inhibited to the same extent as the final saturation step (Figure A1.11).

Because a main effect of monensin in the rumen is inhibition of gram-positive bacteria growth, its impact on biohydrogenation is of interest (Van Nevel and Demeyer, 1995). The primary bacteria (Group A) involved in the conversion of C18:2 to *trans*-

C18:1 is *Butyrvibrio fibrisolvens*, a gram positive bacteria (Harfoot and Hazelwood, 1988). However observations by Zinn (1988) showed steers supplemented with monensin had an increase in *trans*-C18:1 concentrations post-ruminally, which would indicate no inhibition of Group A bacteria. Instead, the observed decrease in stearic acid would indicate an interaction of monensin with group B bacteria. However the primary Group B bacterial species, *Fusocillus* and *Clostridium proteoclasticum*, are gram negative (Harfoot and Hazelwood, 1988); thereby negating a role of monensin on these species. Work conducted by Li et al. (2012) demonstrated most of the bacteria involved in biohydrogenation are uncultured, so assuming all bacteria in group B can be classified as gram negative is inaccurate.

Additional oversight is the absolute classification of bacterial species to Group A or B. *Butyrvibrio fibrisolvens* was classified as Group A by Harfoot and Hazelwood (1988), but recent work by Li et al. (2012) has shown this species involvement in hydrogenating *trans*-C18:1 to C18:0. Therefore the impact of monensin on biohydrogenation is most likely an interaction with uncultured bacterial species or bacteria with multifaceted roles in the pathway.

Research to date on ionophore effects on unsaturated fatty acids has been focused on the inhibition of biohydrogenation. However, Van Nevel and Demeyer (1995) widened this perspective by suggesting that ionophores and other antimicrobials inhibit lipolysis to a greater extent than they inhibit biohydrogenation. In addition to *B. fibrisolvens* role in biohydrogenation it is also a commonly accepted lipolytic species. Therefore, inhibition of *B. fibrisolvens* would hint towards impacts of monensin on lipolysis not simply biohydrogenation. The species has a particularly thin cellular

envelope that is easily affected by rumen environment disruptors, like monensin. However not all antimicrobial additives are effective for inhibiting lipolysis in the rumen. For certain compounds such as Salinomycin sodium, lincomycin hydrochloride, oxytetracycline, virginiamycin, and mentronidazole, an increase in inhibition of lipolysis is paired with an increase in inhibition of VFA production (Table A1.12; Van Nevel and Demeyer, 1995). These findings suggest that these antimicrobial compounds inhibit the rumen microbes themselves rather than shifting energetic pathways. Inhibition of lipolysis by antimicrobial compounds such as amoxicillin, avoparcin, lasalocid sodium, and monensin was not accompanied by a decrease in VFA production (Table A1.12; Van Nevel and Demeyer, 1995). Thus, these four particular compounds helped to increase flows of polyunsaturated fatty acids from the rumen without majorly altering ruminal fermentation.

POST RUMINAL DIGESTION AND ABSORPTION OF PUFA

A survey of published literature found that digestibility of fatty acids can range from 55% to 92%, depending upon factors such as chain length and degree of unsaturation (Demeyer and Doreau, 1999). This survey found that variability in digestibility was not explained by fatty acid concentration in the diet. Contrary to this review, Palmquist and Jenkins (1980) found fat supplementation at moderate levels (3% or 5%) were 80% digested, while dietary fat concentrations greater than that were significantly less (~56%). Assuming a linear response to fat supplementation, the work by Palmquist and Jenkins (1980) would indicate that for each percentage unit above 4% dietary fat concentration, a 3.4% decrease in fat digestibility would be expected (Palmquist and Jenkins, 1980). These observed differences in the effects of dietary fat

concentration between the work by Palmquist and Jenkins (1980) and the survey by Demeyer and Doreau (1999) are suggested to be attributed to the survey's variation in experimental approaches, analysis techniques, and variations in diet formulation (Lock et al., 2000).

Differences in digestibility of isolated fatty acids at similar dietary inclusions are relatively modest, with apparent digestibility values for C18:0, C18:1, C18:2, C18:3 fatty acids determined to be 77%, 85%, 83%, or 76% respectively. As an important note, within this dataset the digestibility value for C18:1 included all of the biohydrogenation isomers, which perhaps exaggerated the digestibility value reported. Additionally, the value for C18:3 may have been inaccurately portrayed due to low dietary inclusions (Glasser et al., 2008). These digestibility values were repeated by Lock et al. (2000) with very similar findings of 72%, 80%, 78% and 77% digestibilities for 18:0 C, 18:1 C, 18:2 C, 18:3 C respectively. Therefore, it can be concluded that the difference in digestibility of differing fatty acids is indeed relatively modest. Zinn et al. (2000) determined that digestibility of fatty acids could be increased by decreasing the extent of biohydrogenation. A linear relationship between proportion of unsaturated fat entering the small intestine and digestibility of saturated fat was determined. This relationship showed a 1% increase in 18:1 escaping biohydrogenation lead to a simultaneous 1% increase in 18:0 absorption (Zinn et al., 2000). Ketels et al. (1989) observed the same effects in broilers.

Although the same effects of unsaturated to saturated fatty acid ratio in digesta exist in both ruminants and non-rumimants, the relatively modest range of digestibility values for individual fatty acids is unique to ruminant animals. Non-ruminants have

shown a wide range of digestibility values for differing fatty acids (Freeman, 1984) with a decrease in digestibility with an increase in number of double bonds (Lessire et al., 1992). These differences between species exist due to differences in lipid material, degree of neutralization of digesta, and source of amphiphile for micelle formation. In ruminants, 80 to 90% of lipid material entering the small intestine is in free fatty acid form, while non-ruminant lipid material is >90% esterified fat (Noble, 1981). Kinetics of rumen digestion and flow create a continuous presentation of digesta to the small intestine of ruminant animal. As digesta enters the small intestine, it stimulates the secretion of bile salts, lecithin, and pancreatic enzymes. Pancreatic enzymes convert lectithin to lysolecthin, the primary amphiphile ruminants utilize to promote micelle formation (Lock et al., 2005). Fatty acid absorption is dependent upon micelle formation and fatty acid incorporation into the core of a micelle molecule. Due to the water insoluble nature of fatty acids combined with the water layer present within intestinal cells, hydrophobic fatty acids are very poorly absorbed from the intestinal lumen without incorporation into a micelle. Before micelle formation, fatty acids must be released from microbes and feed particles by action of lysolectin and bile salts (Moore et al., 1984). Fatty acids flowing out of the ruminant stomach are associated with the particle phase of digesta despite being in free fatty acid form (Doreau and Chilliard, 1997). Because continuously stimulated, pancreatic secretions of ruminants are less concentrated and released in lower quantities at one time. This leads to a lesser degree of neutralization in the duodenum because of lower concentration and amount of bicarbonate secreted. Due to a more acidic pH of digesta in the upper small intestine, the site of digestion is shifted to lower segments of the jejunum than in non-ruminants (Moore et al., 1984). In

response to the lower site of digesta neutralization digesta and absorption, ruminants evolved an alternative system for absorbing fatty acids compared to that of non-ruminants.

Rather than secreting predominantly glycine-conjugated bile acids like non-ruminants, ruminants secrete taurine-conjugated bile acids, which have capability to remain in ionized form despite the acidic nature of digesta (Noble, 1981). Glycine-conjugated bile acids are insoluble at pH 4.5, while taurine-conjugated bile acids remain soluble even at pH 2.5 (Moore et al., 1984). This is significant as the bile salt must be in ionized form to remain in micellar phase and aid in absorption of fatty acids. These bile acids are responsible for solubilizing fatty acids through stabilizing the micelle structure, while the amphiphile or swelling agent (i.e.- lysolecthin) promotes infiltration of unsaturated fatty acids and phospholipids into hydrophopic core of micelle (Davis, 1990). Despite having evolved a more efficient absorption system for fatty acids, total fatty acid digestibility is not 100% but instead averages 74%. Figure A1.14 (Lock et al., 2005) depicts the relationship between duodenal flow of fatty acids (g/d) and daily absorption (g/d). The linear relationship demonstrates for 1 g daily fatty acid flow, only 66% will be absorbed.

Incorporation of PUFA into meat or milk

Digestibility values of less than 100% demonstrate that even if fatty acids escape ruminal biohydrogenation, there is no guarantee that they will be fully utilized by the animal. Because animals are unable to synthesize significant amounts of PUFA at tissue deposition site, all long chain unsaturated fatty acids found in blood stream are of dietary origin or have been mobilized from body stores (Demeyer and Doreau, 1999). Thus

dietary inclusion of PUFA has a direct impact on their concentration in meat or milk products. Regardless of the exact value of digestibility, there is a large amount of variability in the amount of PUFAs deposited, with majority of the variation being explained by diet. In sheep, feeding greater dietary concentrations of concentrate was associated with greater efficiency in utilization of C18:3 of dietary origin (Chilliard et al., 2000; Table A1.13). These data represent the efficiency of intestinal absorption but do not account for the efficiency of incorporation into meat or milk products. This reinforces that the challenge to ruminant nutritionists to manipulate PUFA concentrations in meat and milk products involves more than the manipulation of ruminal biohydrogenation.

As depicted in Figure A1.13, the efficiency of incorporation of C18:3 into milk decreases as supply presented to the small intestine increases (Chilliard et al., 1991; Drackley et al., 1992; Christensen et al., 1994; LaCount et al., 1994; Ottou et al., 1995; Litherland et al., 2005). Long chain fatty acids absorbed in the small intestine are preferentially packaged into HDL. Uptake of HDL differs by tissue; for example adipose tissue takes up HDL much more efficiently than the mammary gland. A portion of PUFA are taken up by the mammary gland to ensure fluidity in milk (Demeyer and Doreau, 1999), but the efficiency of incorporation is not constant. This indicates that the mammary gland is able to selectively incorporate fatty acids in milk fat when larger fatty acid quantities are present. Although efficiency of incorporation decreases there is greater milk fat C18:3 concentration as the amount of C18:3 presented to the small intestine increases.

However, as explained previously, most linolenic and linoleic acid do not reach the small intestine in dietary form. Instead, biohydrogenation alters fatty acid structure to various isoforms referred to commonly as biohydrogenation intermediates. Certain intermediates of linoleic and linolenic acid, including trans-10, cis-12 conjugated linoleic acid (CLA), exert a negative influence on total milk fat yield (Peterson et al., 2003). In a study conducted by Loor et al. (2004), linolenic acid was added to low or high concentrate diets. Both the high concentrate diet (75% inclusion) as well as high concentrate plus linolenic acid decreased milk fat percent and yield. Therefore, management of the amount of biohydrogenation isoforms synthesized is critical in order to avoid milk fat depression when supplementing polyunsaturated fatty acids.

Fatty acids not incorporated into milk fat accumulate in adipose tissue deposits within viscera, subcutaneously, inter- or intra- muscularly. The composition of adipose tissue deposits, specifically those in beef, are directly reflective of the fatty acid profile presented to the small intestine following ruminal digestion (Demeyer and Doreau, 1999). Lipids are selectively deposited in various adipose deposits based on weight and growth stage of the animal. As body weight increases, subcutaneous fat will increase and concentration of unsaturated fatty acids will increase within adipose deposits if presented to small intestine (Moloney, 2002). In finishing cattle, subcutaneous fat is the primary deposition site for unsaturated fatty acids while intramuscular fat is the primary deposition site for saturated fat. To contrast, in lean animals, subcutaneous fat is primarily made up of saturated fats while intramuscular fat accumulates more unsaturated fat. Because lean animals have less body fat, the unsaturated FAs incorporated into cell phospholipid membranes contribute to a greater percentage of total body fat (Demeyer

and Doreau, 1999). No major inhibitory mechanisms for increasing PUFA concentrations in meat are known. Instead, justification for the supplementation of specific dietary FA, especially omega-3 FA, is challenging because omega-3s are deposited within fat depots that trimmed are not directly consumed unless mixed into a ground product.

ECONOMIC BENEFITS OF DIETARY PUFA FOR CATTLE PRODUCERS

The nature of the ruminant biological system presents a challenge to cattle producers to meet demands by modern consumers to provide the appropriate omega-6 to omega-3 ratio. Biohydrogenation, efficiency of absorption, and site of fat deposition all must be considered when aiming to present the product modern consumers desire. As previously described, in finishing cattle unsaturated fat is deposited within subcutaneous fat which is partially trimmed off of the carcass during processing. Therefore, the health benefits associated with balancing omega-3 and omega-6 fats in finishing cattle is not fully obtained within traditional cuts of meat but instead within ground product where trim fat is added back to the product. De Mello et al. (2017) observed that steaks with greater concentration of total PUFAs had greater oxidation and greater surface discoloration. Steers within the study were fed 0%, 15%, or 30% distillers grains plus solubles, leading to significant linear increases in steak omega-6 fatty acids with increasing dietary distillers. No difference in total fat concentration was observed. Therefore, the authors concluded that shifting fat composition without shifting total fat led to a change in meat shelf life that altered consumer desirability due to increases in discoloration with higher concentrations of PUFA.

Despite impacts observed by De Mello (2017) on meat quality, the fat content of full-fat distillers grains provides an excellent source of dietary energy to feedlot cattle. Yet, as corn processing to produce ethanol has changed to extract greater value from the kernel, nutrient composition of distillers grains with solubles has changed. Instead, distillers grains is commonly subjected to further oil extraction; therefore, reduced fat distillers grains with solubles are more commonly produced today. Nelson et al. (2017) examined the effects of feeding 15% full fat distillers (FFDGS), 15%, 30%, or 45% reduced fat distillers (RFDGS) on meat quality. Unlike results from De Mello et al. (2017) with full-fat distillers, reduced-feeding fat distillers showed no difference in retail shelf life for steaks, ground beef, or bologna. The absence of differences in omega-6 content of steaks, ground beef, or bologna between treatments would explain the lack of shelf life differences observed by Nelson et al. (2017). Low levels of dietary PUFA (such as that found in 15% FFDGS) would allow for greater extent of PUFA biohydrogenated in the rumen with less available for incorporation into meat products. As dietary omega-6 fatty acid content increases differences in shelf life stability become apparent, such as those observed at 30% and 45% FFDGS inclusion in work by De Mello et al. (2017). Because of variation present between DGS products, caution should be exercised for basing meat quality conclusions off of dietary distillers grains inclusion without reviewing dietary fatty acid profile.

Benefits of dietary Omega-3 inclusion

Presently, there are no monetary incentives at the packing plant for producers to feed omega-3 fatty acids to cattle. Certain groups in the U.S. are marketing omega-3 rich

beef products; however, these are integrated systems from cattle procurement to cattle feeding and product marketing.

Omega-3 riched products entering the market are regulated by the USDA to avoid broad marketing labels of “enriched” or “enhanced” type claims. Instead, when marketing a product for improved omega-3 fatty acid concentrations, each label must state the milligrams per serving. Thus, the goal as a producer of this type of product is to increase omega-3 concentration per serving to the greatest extent. When feeding flaxseed at a dietary inclusion of 8%, omega-3 fatty acid concentration was increased in the longissimus lumborum from 0.26 g to 0.58 g per 100 g of neutral fat and from 7.42 g to 11.47 g per g phospholipid fat (Maddock et al., 2006). To equate this to 1 serving of 80% lean hamburger, the mg of omega-3 per serving would increase from 165.8 to 283.7 mg when fed without vs with flaxseed respectively. In perspective, this is competitive with grass-fed ground beef, which retails in stores as 200 mg or more of omega-3 content. Table A1.14 shows a simulation of breakeven prices (per bushel) when producers receive a premium above market live weight price for finishing steers on a diet with 8% dietary flaxseed inclusion. Values were calculated using performance data from Maddock et al. (2006). According to the U.S. department of agriculture flaxseed price historical data, the cost of flaxseed on the market for August 2016 was around $8.44 per bushel. To break even with price of flaxseed needed to be purchased for 8% dietary inclusion, producers would need to receive a premium of 42% above live market price (Table A1.14). This increase in price at the packer then translates to an increased cost at retail. Studies conducted from the consumer perspective, have proven an increase in willingness to pay premiums when the food product of interest is perceived as healthier.

McClusky et al. (2005) examined the reason behind consumer willingness to pay for grass-fed beef finding that 24 to 40% of consumers pay premiums for the product due for health associated benefits (McCluskey et al., 2005). It is this segment of the consumer base that a grain fed omega-3 riched product would appeal to, but further research needs to be conducted to determine how consumers would respond to this type of product.

OBJECTIVES FOR CHAPTERS II & III

The objectives of chapters II and III were to examine rumen characteristics associated with diets fed omega-6 and omega-3. Chapter II specifically sought to:

1. Determine the impact of ruminal environment on the passage of omega-3 fatty acids through rumen and
2. Understand differences in ruminal biohydrogenation in grass-fed verses grain-fed cattle.

While Chapter III sought to:

1. Understand how utilization of bio-fuel byproducts impacts rumen environment and fatty acid profile at end point of fermentation and
2. Determine differences in PUFA content at endpoint of fermentation when bio-fuel byproducts are fed to ruminants.

PART II – Effect of manipulation of growth patterns during backgrounding phase on finishing performance and meat quality

INTRODUCTION

A managed growing phase for cattle post-weaning and prior to finishing presents a valuable opportunity to produce more pounds of beef per animal. Commonly known as a growing phase, an accurately timed period of limited energy intake prior to finishing results in larger frame size, ultimately allowing the animal to support more pounds of carcass weight (Perillat et al., 2003). Consuming lower energy causes a delay in the onset of maturity by delaying the onset of fat accumulation. Owens et al. (1993) shows slower rates of growth maintain the same order of deposition of various tissues and sites as rapid rate of growth animals, but the rate of accumulation at any like-point in time differs between these two scenarios. When caloric intake is restored to above maintenance, the animal responds by allocating energy to muscle hypertrophy and growth of various tissues associated with later maturation (Owens et al., 1993). Effects of compensatory growth, such as greater final weight and greater overall feed conversion efficiency, are goals of growth strategies before high-grain finishing[1]. An understanding of how management decisions during the growing phase impact the magnitude of finishing performance and carcass growth is not well understood.

COMPENSATORY GAIN AS MECHANISM OF GROWTH

This period of controlled growth in a growing phase represents an opportunity for maximizing frame growth prior to the deposition of fat. Because accumulation of fat is correlated with carcass maturation, the “framing out” phenomenon that occurs during a

growing period causes a shift in the growth curve, delaying carcass maturity and allowing increases in lean and bone growth (Owens et al., 1993). Prioritized accumulation of lean growth and bone mass over fat is explained through physiological endocrine changes, particularly changes in circulating growth hormone (GH) (Hornick et al., 2000). Lower nutrient availability decreases the hypothalamus' secretion of somatostatin, which is the primary hormone responsible for mediating GH production (Thomas et al., 1990). While a decrease in circulating insulin and thyroid hormones, resulting from lowered glucose intake, leads to a decrease in synthesis of GH receptors (Maes et al., 1983). Limited GH uptake receptors and reductions in somatostatin secretion lower the amount of GH taken up by the liver causing concentrations of circulating GH to accumulate (Thomas et al., 1990). As a result of decreased liver uptake, limited GH is available for IGF-1 synthesis. Because IGF-1 drives anabolic growth, this decrease in liver GH uptake and lower IGF-1 synthesis largely contributes to slowed growth rates during feed restriction (Hornick et al., 2000).

Tissues react differently during periods of reduced growth. Growth rates are reduced for most for viscera, followed by adipose tissue with little effects in muscle (Hornick et al., 2000). Reductions in volume and metabolic activity of the visceral mass leads to a reduction in basal metabolic rate (Yambayamba et al., 1996). This energy-sparing mechanism remains for several weeks even after caloric intake restriction has ended (Ryan et al., 1993). This surplus of energy intake above maintenance needs results in allocation of energy to growth of various tissues whose accumulation is associated with more mature development, particularly muscle hypertrophy and fat accumulation (Owens et al., 1993).

For cattle stocked on or fed forage-based growing diets, an increase in energy required to digest and ruminate fiber based particles as well as an increase in energy losses due to heat of fermentation losses lead to an overall increase in energy demands by the viscera during the growing phase. Owens et al. (1993) described observations of grazing cattle with an increased in DMI entering the finishing phase but a lower feed conversion to gain compared with cattle placed on finishing diet rather than grazing. He attributes this to increased ruminal capacity with increased energetic costs of maintaining a larger visceral mass. However visceral mass, particularly the liver and digestive tract, contracts as cattle adapt to a finishing diet, decreasing metabolic requirements of animal for maintenance of the viscera (Owens et al., 1993). This decrease of energetic needs to maintain the viscera in combination with lower energetic losses from finishing diets result in an increase in energy available for growth. Transitioning from a growing to finishing diet regardless of growing strategy, enhances secretion of insulin, due to an increase in glucose uptake (Blum et al., 1985; Wester et al., 1995). Blum et al. (1985) hypothesized that an increase in insulin concentration serves as an initiating signal for the anabolic growth processes.

GROWING PHASE STRATEGIES

Cattle displaying characteristics of "framing out" due to a lowered plain of nutrition are generally more desirable because of expectations for elevated growth performance (i.e. – compensatory gains). Cattle displaying these characteristics are referred to as "green". An immature or "green" appearance can be achieved by various nutritional strategies during a growing phase. Selection of strategy varies based on feed or land availability and cost-to-benefit ratio for the producer. Weighing benefits across

strategies is challenging as differences in the duration of growing phase, severity of caloric restriction, maturity of animal at harvest, sex, genetic background, and diet composition all play a role in the observed inconsistencies between studies reported in literature.

Effects of stocking and grazing growth strategies are arguably most difficult to effectively evaluate due to an inherent deficiency in knowledge of actual intake or energy intake consumed. Feed intake in ruminants is regulated by two factors: chemostatic or physical. Chemostatic regulation refers to the effects of metabolic signals indicating that the need for nutrients has been satisfied. Physical limitations result from by rumen fill reaching a level where feed particles are not readily leaving the rumen thereby reducing capacity for additional feed to be consumed (slow particle disintegration rate) and/or intake rate limitations (prehension or ease of swallowing constraints; Poppi et al., 1993). Intake control in growing cattle fed ad libitum results from physical limitation. However, due to the variability in caloric density of these diets, intake limitation between growing strategies varies. Grazing cattle are continuous eaters; they maximize the upper limit for eating time (12 hours; Arnold, 1981). A rate of intake limitation occurs when feed is consumed at a slowed rate due to a greater time needed for prehension and a decrease in ease of manipulating feed for swallowing (in case of more mature forages). Forage-fed dry lot cattle consuming ensiled forages are not limited by rate of intake to the same extent as grazing cattle as feed particle size is uniform with consistent moisture content. Although rate of intake may differ, total intake for both grazing cattle and cattle fed harvested forages in a drylot is limited by rumen capacity because of the combined effect of weight and volume on the tension receptors in the reticulorumen (Allen, 2000).

Greater rumen fill leads to an increase in rumen volume in cattle fed forages or grazing. A review by Rohr and Daenicke (1998) found that gut fill ranged from 11 to 17.1% of live weight depending upon various dietary factors. Dietary forage type plays a large part in this variation. Waldo et al. (1990) found that steers fed alfalfa haylage at similar intakes had 28% less gut fill than cattle fed grass silage. Carstens et al. (1991) suggested that much of the compensatory gain response was due to changes in gut fill and body composition of gain. Hogg (1991) suggested that DMI variability in the finishing phase results from differences in dietary energy content of the growing diet, with greater DMI coming cattle grown in forage-based strategies. Sainz et al. (1995) expounded on the suggestion by Hogg (1991) by demonstrating that differences in initial finishing weight gain between cattle grown on forages verses cattle placed in a feedlot post-weaning was not due simply to an increase in visceral mass but instead related to an increase in capacity for dry matter intake. Combined observations by Hogg (1991) and Sainz (1995) conclude that an increase in gut fill capacity for high forage growing strategies can prove beneficial to finishing performance and do not solely represent a loss from an increase in drop weight. However, caution needs to be exercised to ensure the increase in digestive tract weight at slaughter can be compensated by efficiently converting increased intake capacity in finishing phase to increased empty body weight gains.

Growing growth effect on finishing and carcass performance

The ability to precisely predict how growing phase strategies affect finishing and carcass performance is imperative to maximize the benefits of adding a growing phase post-weaning. Across the literature, variable and even conflicting results in performance

have been observed between growing strategies making predictions of performance challenging. Differences in duration of growing phase, severity of caloric restriction, maturity of the animal at harvest, sex, genetic background, and diet composition all played a role in the observed inconsistencies between studies. Growing phase strategies published in the literature represent the range from low caloric density dry lot diets to grazing systems on native range. Each strategy presents a unique management decision especially when considering possible interactions of dietary energy intake with number of days on feed. As experiments within the literature have led to conflicting observations on finishing and carcass performance, certain research groups have turned to a meta-analysis approach to better understand the effects of growing strategies on finishing (Lancaster et al., 2014; Klopfenstein et al., 1999). Lancaster et al. (2014) was able to generate prediction equations for finishing and carcass performance estimates. Average daily gain in growing phase and body weight at the end of the growing phase were primary predictors of finishing and carcass performance characteristics (i.e. finishing ADG, finishing DMI, HCW, 12^{th} rib fat thickness, LMA, ect.). However, no correction was made for degree of maturity of the animal at slaughter, instead HCW and fat thickness were simply used as terms within the prediction equation to correct the carcass performance data. This presents a challenge when interpreting the data because observations were not compared at same degree of maturity, so systematic biases exist within the predicted data. Additionally, growing strategy utilized in the experiment was not modeled, and because DMI was not used as an independent variable, it is challenging to understand intake capacity differences that stem from different dietary forage content. In their review of grazing cattle prior to a finishing phase, Drouillard and Kuhl (1999)

state the imperative nature of standardizing gut fill when comparing across strategies. Therefore, there is a need for strategy-corrected prediction equations to better extrapolate equations to particular situations common to the industry.

McMeniman et al. (2010) and Galyean et al. (2011) were able to predict finishing performance based on initial performance as animals came into the finishing phase. The two primary predictors they identified for finishing and carcass performance were initial body weight (at the start of finishing) and dry matter intake for days 8 to 28 of the finishing period. Therefore, demonstrating the importance of an increase in potential to eat but also the amount they gained prior to finishing phase. Heavier weights coming into finishing correlated to an increase in DMI, an increase in final body weight, an increase in ADG, and an increase in HCW according to prediction equations presented by McMeniman et al. (2010). Strong correlations between the initial weight at the start of finishing and final performance characteristics lend to the idea that the extent of gain in the growing period (relating to duration of days on feed and daily gain) may have significant impacts on finishing and carcass performance.

Taylor et al. (2015) examined the interaction of days on feed and a target average daily gain by varying both ADG and DOF to achieve same final body weight of cattle entering the feedlot. Increased final BW, HCW, ADG, DMI and decreased marbling scores were observed for animals with lower growing ADG and longer DOF. Demonstrating the differences in magnitude of compensatory gain responses amongst backgrounded cattle (both frame size and delayed maturity) correlate to carcass quality attributes. Despite the large amount of research completed on growing cattle, there is still

a lack of understanding of the interactions of duration of time in the growing phase and rate of gain growing.

GROWING PHASE IMPACTS ON ECONOMICS

Although the growing phase is considered a low input situation, as this period is extended, the animal's maintenance requirement costs contribute a greater portion of total production costs (Drouillard and Kuhl, 1999). If compensatory gains are expressed when market cost of inputs are greatest (i.e. - during finishing), there is the possibility to offset inefficient performance during the growing phase. As apparent from figure A1.21, implementing a growing phase has the ability to increase final weights of steers; however, this comes at a cost as days on feed. Therefore, the risk of adding a growing phase must also be considered. Adding days on feed, depending upon how the market shifts, may push the slaughter date to a point in the market were animals are less profitable.

Using growth patterns from Sainz et al. 1995 (Figure A1.21), economic scenarios can be examined to demonstrate gains and losses from cattle requiring extended days on feed due to a growing phase. Figure A1.22 shows cattle price by month for 2012 to 2016. Using these values, if cattle raised without a growing phase were harvested at 450 kg in July 2014 for $3.49/kg with yard cost of $0.37/d, gross profit (minus yardage) would yield $1,521 per animal. Instead, if those cattle were raised with a growing period that extended the feeding period by 60 days, final weight would increase to 500 kg. Price on September 2014 price (60 d after July price referenced earlier) for cattle was $3.52/kg and assuming the same yardage costs per day, gross profit minus yardage would be $1,684, $163 more per animal. However, this example is contingent upon price/kg being

relatively stable. On the opposite end of the spectrum, in July to September 2016, when prices were low and continued to fall, a drop in price from $2.58/kg (July 2016) to $2.34/kg (September 2016) would cause cattle raised with a growing phase to yield $14 less than if those cattle were raised without the growing phase and sold in July 2016 (Table A1.21). If this example is applied to all possible combinations of 60 d increments year round from 2012-2016, cattle raised with a growing phase averaged $120 more per animal than cattle raised without growing phase. During these years, profit from cattle raised with a growing phase ranged from $90 less to $290 more than cattle without, demonstrating the obvious risk involved with adding a growing phase to a production system. However, on average, positive economic benefits compensate for greater days on feed required by cattle raised with a growing period.

OBJECTIVES OF CHAPTER IV

A meta-analysis approach was utilized to understand interactions of growing phase performance on finishing performance and at constant maturity at harvest, sex, genetic background, and dietary strategy. The objectives of this analysis were to understand:

1. Impact of growing nutritional strategy on finishing and carcass performance
2. Understand the impact of calorie consumption and form of calories in the growing phase on finishing and carcass performance
3. Identify interactive terms from the growing phase that correlated to finishing and carcass performance

Chapter II.

RUMINAL DIGESTION FACTORS THAT MAY AFFECT INCREASING ALPHA LINOLENIC FATTY ACID PASSAGE RATE IN GRAIN-FED CATTLE AS COMPARED WITH GRASS-FED CATTLE

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INTRODUCTION

Because there is a consumer desire to understand fat composition of meat presented on retail shelves, an increasing demand for grass-fed beef has developed. The beef consumer index from March 2014 found that 69% of the 19% of consumers not consuming beef were doing so because of nutritional reasons. Nutritional awareness is suspected to be a primary driver behind the 20% annual increase in cattle raised in alternative systems (organic, natural, and grass-fed) in recent years. Arguably, a certain percentage of this increase is due to misinformed consumer perceptions that alternative beef production systems provide a more ideal animal environment, a concern voiced by 9% of consumers not consuming beef. However, the majority of concerns expressed were in direct relation to human health when consuming beef products.

The primary marketing approach for grass-fed beef is the advertisement of improved omega-3 to omega-6 fatty acid ratio due to an increase in omega-3 content relative to grain-fed beef. The most common omega-3 is alpha linolenic acid (ALA;

C18:3) which serves as a precursor for eicosapentaenoic acid (EPA; 25:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). These polyunsaturated fatty acids are functional foods because they serve primary roles in human anti-inflammatory immune responses (Cherfaoui et al., 2011). This desirable shift in nutritional composition of grass-fed beef has led to increases in consumers' willingness to pay up to $3.44 premium per pound for grass-fed beef (Abidoye et al., 2011). However, finishing animals on grass comes at a cost. Grass finishing animals requires a 21% longer feeding period compared with grain-fed animals (Berthiaume et al., 2006), greenhouse gas production (particularly methane) is greatest per kg beef produced for grass-fed cattle (Pelletier et al., 2010), and cattle on grass require more extensive land use. Therefore, within the beef industry, there is an opportunity to better understand alternatives to grass-feeding systems that maintain the health benefits of grass-fed beef while producing product in a more sustainable and efficient manner.

Studies have shown that omega-3 concentrations in meat and milk are directly related to dietary omega-3 fatty acid content of the animal (Wood et al., 2004). Grass-fed beef tends to have a greater omega-3 to omega-6 ratio compared with grain-fed beef. This is expected as alpha linolenic acid is the primary fat found in grasses and grains are generally high in linoleic acid (an omega-6 fatty acid). However, the objective of this study was to determine whether dietary concentrations of omega-3 fatty acids were identical between grass- and grain-fed animals. Also, how does the rumen environment alter the amount of non-hydrogenated PUFA to the animal for incorporation into meat or milk products? It was hypothesized that the rumen environment of a grain-fed animal, due to time spent at lower pH throughout the day, would decrease PUFA transformation,

in turn increasing unsaturated alpha linolenic acid present at the endpoint of fermentation. The objectives were to:

1. Examine the influence of rumen environment on fatty acid profile at the end of fermentation for grass- vs. grain-based diets,
2. Determine differences in flow rates of fatty acids within three different digesta phases for grain- vs. grass-based diets, and
3. Understand rumen microbial activity and efficiency of fermentation when cattle were fed grass- vs. grain-based diets.

MATERIALS AND METHODS

The experiment was conducted at the University of Minnesota Beef Research Facility in Rosemount, MN. All animals involved in this experiment were cared for according to guidelines of the University of Minnesota Institutional Animal Care and Use Committee (IACUC). All experimental procedures, including diets, sampling, and cannulation were reviewed and approved by IACUC (protocol #1507-32760A).

Animals, Experimental Design, and Dietary Treatment

Six ruminally cannulated Holstein steers (300 ± 33 kg) were utilized in a 3 x 3 replicated Latin square design (n = 2 per period). Periods were 28 d with 21 d of adaptation and a 7-d sampling period. Cattle were housed in tie stalls with individual feed bunks and waters. Body weights were measured on the last day of the sampling period after an overnight fast.

Dietary treatments consisted of a grass-based diet (GRASS), a grain-based diet with flax (FLAX), or a grain-based diet with corn oil (CORN) (Table 2.1). All diets were formulated to be isonitrogenous and provide 300 mg/hd/d of monensin. Omega-3 content

of grass sources and flax seed oil was measured prior to the start of the experiment. Based on these values, GRASS and FLAX were formulated to provide the same amount (g/d) of alpha linolenic acid (ALA). Within grain-based diets, flax seed oil and corn oil were included at the same concentration to ensure the same fat content between grain-based diets. Steers were fed for zero refusals with orts recorded and sampled daily.

Diets were mixed individually on a daily basis using a concrete mixer. Individual diet ingredient samples were collected daily during sampling week and weekly throughout the adaptation period. Grass based diets utilized a high and low quality harvested green chop. Grass consisted primarily of orchard grass and reed canary grass harvested from pastures at the Rosemount Research and Outreach Center in late August and chopped using a John Deere forage harvester with a haylage head to achieve approximately 5.08 cm particle length. Once harvested, grass was stored in 189 L bags and frozen at -20° C. Bags were removed from the freezer the night prior to feeding to allow to thaw at room temperature. This harvest method allowed for preservation of nutrient composition of grass-fed throughout the Latin square design and allowed for confinement of steers for continuous infusions for flow rate data.

Marker infusion

The triple marker method, developed by France and Siddons (1986), was utilized to determine digesta flow rate. Markers selected for this study were $YbCl_3$ (modified from Siddons et al., 1985) and CoEDTA (Udén et al., 1980) as external markers, and iNDF as an internal marker (Ahvenjärvi et al., 2003). Use of 3 markers allows digesta to be divided into small particle phase, large particle phase, and fluid phase, marked by Yb, i

NDF, and Co respectively. Cobalt-EDTA and $YbCl_3$ were dissolved in distilled water to create a marker solution for continuous infusion into the rumen.

On the first day of the sampling period, steers were fitted with an infusion cannula plug which allowed for continuous infusion of the flow rate marker solution using a peristaltic pump (Masterflex). Cobalt and Yb were infused continuously at a rate of 0.44 g/d, 0.49 g/d, and 0.10 g/d respectively. Prior to the start of continuous infusion, a spot sample of ruminal contents was collected to establish natural abundancy of Yb and Co present. This sample was frozen at -20º C until lyophilization. Immediately prior to the beginning of infusion, a 2 L priming dose of the marker solution was delivered through the cannula opening and thoroughly mixed by hand into ruminal contents. The marker solution was continuously infused into the rumen of the steer for the entirety of the 7 d sampling period, with the first 3 d representing a plateau period for the concentration of markers within the rumen.

Sample collection

On d 4, d 5, and d 6 of the sampling period, samples were collected in 8 h increments. Eight h sampling periods were offset so each day represented a different 8 h time point relative to feeding to create a 24 hour composite. Within each 8 h period, samples of ruminal and omasal fluid were collected every 4 h and composited to represent a 24-h period. Spot fecal samples were collected at every natural defecation during each 8 h period and all samples were composited from the 3, 8 h periods. Omasal samples were collected utilizing the procedure developed by Huhtanen et al. (1997) modified by Ahvenjärvi et al. (2000). A reinforced 1.9 cm diameter hose was manually placed in the omasum of each steer at time of sampling. The hose was connected to a

dual flow, vacuum pump and air compressor to ensure that placement of the hose remained within the omasum and clogs in the line could be easily be unblocked with a low pressure air compressor. Bi-hourly ruminal and omasal samples were divided into 3 subsamples at the time of sample collection: 24 h composite (200 mL), 24 h composite acidified to pH 2.0 with sulfuric acid (75 mL), and bacterial isolation sample (50 mL). Samples were kept on ice during sample collection and stored at -20º C immediately after samples were collected until further processing, with the exception of bacterial samples which were processed immediately following sample collections.

On d 7 of the sample collection period, rumen evacuations were conducted. Animals access to feed and water were restricted and ruminal contents were emptied manually via the rumen fistula opening into a 90 L tub. Once a majority of solid particles were removed, a vacuum pump was used to remove the liquid fraction within the rumen to ensure consistent emptying. Tubs containing ruminal contents were weighed, mixed and subsampled in triplicate. Samples of ruminal contents were frozen at -20º C until further processing and remaining contents were immediately placed back into the rumen via the fistula. Caution was exercised to complete the entire rumen evacuation process in less than 30 min to minimize any detrimental impact on the ruminal microbe population or animal hydration. Access to feed and water was restored immediately following rumen evacuation.

Sample processing and chemical analysis

Composites, acidified composites, and rumen evacuation samples were all frozen immediately post collection at -20º C, while bacterial samples were processed immediately.

Bacterial samples were first strained through 2 layers of cheese cloth. The fluid portion was used to isolate the fluid associated bacteria (FAB) fraction (Firkins et al., 1984), while the solid fraction separated by cheese cloth was used to isolate solid associated bacteria (SAB) following methods outlined by Whitehouse et al. (1994). The fluid portion was centrifuged at 1,000 x *g* for 10 min to remove feed and protozoal contamination. Supernatant was saved for further centrifugation at 20,000 x *g* for 20 min. The supernatant was discarded, while the pellet was saved to represent the FAB fraction and frozen at -20º C until lyophilization. The solid fraction separated from cheese cloth was incubated in 0.8% methylcellulose-saline solution for 1 h in a 39º C water bath. After a 1-h incubation, the solution containing the solid fraction was refrigerated at 4º C for 24 h. After 24 h at 4º C, the sample was mixed for 1 min using an omni-mixer. Solids and methylcellulose-saline solution were then strained through 2 layers of cheese cloth with the solid fraction rinsed with an additional 100 mL of saline. The separated fluid then underwent centrifugation at 1,000 x *g* followed by 20,000 x *g*, as previously described for FAB isolation. The isolated pellet represented the SAB fraction and was stored at -20º C until lyophillization. Bacterial isolations were only conducted for omasal contents.

Omasal and rumen composite samples were thawed at room temperature before further separation. Total sample was weighed and homogenized using an overhead mixer. Prior to separation of digesta phases, a subsample of the homogenized composite sample was collected and saved as a reference sample of the digesta composite. To begin phase separations, samples were strained through 4 layers of cheese cloth. Solids were weighed and placed in a separate storage container to be frozen at -20º C until

lyophilization. This sample represented the large particle fraction of digesta (LP). The fluid fraction remaining after LP was removed was then centrifuged at 10,000 x *g* for 15 min. The supernatant was poured off into a separate container, weighed and stored at -20º C until lyophilization. The supernatant fraction represented the fluid phase of digesta (FP). The pellet formed after centrifugation represented the small particle phase of digesta (SP) and was frozen at -20º C until lyophilization. The weight of the SP phase was determined via subtraction of the weights of FP and LP phases from total original sample weight.

Nutrient analysis and digestibility

Bacterial isolates (FAB and SAB), a subsample of the fecal composites, rumen digesta phases (FP, SP, LP), and omasal digesta phases (FP, SP, LP) were frozen on dry ice prior to lyophilization to ensure samples were solidified and prevent boiling of the sample within the lyophilizer. Subsamples of feed samples from the last 4 d of the sampling period were also frozen on dry ice prior to lyophilization.

Feed samples and fecal samples (minus subsamples for lyophilization) were dried in a conventional drying oven at 55º C. Once dry, samples were ground through a 2-mm screen using a Thomas Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ). Samples were then analyzed for nutrient composition (DM, OM, CP, and NDF/ADF). Feed ingredients were analyzed individually and mathematically re-combined for diet composition. Dry matter was determined using fresh and dry weights from the initial 55º C drying (DM1) and then later corrected using an additional 100º C oven drying of 1 gram subsample (DM2). To measure ash, 1 g of sample was weighed into a crucible and placed in an ashing oven for 12 h at 450º C. Organic matter (OM) was determined using

1 minus ash content of sample. Crude protein (CP) was determined via steam distillation Kjeldahl technique (Kjeltec 2300). A sample of 0.5 g was weighed into a Kjeldahl tube, along with 1 $CuSO_4$ kjeltab and 10 mL H_2SO_4. The tube contents were digested at 410º C for 1 h, after which they were steam distilled using NaOH as an alkali addition, boric acid as an indicator solution, and 0.1 M HCl as titrant (all titrations done automatically by Kjeltec 2300). Using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), neutral detergent fiber (NDF) was determined via 60 min extraction in 100º C neutral detergent solution with additions of sodium sulfite and heat stable alpha-amylase. Samples high in fat have shown great variation in NDF results due to bound fat within the fiber matrix. To reduce variability between replicates, samples with greater than 5% fat underwent a biphasic fat pre-extraction procedure prior to NDF analysis using diethyl ether and petroleum ether (modified from Bremer et al., 2010). After NDF analysis, samples were gently shaken in 400 mL of acetone, allowed to air dry for 2 h and then placed in a 100º C oven overnight to obtain a hot weight. This final weight along with a hot bag weight and a sample weight were used to calculate NDF percent of the sample. After a final hot weight was collected, the sample then underwent acid detergent fiber (ADF) analysis using a 60 min 100º C extraction procedure in an ANKOM 200 fiber analyzer, with ADF solution. After ADF analysis, samples were placed in 100º C oven overnight to obtain a hot weight. This final ADF weight along with the final NDF hot weight were used in the calculation of ADF percent of the sample.

Subsamples of feed as well as omasal phases were all lyophilized using a Virtis shelf freeze drier. Approximately 500 g of sample were placed in 22.9 x 33.0 x 5.1 cm aluminum pans spread less than 2 cm thick and covered with a standard hair net. The

hair net prevented transfer of small particles or volatile compounds into the oil of the lyophilizer. Once in aluminum pans, samples were placed on dry ice overnight until the sample was completely frozen. This is critical for rumen and omasal fluid samples to prevent boiling of any liquid within the sample during the sublimation process. The lyophilized subsample of feed was analyzed for fatty acid (FA) profile. Analysis was completed using Jenkins 1-step direct methylation procedure, with internal standards of Tridecanoic acid (C13:0) and Heptadecenoic acid (C17:1). A 0.5-g sample was weighed into screw cap borosilicate tube and 0.5 mL of 2 mg/mL internal standard:toluene and 3 mL 5% Methanolic HCl were added directly to the tube. The tube was vortexed and incubated in 70º C shaking water bath for 2 h. Following incubation, 7.5 mL 6% K_2CO_3 and 1 mL Hexane were added. The solution was centrifuged at 286 x *g* for 8 min at 4º C. The organic layer was transferred to a separate tube and one scoop (~0.4g) of charcoal was added. The solution was vortexed and allowed to sit for 1 h, after which the sample was re-centrifuged at 418 x *g* for 5 min. The top layer of solution was pipetted into a gas chromatography (GC) vial and refrigerated until analyzed for fatty acid composition. Fatty acid methyl esters were measured using GC using an Agilent 7890B gas chromatograph. Samples were run through a capillary column (100 m x 0.25 mm i.d. with 0.2 um film thickness) with fame-ionization detector with hydrogen as carrier gas. Pure methyl ester standards (GLC 60; Nu Chek Prep Inc., Elysian, MN) we used to identify methyl ester peaks.

Separated phases of omasal digesta were also analyzed for DM (via lypohilization pre and post-weights), OM, NDF/ADF, CP, and fatty acids using the same procedures described for feed analysis with the exception of fatty acid analysis of omasal digesta.

Feed was analyzed for fatty acid composition using Jenkins-1 step direct methylation procedure, while omasal digesta phases were analyzed via Jenkins-2 step methylation procedure. The primary difference between these two procedures is that the Jenkins 2-step adds 2 ml 0.5 M Sodium Methoxide to the sample and incubates it at 50º C for 10 min prior to addition of 5% Methanolic HCL. The remainder of preparation steps proceed in accordance with those described for feed analysis.

Lyophilized digesta phases were also measured for Yb, Co, and iNDF as markers for passage rate determinations. Samples were prepared for Yb and Co analysis following the procedure of Ellis et al. (1982). Omasal digesta phases (LP, SP, and FP), and fecal samples were ashed and digested in an acid mixture of 3M HCL and 3M HNO_3 for 12 h. Samples were filtered through a Whatman #1 filter and diluted using deionized water and a 6% KCl solution. Natural abundance of markers (particularly Co) were determined by measuring Yb and Co in lyophilized rumen spot samples collected prior to the infusion of marker solution. Concentration of Yb and Co were determined using Inductively Coupled Plasma (ICP) spectrometry at UF/IFAS Analytical Services Laboratory (Gainesville, FL). Both SP and LP omasal digesta phases, in addition to fecal samples and rumen evacuation samples were analyzed for iNDF (Van Soest et al., 1991), with iNDF representing the large particle passage rate of digesta. The fluid phase was not analyzed for iNDF as it was assumed that no indigestible fiber was present in this phase. A 0.5-g sample was weighed into a 125-mL Erlenmeyer flask, in duplicate. Samples were inoculated with 40 mL of pre-warmed in vitro “day of inoculation” solution (mixture of in vitro rumen buffer, in vitro macromineral solution, and in vitro micromineral solution plus Trypticase and a reducing solution and indicator), and 10 mL

of rumen fluid previously strained through 4 layers of cheese cloth. Samples were placed in a 39º C water bath continuously gassed with CO_2 with pressure monitored using a barometer to maintain approximately 5 cm water displacement. All flasks were incubated for 240 h and then stored at 4º C until analyzed. Contents of each fermentation bottle, including blanks, were analyzed for NDF content using Van Soest reflux apparatus method with same solution as NDF method described earlier (Van Soest et al., 1991). Neutral detergent fiber remaining after 240 h incubation was considered indigestible (iNDF). This procedure was repeated until there were 4 observations per sample with 2 different fluid inoculation time points. In addition to the analysis of the 3 markers for digesta passage rate (Yb, Co, iNDF), a microbial marker was also analyzed to determine microbial contribution to nutrient flow. Purines were used as internal microbial marker and measured in both the microbial isolates as well as omasal digesta phases using the procedure developed by Zinn and Owens (1986). Pure torula yeast RNA was utilized as a standard to generate the standard curve using known concentrations of pure bacterial purines. All samples and standards were digested in 70% perchloric acid at 90º C for 1 h after which 0.0285M Ammonium Phosphate buffer was added to solution to help break up the charred mass formed from perchloric acid digestion of the sample. Solution was vortexed and incubated at 90º C for an additional 15 min. Samples were immediately filtered through a Whatman 54 filter. Filtered solution (0.5 mL) was combined with 0.5 mL of silver nitrate in a screw-cap centrifuge tube, along with 9 mL of 0.2M ammonium phosphate buffer. Samples and solution were then stored in a dark refrigerator overnight. After overnight incubation, samples were centrifuged at 10,000 x *g* for 10 min, supernatant was discarded and pellet washed with 10 mL of deinonized water that was

adjusted to pH 2 with sulfuric acid. Samples were re-centrifuged and supernatant was discarded once again. Ten mL of 0.5 M HCl were then added to the pellet and vortexed to suspend the pellet in solution. Samples were then incubated for 30 min at 90º C, and re-centrifuged. Supernatant was pipetted into a UV transparent 96 well plate and read at 260 nm using a Biotek Synergy plate reader. Absorption values were converted to mg/mL using the standard curve created with torula yeast.

Samples of rumen and omasal fluid that were acidified and left in the collected form were thawed and analyzed for ammonia-N and VFA. In preparation for ammonia-N analysis, samples were first centrifuged at 5,000 x *g* for 15 min to remove feed particles from solution. Then, 15 mL of sample were pipetted into a Kjeldahl tube containing 2g MgO and 15 mL of distilled water. This solution was then analyzed via steam distillation in a Kjeltec 2300 for N content, representing ammonia-N concentration of the fluid sample. Acidified rumen and omasal fluid were also analyzed for selected VFA: acetate, propionate, butyrate, valerate, isobutyrate, isovalerate and 2-methylbutyrate. Five-point calibration curves were built for each of these VFA with known amounts of standard solutions. Calibration curves allow a correlation to be constructed between the area under the curve value provided from gas chromatograph and the concentration of VFA present. Samples were prepared for GC analysis following a modified procedure by Erwin et al. (1961) for ruminal fluid VFA analysis. Samples were prepared using a similar centrifugation process to that used for ammonia-N analysis to remove feed particles. A 2.0 mL sample of supernatant was added to a solution of 25% meta-phosphoric acid/2-Ethyl-Butyrate (2-EB) which then underwent a series of centrifugation and freeze and thaw steps. During these steps, meta-phosphoric acid aids in protein

precipitation while 2-EB serves as an internal standard for GC analysis. Supernatant was filtered through 0.45 μm polyethersulfone micropore-filter and added to distilled water. One N NaOH was utilized to adjust pH of the solution between 6 and 7 to prevent any damage to the GC column packing. Prior to transferring to a GC vial for analysis, 0.03% oxalic acid was added to solution to prevent "ghost effects" and maintain column performance by degrading to formic acid during injection (Fussell and McCalley, 1987). Samples were then transferred to a GC vial and frozen at -20º C until analysis was conducted. Prepared sampled were thawed for 1 h prior to analysis in Hewlet-Packard HP6890 Gas Chromatograph. Samples were run through 2 m x 0.64 cm x 2 mm carbopack glass column (SUPELECO) with 40 min run time: 27 min initial run time at 175º C, 9 min ramp time to 225º and 4 min post run.

Calculations

Calculated reconstitution of true omasal digesta, devoid of sampling error, was determined using equations outlined by France and Siddons (1986).

Statistical Analyses

Data were analyzed using the mixed procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Intake, fermentation parameters and flow data were analyzed as a Latin square with experimental design effects included as random factors. The statistical model was as followed:

$$Y_{ijkl} = \mu + \alpha_i + S_k + P_l + \varepsilon_{ijkl}$$

μ = population mean
α = i^{th} effect of treatment
S = Random k^{th} effect of steer
P = Random l^{th} effect of period in time
ε = residual error

Hourly pH measurements were analyzed within a similar model with hour as a repeated measure, and subject of steer within period with variance modeled using Toeplitz covariance structure.

RESULTS AND DISCUSSION

Intake, rumen volume and flow rate

Grass-fed cattle had greater ($P < 0.01$) as-fed intakes (AFI) relative to grain-fed cattle, but lower dry matter intakes (DMI; $P < 0.01$; Table 2.3) relative to grain-fed treatments. Differences in intake can be explained by extreme differences in DM % of the diet (Table 2.2). However this inverse relationship between AFI and DMI is important to consider when interpreting weight of ruminal contents. No differences were observed ($P = 0.30$) between treatments for DM ruminal contents, but increased ($P < 0.001$) as-is ruminal content weights were observed for grass compared with grain treatments (Table 2.3). Droulliard and Kuhl (1999) stated that ruminal fill on a DM basis has limited use and application because there is no consideration of variation in DM content of the diet. Because grass-fed cattle consumed less feed on a DM basis but had no difference in DM ruminal contents it can be concluded that these animals had a longer rumen retention time than grain-fed counterparts. This conclusion is reinforced by greater as-is ruminal contents observed for the grass-fed treatment. This slowed passage out of the rumen paired with greater as-is ruminal contents lead to the conclusion that grass-fed cattle had greater rumen capacity. Greater rumen volumes were expected for grass-fed cattle as greater as-fed intakes are generally required in order to consume calories necessary for energy requirements. A review by Rohr and Daenicke (1984) found that gut fill ranged from 11 to 17.1% of live weight depending upon various dietary

factors. Dietary forage type plays a large part in this variation. Feeding forages typically leads to an increase in passage rate if the forage consumed is high quality. Contrary to this, less true digesta flow (kg/d) was observed ($P = 0.05$; Table 2.3) for grass-fed cattle compared with grain-fed cattle despite a high quality orchard grass-mix being fed. This may be a consequence of experimental execution. Because cattle were fed once a day in bunks, feedlot-like eating behaviors were exhibited. Larger meals were consumed earlier in the day with all feed consumed before feeding the following day. This behavior contradicts normal grazing which would be multiple small meals throughout the day to seemingly "push" feed through the rumen at faster rate. Instead, longer retention times were observed ($P = 0.03$; Table 2.3) for grass-fed compared with grain-fed cattle.

Fermentation parameters and rumen environment

Ruminal volatile fatty acid (VFA) concentration was greater ($P = 0.05$) for grain-fed cattle compared with grass-fed (Table 2.4). However when extrapolated to moles of VFA in the rumen, there were no differences between treatments ($P = 0.53$; Table 2.4), demonstrating the importance of rumen volume when comparing different feeding systems (i.e.- grain vs. grass-fed cattle). Despite similar VFA totals in the rumen, grass-fed cattle had a higher ruminal pH ($P < 0.01$) , with an average ruminal pH of 6.6 (GRASS) vs 6.0 (FLAX) and 6.0 (CORN) in grain-fed treatments (Figure A2.1; Table 2.5). Less severe pH declines are partially explained by a greater ($P < 0.01$) acetate to propionate ratio in grass compared with grain-fed cattle (Table A2.4). Greater pKa of propionate leads to more severe pH impacts on the rumen compared with acetate (van Houtert, 1993). Additionally, production of acetate is associated with microbial

breakdown of forages. Fiber digestion by ruminal bacteria occurs after a lag period leading to a delay in digestion rate. This slowed rate of digestion in combination with a less severe pKa of the primary VFA produced, acetate, offers an explanation for the higher ruminal pH of the grass-fed treatment while maintaining the same total volume of VFAs as grain-fed treatments. Due to an increase in mastication and rumination with high forage-fed ruminants, there is also an increase in buffering capacity of ruminal contents due to larger quantities of saliva production.

Nitrogen Flow

Because of challenges in balancing a grain-based finishing ration to match the high CP content of the orchard grass mixture utilized, diets were not isonitrogeous. The grass-fed diet was highest in CP (19.1 for GRASS, 17.5 for FLAX and 17.5% for CORN; Table 2.2) of all treatments. However on a g of N intake basis, grass-fed cattle had the lowest N intake ($P < 0.01$; Table 2.6) due to lower DMI ($P < 0.01$; Table 2.3), demonstrating that a balanced total N flow would have to be achieved by formulating rations with known DM intakes.

Lower intake of N in GRASS was accompanied by lower ($P < 0.01$) g of non-ammonia non-microbial nitrogen (NANMN) passage as a percent of total N flow (Table 2.6), indicating greater ruminal digestion of dietary CP compared with grain-fed cattle. This greater ruminal digestion could be due to differences in ruminal degradable protein between diets (which was not tested), or due to the slower passage rate which allowed for longer retention of feed in the rumen. If the latter, better understanding the impact of forage eating behavior on digestibility is important in extrapolating these results back to grazing cattle from those fed green-chop in a bunk as in this study.

Greater dietary CP degradability in the rumen of less readily available N source can explain a higher (P = 0.02; Table 2.6) ruminal ammonia-N flow in GRASS compared with CORN and FLAX. Bacterial-N as a percent of total N flow was also higher (P = 0.01) for grass-fed compared with grain-fed treatments. Reis and Combs (2000) observed a decrease in ruminal ammonia-N when concentrate was fed to grazing dairy cows. They concluded that adding fermentable substrate to provide energy to microbes improved utilization of N in pasture-grazed ruminants. Although an increase in ammonia-N can indicate inefficient utilization of dietary N, ammonia as a substrate serves as the primary N source for fiber digesting bacteria, especially cellulolytic bacteria (Burroughs et. al., 1951). Therefore, an increase in ammonia accompanied by an increase in bacterial-N flow may indicate an ideal environment for fiber digesting bacteria to flourish. This ideal environment may partially explain the increase (P = 0.01; Table 2.6) in percent bacterial-N flow.

The NRC (1996) MCP synthesis model with a forced (0, 0) intercept, predicts digesta flow of finishing steers to contain approximately 816 g MCP/d, while digesta from growing steers fed high forage were estimated at 609 g MCP/d. The NRC (2017) MCP synthesis model with no forced intercept, predicts finishing steers flow to be approximately 822 g MCP/d while growing steers were estimated at 450 g MCP/d. Within the current experiment, using FLAX as comparison for finishing steers and GRASS as comparison for growing cattle, values of 899 g MCP/d and 658 g MCP/d respectively were determined. An overestimation of MCP was expected because purines were used to analyze bacterial content (Obispo and Dehority, 1999). Despite the overestimation, from the comparison of these data to the NRC models, the NRC (1996)

model was determined to be a better predictor of MCP synthesis. The difference in MCP synthesis can be explained by flow rate differences in cattle fed these types of diets. A lower quantity of bacteria within digesta flow was expected for treatments with lower total digesta flow, simply due to longer retention times in the rumen. Initially higher (P = 0.05; Table 2.3) OM digestibility for GRASS supports the concept that more substrate was available for microbes because of a larger rumen capacity. The lower quantity of MCP makes sense because total flow (kg/d) was lower (P = 0.05; Table 2.3) for GRASS. On a percent basis, there is an increase in bacterial contribution to N flow for GRASS (P = 0.02; Table 2.6).

Fatty acid profile transformation

In general, g of stearic acid in the flow of digesta were higher than dietary stearic acid intake due to biohydrogenation of C18 unsaturated fatty acids by rumen microbes. Results from the current experiment show an increase (P = 0.04) in the ratio of g of stearic acid to g of C18:2 and C18:3 fatty acid intake for GRASS compared with either FLAX or CORN. This may be due to the fact that C18:3 and C18:2 make up a large majority of the total fatty acids in the diet of grass-fed cattle and therefore an increase in ruminal availability leads to a greater contribution to stearic acid formation. Linolenic (C18:3) and Linoleic (C18:2) acids are also preferentially biohydrogenated over fatty acids with less unsaturated bonds like C18:1. Doreau and Ferlay (1994) noted an increase in the amount of C18:3 biohydrogenation compared with C18:2 when added as a substrate to the same rumen environment.

This study hypothesized that grain fed cattle may have lowered biohydrogenation of polyunstaturated fatty acids because of an increase in time spent below pH 5.7.

However this is difficult to discern due to DMI differences across treatments in g of fatty acids consumed. No differences ($P = 0.88$) in g of omega-3 fatty acid transformed were observed across treatments while lower g/d omega-6 fatty acid transformation was observed for grass compared with grain-based diets ($P = 0.01$; Table 2.7). Oleic acid showed a trend ($P = 0.07$) for increased transformation when cattle were fed grass compared with grain-based diets (Table 2.7). The lack of consistent response in fatty acid transformation across the major C18 PUFA suggests that ruminal environment effects transformation differently for different fatty acids. It is important to note that the trend for an increase in biohydrogenation of fatty acids with increased unsaturated bonds first observed by Doreau and Ferlay (1994) is also found in the current experiment regardless of treatment. Linolenic acid had the greatest numeric g of fatty acids transformed per g dietary fatty acids intake, followed by linoleic and oleic acid respectively (Table 2.7).

Russel and Dombrowski (1980) indicated that ruminal pH had a strong impact on reducing enzyme activity involved in the biohydrogenation process. However with inconsistent transformation responses to treatment observed across C18:1, C18:2, and C18:3 unsaturated FA, the question becomes whether biohydrogenation processes are more effected by ruminal pH, dietary monensin inclusion or a combination of the two? Because monensin was added to both grass-fed and grain-fed diets, it is not possible to measure this in the current experiment. No differences were observed in omega-3 biohydrogenation across treatments, so it can be concluded that pH changes in the presence of monensin, specifically time spent below 5.7, do not have a large impact on the extent of biohydrogenation of omega-3 fatty acids. Contrary to what was hypothesized, grass-fed cattle elicited a decrease in biohydrogenation of C18:2 fatty

acids. Oleic acid was the only fatty acid that showed a trend for higher ($P = 0.07$) transformation in grass compared with grain-based diets. Differences in transformation are most likely related to dietary fatty acid profile and abundance of certain fatty acids within the rumen. Linoleic acid (C18:2) accounts for 18% the fatty acids in FLAX and 51% of the fatty acids in CORN, but only contributes 7% of fatty acids in GRASS. Therefore C18:2 would be more concentrated in fat fraction in rumen of grain fed animals than grass fed, which would change probability of biohydrogenation microbes having contact with C18:2 in different rumen conditions. Further research will need to be conducted to fully understand the effect of monensin and ruminal pH on biohydrogenation across grain and grass-fed diets.

Despite no differences in transformation percent, greater fatty acid intake stimulated greater omega-3 fatty acids in the digesta of FLAX cattle. Because USDA regulations for omega-3-rich meat require suppliers to list the mg of omega-3 present in the product, increasing total g of omega-3 in the meat using flax supplementation could be an economically and sustainably viable alternative to grass-feeding systems for producing beef products.

Unpublished data by Bauchart and Poncet referenced in Chilliard et al. (2000) shows differences in efficiency of utilization of omega-3 fatty acids under different rumen environmental conditions. The lowest efficiency of utilization of omega-3 was in fresh grass based diets, however in this study fresh grass also had the greatest amount of C18:3 intake. Numerous studies (Chilliard et al., 1991; Drackley et al., 1992; Christensen et al., 1994; LaCount et al., 1994; Ottou et al., 1995; Litherland et al., 2005) determined a linear decrease in transfer efficiency of C18:3 from the intestine to deposition in milk when increasing C18:3 amounts are presented to the small intestine. Bauchart and Poncet (unpublished data)

indicated that an increase in C18:3 presented to the small intestine may have negatively affected the efficiency of utilization of C18:3 for grass-fed cattle (Chilliard et al., 2000). However this study also compared efficiency of C18:3 utilization for diets containing hay to concentration ratios of 75:25 and 30:70. It is interesting to note the increase in efficiency of utilization from 4.71% in 75:25 diet to 9.05% in 30:70 diet despite very similar C18:3 grams presented to the small intestine. This increase in efficiency indicates that grain-fed cattle fed diets may have an improved ability to utilize C18:3 for incorporation into meat products. An increase in efficiency of C18:3 utilization presents an added benefit of utilizing flax supplementation in grain-based systems to obtain omega-3 rich meat products to meet the demand of the modern-day consumer.

CONCLUSIONS

Diet is a primary driver in characterizing the ruminal environment. Rumen volume and passage rate were characterized both by diet as well as eating behavior. Grass-fed cattle exhibited larger as-fed intakes but lower DM ruminal contents because of the high moisture content of the diet. Despite larger as-fed intakes, grass-fed cattle also had slower passage rates compared with grain-based treatments likely due to once-a-day, slick-bunk management rather than traditional continuous feeding in grazing environment. Slower passage rate in grass-fed cattle increased OM digestibility and % bacterial N flow in digesta. An increase in ruminal ammonia and decrease in RUP in omasal true digesta of grass-fed cattle compared with grain-based treatments demonstrated an inefficiency of utilization of N sources, most likely as an alternative energy source for microbial growth. The magnitude of transformation of unsaturated fatty acids was directly related to the contribution of that fatty acid to the dietary inclusion. Fatty acids with the greatest number of unsaturated bonds found in the highest abundance were transformed preferentially. Despite no differences in percent omega-3

transformation across treatments, cattle on the FLAX treatment demonstrated the greatest g of omega-3 fatty acid flow escaping biohydrogenation due to an increase in intake of dietary omega-3 fatty acids. From these conclusions it can be implied that providing grain-fed cattle with a dietary source of omega-3 FA, like flax oil, is an economically and sustainably favorable alternative to grass-fed systems for producing beef with increased mg of omega-3 fatty acids.

Table 2.1 Dietary ingredient composition of grass-fed and grain-fed treatments with either a flax oil or corn oil supplement

Inclusion, % DM	**GRASS**	**FLAX**	**CORN**
Green chop grass[1]	95	.	.
Rye grass silage	.	12	12
Soybean meal	.	14	14
Dry rolled corn	.	75	75
Flax oil	.	2	.
Corn oil	.	.	2
Liquid supplement[2]	5	.	.
Liquid supplement[3]	.	4	4

[1]Green chop grass was harvested at one date, frozen and thawed and fed fresh daily

[2,3]Liquid supplement - provides increased RDP in form of urea and formulated to supply 300 mg/hd/d monensin

[2]Liquid supplement = low protein concentration

[3]Liquid supplement = high protein concentration

Table 2.2 Nutritional composition of dietary treatments for grass-fed and grain-fed cattle with either a flax oil or corn oil supplement

	GRASS	**FLAX**	**CORN**
DM, %	27.3	79.5	79.6
OM, % DM	86.3	96.0	96.0
CP, % DM	19.1	17.5	17.5
NDF, % DM	53.6	21.1	21.0
ADF, % DM	27.1	8.9	8.9
TDN[1], % DM	70.2	89.7	89.7
NEm, Mcal/kg [2]	1.74	2.31	2.31
NEg, Mcal/kg [3]	1.03	1.54	1.54
Fatty Acids, % DM	2.7	6.1	5.9
C18:0, % FA	0.1	0.2	0.1
C18:1, % FA	0.2	0.5	0.5
C18:2, % FA	0.2	1.1	3.0
C18:3, % FA	1.0	2.5	0.3

[1]calculated from TDN contribution of each ingredient calculated from ADF to TDN equation for that particular ingredient class
[2] NEm: Mcal/kg = (TDN % x 0.01318) - 0.132
[3] Neg: Mcal/kg = (TDN % x 0.01318) - 0.459

Table 2.3 Intake, digestibility, flow, and rumen volume measurements for grass-fed and grain-fed cattle with either flax oil or corn oil supplement

	GRASS	**FLAX**	**CORN**	**SEM[1]**	***P*-value**
As-fed intake, kg/d	17.84 [a]	10.68 [b]	11.18 [b]	0.50	< 0.01
DM intake, kg/d	5.04 [a]	8.50 [b]	8.80 [b]	0.36	< 0.01
True digesta Flow, kg/d	6.42 [a]	8.15 [b]	8.24 [b]	0.69	0.05
True OM digestibility[2] , %	68.18 [a]	59.42 [b]	54.57 [b]	5.25	0.05
Flow rate, % of rumen volume/h	3.40	5.10	5.33	0.51	0.06
Retention time, h	30.37 [a]	21.38 [b]	19.46 [b]	2.45	0.03
Ruminal contents DM, kg	8.12	6.93	6.72	0.74	0.30
Ruminal contents DM, % SBW	2.79	2.43	2.15	0.20	0.09
Ruminal contents as-is, kg	59.23 [a]	39.57 [b]	38.79 [b]	2.47	< 0.01
Ruminal contents as-is, % SBW	20.7 [a]	13.9 [b]	12.8 [b]	1.2	< 0.01

[1]SEM = Standard error of the mean
[2]calculated: kg OM intake – (kg OM flow – kg OM bacteria in flow)
[ab]Unlike superscripts differ ($P < 0.05$)

Table 2.4 Ruminal VFA concentration and composition as affected by grass-fed and grain-fed cattle with either a flax oil or corn oil supplement

	GRASS	FLAX	CORN	SEM[1]	*P*-value
Total, mM	136 [b]	182 [a]	189 [a]	22	0.05
Total Mol in rumen volume[2]	7,056	8,020	7,331	732	0.53
acetate, %	70.0 [b]	46.6 [a]	48.5 [a]	1.2	< 0.01
propionate, %	19.6 [b]	39.1 [a]	36.5 [a]	2.5	< 0.01
butyrate, %	7.1	9.8	9.7	1.7	0.45
valerate, %	0.6 [b]	2.1 [a]	2.0 [a]	0.2	< 0.01
isobutyrate, %	0.9	0.8	0.8	0.1	0.54
isovalerate, %	0.6	0.6	0.7	0.1	0.57
2-MB[3], %	1.1	1.0	1.7	0.3	0.17
Acetate : Propionate	3.6[b]	1.2[a]	1.4[a]	0.2	< 0.01

[1]SEM = standard error of the mean
[2]Total mol in total rumen volume = mM * kg as-is weight in rumen
[3]2-MB = 2-methylbutyrate
[ab]Unlike superscripts differ ($P < 0.05$)

Figure 2.1 Hourly differences in ruminal pH post feeding as affected by grass-fed and grain-fed cattle with either a flax oil or corn oil supplement

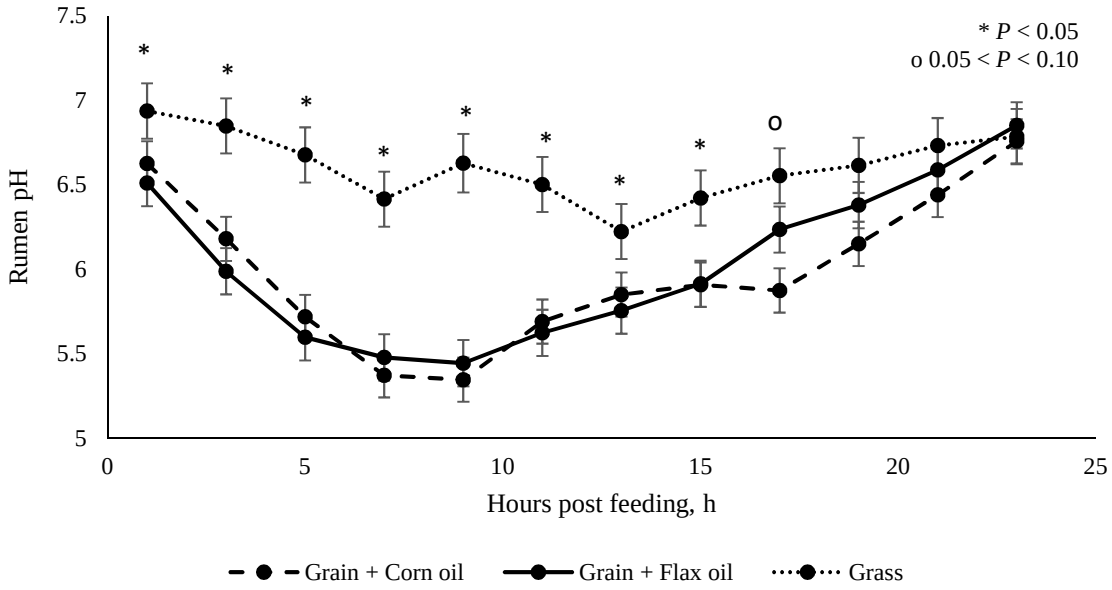

Table 2.5 Effects of grass-fed and grain-fed cattle with either a flax oil or corn oil supplement on average ruminal pH and time ruminal pH spent below ruminal pH 5.7

	GRASS	**FLAX**	**CORN**	**SEM[1]**	***P*-value**
Average pH	6.6 [a]	6.0 [b]	6.0 [b]	0.1	< 0.01
Time below 5.7, h	0.0 [a]	7.5 [b]	8.8 [b]	2.0	0.02

[1]SEM = standard error of the mean
[ab]Unlike superscripts differ ($P < 0.05$)

Table 2.6 Omasal true digesta N flow for grass-fed verses grain-fed cattle with either a flax oil or corn oil supplement

	GRASS	FLAX	CORN	SEM[1]	*P*-value
N Intake, g/d	141.4 [b]	251.2 [a]	253.5 [a]	9.9	< 0.01
NH_3-N[3], mg/dL	6.3 [b]	3.4 [a]	3.6 [a]	1.3	0.01
Total N Flow, g	146.6 [b]	250.7 [a]	251.3 [a]	15.2	< 0.01
NH_3-N[3], g/d	1.2 [b]	0.8 [a]	0.8 [a]	0.3	0.02
Bacterial N, g/d	105.3 [b]	143.9 [a]	155.0 [a]	9.4	0.01
NANMN[2], g/d	40.1 [b]	106.1 [a]	95.1 [a]	10.4	< 0.01
NH_3-N[2], % of total N	4.1 [b]	1.4 [a]	1.5 [a]	0.8	< 0.01
Bacterial-N, % of total N	69.5 [b]	57.3 [a]	61.2 [a]	2.9	0.02
NANMN[3], % of total N	26.4 [b]	41.4 [a]	37.4 [a]	3.0	< 0.01

[1]SEM = standard error of the mean
[2]NH_3-N = Ammonia-N
[3]NANMN = non-ammonia, non-microbial nitrogen
[ab]Unlike superscripts differ ($P < 0.05$)

Table 2.7 Fatty acid composition of intake, omasal true digesta flow, and percent dietary fatty acid transformed in the rumen for grass-fed and grain-fed cattle with either a flax oil or corn oil supplement

	GRASS	FLAX	CORN	SEM[1]	*P*-value
Intake, g/d					
C18:0	4.8 [b]	16.2 [a]	12.5 [c]	0.6	< 0.01
C18:1	9.9 [b]	40.3 [a]	43.3 [a]	1.5	< 0.01
C18:2	9.0 [b]	210.7 [a]	260.6 [c]	7.3	< 0.01
C18:3	50.3 [b]	90.0 [a]	22.4 [c]	3.2	< 0.01
C18:2 + C18:3	59.3 [b]	300.7 [a]	283.1 [a]	10.2	< 0.01
Flow, g/d					
C18:0	22.9 [b]	52.2 [a]	66.3 [a]	7.8	< 0.01
C18:1	2.9 [b]	23.1 [a]	22.4 [a]	3.7	< 0.01
C18:2	1.0 [b]	8.9 [a]	6.8 [c]	0.6	< 0.01
C18:2 t10c12	0.1	0.7	0.6	0.2	0.18
C18:2 c9t11	0.5	0.7	0.6	0.2	0.67
C18:3	1.1 [b]	1.5 [a]	0.4 [c]	0.1	< 0.01
C18:2 + C18:3	2.1 [b]	10.4 [a]	7.1 [c]	0.6	< 0.01
g in digesta flow / g of intake					
C18:0 / C18:0	503.1	321	511.9	139.4	0.16
C18:0 / C18:2 + C18:3	0.4 [b]	0.2 [a]	0.2 [a]	0.1	0.04
g transformed[2] / g of intake					
C18:1	0.70	0.44	0.50	0.08	0.07
C18:2	0.89 [b]	0.96 [a]	0.97 [a]	0.02	0.01
C18:3	0.98	0.98	0.98	0.005	0.88
C18:2 + C18:3	0.97 [b]	0.96 [a]	0.98 [b]	0.003	0.04
g in digesta flow/ g transformed[2]					
C18:2 t10c12 / C18:2 + C18:3	2.3	2.3	2.1	0.8	0.99
C18:2 c9t11 / C18:2 + C18:3	8.4 [b]	2.2 [a]	1.8 [a]	1.2	0.01
True Digesta Flow Ratio C18:2 to C18:3	1.2 [b]	6.0 [a]	16.2 [c]	0.9	< 0.01

[1]SEM = standard error of the mean

[2]g transformed = g/d of intake – g/d in digesta

[abc]Unlike superscripts differ ($P < 0.05$)

Chapter III.

EFFECT OF INCLUSION OF DISTILLERS GRAINS WITH SOLUBLES AND CRUDE GLYCERIN IN BEEF CATTLE FINISHING DIETS ON RUMINAL FERMENTATION AND FATTY ACID BIOHYDROGENATION

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INTRODUCTION

Within modern grain-based cattle feeding systems, an increase in linoleic acid concentration within diets has become a topic of interest because of feeding of ethanol byproducts such as distillers grains and corn oil. Corn-based ethanol is produced via fermentation of starch found within the grain. Byproducts from this process consist of the remaining fractions of corn grain and are commonly utilized as feed sources for livestock. With the starch fraction removed from corn grain, the concentration of fat within remaining byproducts is much higher than in corn grain itself. Unsaturated fatty acids make up over 80% of the fat composition of corn, with over 50% of unsaturated fatty acids being linoleic acid. Henderson (1973) demonstrated in pure culture that unsaturated fatty acids exhibited greater negative impacts on the growth of cellulolytic bacteria than saturated fats. Several studies have reported a shift in ruminal fermentation with an increase in the supplementation of dietary fat concentrations (Czerkawski 1966; Clapperton et al., 1969; Dinius et al., 1974; Zinn, 1989). A decrease in ruminal acetate production with an increase in ruminal propionate and a decrease in methane production

are consistent responses to addition of dietary fat (Clapperton et al., 1969). A decrease in growth of cellulolytic bacteria in response to supplementation of dietary fat explains the decrease in ruminal acetate concentration and lowered methane production (Rasmussen and Harrison, 2011). An increase in ruminal propionate concentration can be explained by increased lipolysis of triglycerides freeing glycerol backbones which ferment to propionate in the rumen (Wang et al., 2009).

To avoid detrimental impacts of high concentrations of dietary fatty acids on ruminal fermentation but still maintain an energy-dense feed ingredient, investigations into alternative byproducts, such as glycerin, have been conducted. Glycerin is a byproduct of the biodiesel industry and is comprised of the glycerol backbone of a triglyceride fat. Glycerol has been suggested to ferment directly to propionate (Wang et al., 2009). However, dietary glycerin, similar to unsaturated fatty acids, has been shown to have negative effects on fiber-digesting bacteria (*Butyvibrio fibrosolvens* and *Selenomonas ruminantium*; AbuGhazaleh et al., 2011). A similar decrease in ruminal acetate and increase in ruminal propionate would be expected when feeding glycerin or supplementing unsaturated fat.

De Mello et al. (2017) reported an increase in concentrations of total polyunsaturated fatty acids (PUFA) within steaks resulted in greater oxidation of beef product in a retail case as well as greater surface discoloration. Steers within the study were fed 0, 15, or 30% of their dietary DM as distillers grains plus solubles, which resulted in significant increases in steak omega-6 fatty acids with increasing dietary distillers inclusion. However, there was no difference in overall fat percentage of the steak. This suggests that shifting fat composition without shifting total fat leads to

changes in meat shelf life. This change in shelf life could potentially alter consumer desirability due to increased discoloration of beef from cattle fed increased concentrations of PUFAs. Despite the impact on meat quality, fat content of distillers grains provides an excellent source of dietary energy to feedlot cattle. As the ethanol process has been refined, distillers grains with solubles are no longer the primary byproduct created. Instead, as fat extraction of distillers grains has become more common, reduced-fat distillers and extracted corn oil are the primary byproducts formed. Nelson et al. (2016) examined the effects of feedlot cattle diets containing 15% full fat distillers (FFDGS) compared with 15, 30, and 45% reduced fat distillers (RFDGS) on meat quality. In contrast with De Mello et al. (2017), there was no difference in retail shelf life for steaks, ground beef, or bologna. This is likely explained by lack of differences in omega-6 content of the beef between treatments. This is consistent with the literature that reports low concentrations of dietary PUFA (such as that found in 15% FFDGS) have no impact on meat quality as majority of PUFA will be biohydrogenated within the rumen. However, as dietary inclusion reaches 30-40% FFDGS differences in shelf life stability are noticed (De Mello et al., 2017).

Based on the previous literature it was hypothesized that glycerin or distillers grains could partially replace corn as a dietary energy source in cattle diets. However, supplementation of byproducts can alter post-ruminal unsaturated fatty acid content of the digesta, which can change shelf life stability of the final beef product. Therefore the objective of this study was to examine the impact the rumen plays on altering the fatty acid profile of digesta when distillers grains (DGS) and/or glycerin (GLY) replaced steam flaked corn (SFC) in the diet. Specific objectives were to:

1. Understand how utilization of bio-fuel byproducts impacts the ruminal environment and fatty acid profile at end point of fermentation
2. Determine differences in PUFA content at endpoint of fermentation when bio-fuel byproducts are fed to ruminants.

MATERIALS AND METHODS

The experiment was conducted at the University of Minnesota Beef Research Facility in Rosemount, MN. All animals involved in this experiment were cared for according to guidelines of the University of Minnesota Institutional Animal Care and Use Committee (IACUC). All experimental procedures, including diets, sampling, and cannulation were reviewed and approved by IACUC (protocol #1507-32777A).

Animals, Experimental Design, and Dietary Treatments

Four ruminally cannulated Holstein steers (371 ± 5 kg) were utilized in a 4 x 4 Latin square design (n = 1 per period). Periods were 21 d with 14 d of adaptation and a 7-d sampling period. Cattle were housed in individual tie stalls with individual feed bunks and water troughs. Body weights were measured on the last day of the sampling period after an overnight fast.

Treatments were applied as a 2 x 2 factorial using modified distillers grains with solubles (DGS) and crude glycerin (GLY) replacing steam flaked corn (SFC) in the basal diet. Treatments were a SFC-based diet (DGS-N GLY-N) with 40% replacement of SFC by DGS (DGS-Y GLY-N), or 10% replacement of SFC with GLY (DGS-N GLY-Y), or 40% replacement of SFC by DGS with an additional 10% replacement with GLY (DGS-Y GLY-Y; Table 3.1). All diets were formulated for similar NEg content and provided 300 mg/hd d^{-1} monensin. Diets were fed for zero refusals, with orts recorded and

sampled daily. Diets were mixed individually on a daily basis using a concrete mixer. Individual diet ingredient samples were collected daily during the sampling period and weekly throughout the adaptation period.

Marker infusion

The triple marker method, developed by France and Siddons (1986), was utilized to determine digesta flow rate. Markers selected for this study were $YbCl_3$ (modified from Siddons et al., 1985) and CoEDTA (Udén et al., 1980) as external markers, and iNDF as an internal marker (Ahvenjärvi et al., 2003). Use of three markers allows digesta to be divided into small particle phase, large particle phase, and fluid phase, marked by Yb, iNDF, and Co respectively. Cobalt-EDTA and $YbCl_3$ were dissolved in distilled water to create a marker solution for continuous infusion into the rumen.

On the first day of the sampling period, steers were fitted with an infusion cannula plug which allowed for continuous infusion of the flow rate marker solution using a peristaltic pump (Masterflex). Cobalt and Yb were infused continuously at a rate of 0.44 g/d, 0.49 g/d, and 0.10 g/d respectively. Prior to the start of continuous infusion, a spot sample of ruminal contents was collected to establish natural abundance of Yb and Co present. This sample was frozen at -20º C until lyophilization. Immediately prior to the beginning of infusion, a 2 L priming dose of the marker solution was delivered through the cannula opening and thoroughly mixed by hand into ruminal contents. The marker solution was continuously infused into the rumen of the steer for the entirety of the 7 d sampling period, with the first 3 d representing a plateau period for the concentration of markers within the rumen.

Sample collection

On d 4, d 5, and d 6 of the sampling period, samples were collected in 8 h increments. Eight h sampling periods were offset so each day represented a different 8 h time point relative to feeding to create a 24 h composite. Within each 8 h period, samples of ruminal and omasal fluid were collected every 2 h and composited to represent a 24 h period. Spot fecal samples were collected at every natural defecation during each 8 h period and all samples were composited from 3, 8 h periods. Omasal samples were collected utilizing the procedure developed by Huhtanen et al. (1997) and modified by Ahvenjärvi et al. (2000). A reinforced 1.9-cm diameter hose was manually placed in the omasum of each steer at time of sample collection. The hose was connected to a dual flow vacuum pump and air compressor to ensure that placement of the hose remained within the omasum and clogs in the line could be easily unblocked with the low-pressure air compressor. Bi-hourly ruminal and omasal samples were divided into 3 subsamples at the time of sample collection: 24 hour composite (200 mL), 24 hour composite acidified to pH 2.0 with sulfuric acid (75 mL), and bacterial isolation sample (50 mL). Samples were kept on ice during sample collection and stored at -20^{o} C immediately after samples were collected until further processing with the exception of bacterial samples which were processed immediately following sample collections.

On d 7 of the sample collection period, rumen evacuations were conducted. Access to feed and water was restricted and ruminal contents were emptied manually via ruminal fistula into a 90 L tub. Once a majority of solid particles had been removed from the rumen, a vacuum pump was used to remove the remaining contents to ensure consistent emptying. Tubs containing ruminal contents were weighed, mixed, and

subsampled in triplicate. Samples of ruminal contents were frozen at -20º C until further processing and remaining contents were immediately placed back into the rumen. Caution was exercised to complete the entire rumen evacuation process in less than 30 min to minimize any detrimental impact on the ruminal microbe population or animal hydration. Access to feed and water was restored immediately following rumen evacuation.

Sample processing and chemical analysis

Composites, acidified composites, and rumen evacuation samples were all frozen immediately post-collection at -20º C, while bacterial samples were processed immediately.

Bacterial samples were first strained through 2 layers of cheese cloth. The fluid portion was used to isolate the fluid associated bacteria (FAB) fraction (Firkins et al., 1984), while the solid fraction was used to isolate solid associated bacteria (SAB; Whitehouse et al., 1994). The fluid portion was centrifuged at 1,000 x *g* for 10 min to remove feed and protozoal contamination. Supernatant was saved for further centrifugation at 20,000 x *g* for 20 min. The supernatant was discarded, while the pellet was saved to represent the FAB fraction and frozen at -20º C until lyophilization. The solid fraction separated from cheese cloth was incubated in 0.8% methylcellulose-saline solution for 1 hour in a 39º C water bath. After the 1-hour incubation, the solution containing the solid fraction was refrigerated at 4º C for 24 hours. After 24 hours at 4º C, the sample was mixed for 1 min using an omni-mixer. Solids and methylcellulose-saline solution were then strained through 2 layers of cheese cloth with the solid fraction rinsed with an additional 100 mL of saline. The separated fluid then underwent centrifugation

at 1,000 x *g* followed by 20,000 x *g* as previously described for FAB isolation. The isolated pellet represented the SAB fraction and was stored at -20º C until lyophillization. Bacterial isolations were only conducted for omasal contents.

Omasal and ruminal composite samples were thawed at room temperature before further separation. The total sample was weighed and homogenized using an overhead mixer. Prior to separation of digesta phases, a subsample of the homogenized composite sample was collected and saved as a reference sample of the digesta composite. To begin phase separations, samples were strained through 4 layers of cheese cloth. Solids were weighed and placed in a separate storage container to be frozen at -20º C until lyophilization. This sample represented the large particle fraction of digesta (LP). The fluid fraction remaining after the LP was removed was then centrifuged at 10,000 x *g* for 15 min. The supernatant was poured off into a separate container, weighed, and stored at -20º C until lyophilization. The supernatant fraction represented the fluid phase of digesta (FP). The pellet formed after centrifugation represented the small particle phase of digesta (SP) and was frozen at -20º C until lyophilization. The weight of the SP phase was determined via subtraction of the weights of FP and LP phases from total original sample weight.

Nutrient analysis and digestibility

Bacterial isolates (FAB and SAB), a subsample of the fecal composites, ruminal digesta phases (FP, SP, LP), and omasal digesta phases (FP, SP, LP) were frozen on dry ice prior to lyophilization to ensure samples were solidified and to prevent boiling of the sample within the lyophilizer. Subsamples of feed samples from the last 4 days of the sampling period were also frozen on dry ice prior to lyophilization.

Feed samples and fecal samples (minus subsamples for lyophilization) were dried in a conventional drying oven at 55º C. Once dry, samples were ground through a 2 mm screen using a Thomas Model 4 Wiley Mill (Thomas Scientific, Swedesboro, NJ). Samples were then analyzed for nutrient composition (DM, OM, CP, and NDF/ADF). Feed ingredients were analyzed individually and mathematically re-combined for diet composition. Dry matter was determined using fresh and dry weights from the initial 55º C drying (DM1) and then later corrected using an additional 100º C oven drying of 1 gram subsample (DM2). To measure ash, 1 g of sample was weighed into a crucible and placed in an ashing oven for 12 h at 450º C. Organic matter (OM) was determined using 1 minus ash content of sample. Crude protein (CP) was determined via a steam distillation Kjeldahl technique (Kjeltec 2300). A 0.5 g sample was weighed into a Kjeldahl tube, along with 1 $CuSO_4$ kjeltab and 10 mL H_2SO_4. The tube contents were digested at 410º C for 1 hour, after which they were steam distilled using NaOH as an alkali addition, Boric acid as an indicator solution, and 0.1 M HCl as titrant (all titrations were conducted automatically by the Kjeltec 2300). Using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), neutral detergent fiber (NDF) was determined via 60 min extraction in 100º C neutral detergent solution with additions of sodium sulfite and heat stable alpha-amylase. Samples high in fat have shown great variation in NDF results due to bound fat within the fiber matrix. To reduce variability between replicates, samples with greater than 5% fat underwent a biphasic fat pre-extraction procedure prior to NDF analysis using diethyl ether and petroleum ether (modified from Bremer et al., 2010). After NDF analysis, samples were gently shaken in 400 mL of acetone, allowed to air dry for 2 h and then placed in 100º C oven overnight to

obtain a hot weight. This final weight along with a hot bag weight and a sample weight were used to calculate NDF percent of the sample. After a final hot weight was collected, the sample then underwent ADF analysis using a 60 min 100º C extraction procedure in an ANKOM 200 fiber analyzer, with ADF solution. After ADF analysis, samples were placed in 100º C oven overnight to obtain a hot weight. This final ADF weight along with the final NDF hot weight were used in the calculation of ADF percent of the sample.

Subsamples of feed as well as omasal phases were all lyophilized using a Virtis shelf freeze drier. Approximately 500 g of sample were spread less than 2 cm thick in a 22.9 x 33.0 x 5.1 cm aluminum pan and covered with a standard hair net. The hair net prevented transfer of small particles or volatile compounds into the oil of the lyophilizer. Once in aluminum pans, samples were placed on dry ice overnight to ensure the sample was completely frozen which is critical for ruminal and omasal fluid samples to prevent boiling of any liquid within the sample during the sublimation process. The lyophilized subsample of feed was analyzed for fatty acid profile. Analysis was completed using Jenkins 1-step direct methylation procedure, with internal standards of Tridecanoic acid (C13:0) and Heptadecenoic acid (C17:1). A 0.5 g sample was weighed into a screw-cap borosilicate tube. After the sample was placed in the tube, 0.5 mL of 2 mg/mL internal standard:toluene and 3 mL 5% Methanolic HCl were added directly to the tube. The tube was vortexed and incubated in a 70º C shaking water bath for 2 hours. Following incubation, 7.5 mL 6% K_2CO_3 and 1 mL Hexane were added. The solution was centrifuged at 286 x *g* for 8 min at 4º C. The organic layer was transferred to a separate tube and one scoop (~ 0.4 g) of charcoal was added. The solution was vortexed and allowed to sit for 1 h, after which the sample was re-centrifuged at 418 x *g* for 5 min.

The top layer of solution was pipetted into a gas chromatography (GC) vial and refrigerated until analyzed for fatty acid composition. Fatty acid methyl esters were measured using GC using Agilent 7890B gas chromatograph. Samples were run through a capillary column (100 m x 0.25 mm i.d. with 0.2 um film thickness) with fame-ionization detector with hydrogen as carrier gas. Pure methyl ester standards (GLC 60; Nu Chek Prep Inc., Elysian, MN) we used to identify methyl ester peaks.

Separated phases of omasal digesta were also analyzed for DM (via lypohilization pre- and post-weights), OM, NDF, ADF, CP, and fatty acids using the same procedures described for feed analysis with the exception of fatty acids analysis of omasal digesta. Feed was analyzed for fatty acids composition using Jenkins-1 step direct methylation procedure, while omasal digesta phases were analyzed via Jenkins-2 step methylation procedure. The primary difference between these two procedures is that the Jenkins 2-step adds 2 ml 0.5 M Sodium Methoxide to the sample and incubates it at 50º C for 10 min prior to addition of 5% Methanolic HCL. The remainder of preparation steps proceed in accordance with those described for feed analysis.

Lyophilized digesta phases were also measured for Yb, Co, and iNDF as markers for passage rate determinations. Samples were prepared for Yb and Co analysis following the procedure of Ellis et al. (1982). Omasal digesta phases (LP, SP, and FP), and fecal samples were ashed and digested in an acid mixture of 3 M HCL and 3 M HNO_3 for 12 hours. Samples were filtered through a Whatman #1 filter and diluted using deionized water and a 6% KCl solution. Natural abundance of markers (particularly Co) was determined by measuring Yb and Co in lyophilized rumen spot samples collected prior to the infusion of marker solution. Concentrations of Yb and Co were determined

using Inductively Coupled Plasma (ICP) spectrometry at UF/IFAS Analytical Services Laboratory (Gainesville, FL). Both SP and LP omasal digesta phases, in addition to fecal samples and rumen evacuation samples were analyzed for iNDF (Van Soest et al., 1991) with iNDF representing the large particle passage rate of digesta. The fluid phase was not analyzed for iNDF as it was assumed that no indigestible fiber was present in this phase. A 0.5 g sample was weighed into a 125 mL Erlenmeyer flask, in duplicate. Samples were inoculated with 40 mL of prewarmed in vitro "day of inoculation" solution (mixture of in vitro rumen buffer, in vitro macromineral solution, and in vitro micromineral solution plus Trypticase and a reducing solution and indicator), and 10 mL of ruminal fluid previously strained through 4 layers of cheese cloth. Samples were placed in a 39º C water bath continuously gassed with CO_2 with pressure monitored using a barometer to maintain approximately 5 cm water displacement. All flasks were incubated for 240 hours and then stored at 4º C until analyzed. Contents of each fermentation bottle, including blanks, were analyzed for NDF content using the Van Soest reflux apparatus method with same solution as NDF method described earlier (Van Soest et al., 1991). Neutral detergent fiber remaining after 240 h incubation was considered indigestible (iNDF). This procedure was repeated until there were 4 observations per sample with 2 different fluid inoculation time points. In addition to the analysis of the three markers for digesta passage rate (Yb, Co, iNDF), microbial markers were also analyzed to determine microbial contribution to nutrient flow. Purines were used as internal microbial marker and measured in both the microbial isolates as well as omasal digesta phases using the procedure developed by Zinn and Owens (1986). Pure Torula yeast RNA was utilized as a standard to generate the standard curve using a

known concentrations of pure bacterial purines. All samples and standards were digested in 70% perchloric acid at 90º C for 1 hour after which 0.0285 M Ammonium Phosphate buffer was added to the solution to help break up the charred mass formed from perchloric acid digestion of the sample. The resulting solution was vortexed and incubated at 90º C for an additional 15 min. Samples were immediately filtered through a Whatman 54 filter. One half mL of filtered solution was combined with 0.5 mL silver nitrate in a screw-cap centrifuge tube, along with 9 mL of 0.2 M ammonium phosphate buffer. Samples and solution were then stored in a dark refrigerator overnight. After overnight incubation, samples were centrifuged at 10,000 x *g* for 10 min, supernatant was discarded and the pellet washed with 10 mL deinonized water that was adjusted to pH 2 with sulfuric acid. Samples were re-centrifuged and supernatant was discarded once again. Ten mL of 0.5 M HCl were then added to the pellet and vortexed to suspend the pellet in solution. Samples were then incubated for 30 min at 90º C, and re-centrifuged. Supernatant was pipetted into a UV transparent 96 well plate and read at 260 nm using a Biotek Synergy plate reader. Absorption values were converted to mg/mL using the standard curve created with Torula yeast.

Samples of rumen and omasal fluid that were acidified and left in the collected form were thawed and analyzed for ammonia-N and VFA. In preparation for ammonia analysis, samples were first centrifuged at 5,000 x *g* for 15 min to remove feed particles from solution. Then, 15 mL of sample were pipetted into a Kjeldahl tube containing 2g MgO and 15 mL of distilled water. This solution was then analyzed via steam distillation in a Kjeltec 2300 for N content, representing ammonia concentration of the fluid sample. Acidified ruminal and omasal fluid were also analyzed for selected VFA: acetate,

propionate, butyrate, valerate, isobutyrate, isovalerate and 2-methylbutyrate. Five-point calibration curves were built for each of these VFA with known amounts of standard solutions. Calibration curves allow a correlation to be constructed between the area under the curve value provided from gas chromatograph and the concentration of VFA present. Samples were prepared for GC analysis following a modified procedure by Erwin et al. (1961) for ruminal fluid VFA analysis. Samples were prepared using a similar centrifugation process to that used for ammonia-N analysis to remove feed particles. A 2 mL sample of supernatant was added to a solution of 25% meta-phosphoric acid/2-Ethyl-Butyrate (2-EB) which then underwent a series of centrifugation and freeze and thaw steps. During these steps, meta-phosphoric acid aids in protein precipitation while 2-EB serves as an internal standard for GC analysis. Supernatant was filtered through a 0.45 μm polyethersulfone micropore-filter and added to distilled water. One N NaOH was utilized to adjust pH of the solution to between 6 and 7 to prevent any damage to GC column packing. Prior to transferring to a GC vial for analysis, 0.03% oxalic acid was added to the solution to prevent "ghost effects" and maintain column performance by degrading to formic acid during injection (Fussell and McCalley, 1987). Samples were then transferred to a GC vial and frozen at -20º C until analysis was conducted. Prepared sampled were thawed for 1 hour prior to analysis in Hewlett-Packard HP6890 Gas Chromatograph. Samples were run through a 2 m x 0.64 cm x 2 mm carbopack glass column (SUPELCO) with 40 min run time: 27 min initial run time at 175º C, 9 min ramp time to 225º C and 4 min post-run.

Calculations

The calculations for reconstitution of true omasal digesta, devoid of sampling error, were completed using equations outlined by France and Siddons (1986).

Statistical Analyses

Data were analyzed using the mixed procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Intake, fermentation parameters and flow data were analyzed as a 2 x 2 factorial design with the main effects of the Latin square design included as fixed effects and steer and period as random factors. The statistical model was as followed:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \alpha*\beta_{i(j)} + S_k + P_l + \varepsilon_{ijkl}$$

μ = population mean
α = i^{th} effect of dietary distillers grains inclusion
β = j^{th} effect of dietary glycerin inclusion
$\alpha*\beta$ = interaction between i^{th} effect of distillers grains and j^{th} effect of glycerin
S = Random k^{th} effect of steer
P = Random l^{th} effect of period in time
ε = residual error

Hourly pH measurements were analyzed within a similar model with hour as a repeated measure, and subject of steer within period with variance modeled using Toeplitz covariance structure.

RESULTS AND DISCUSSION

Intake, rumen volume, and flow rate

The interaction of DGS and GLY did not impact dry matter intact. Dry matter intake was increased ($P = 0.04$) by 0.4 kg for the main effect of DGS compared with no difference ($P = 0.64$) in DMI observed for the main effect of glycerin (Table 3.5). Nuttelman et al. (2011) found differences in intake between types of DGS with dried or modified DGS having greater intakes compared with wet DGS. Dietary inclusion rates of

20, 30, and 40% wet DGS all had increased intake compared with a negative control (Nuttelman et al., 2011). However, Veracini et al. (2013) found a 0.93 kg decrease in DMI over a 244-d feeding period when modified DGS were fed at 40% dietary inclusion. Unlike modified DGS utilized in the current trial, Veracini et al. (2013) utilized full-fat DGS, which may have influenced the decrease in DMI through chemostatic energy intake mechanisms or through effects of unsaturated fat on rumen microbes. Variation in DMI observed within the literature is assumed to be from inaccuracies when reporting nutrient composition of the byproduct utilized and byproduct manufacturing differences.

Similar to DGS, previous research on the effects of glycerin has also had variable DMI results. Hales et al. (2013) published a series of experiments with pure glycerin in receiving cattle in which there were both neutral and negative intake responses. Observations of steer eating behavior within the current study noted a hesitation to consume glycerin-containing diets. However this is purely observation as no incremental feed refusals were recorded and there were no differences in DMI ($P = 0.64$). Carvalho et al. (2011) also was able to determine a change in feeding behavior with dietary inclusion of glycerol in dairy cattle. Glycerol inclusion at 11.5% (pre-partum) and 10.8% (post-partum) of the diet resulted in decreased sorting behavior and shifted a larger portion of intake to later in the day rather than immediately after feeding. Carvalho et al. (2011) observed a 21% decrease in DMI for the 4 hours immediately post-feeding with no different in total DMI over course of the entire day. Within the current study, since no interactive effects were seen for DGS and GLY for ruminal contents or digesta flow variables, the main effect of glycerin can be used to describe this potential shift in feeding pattern. The feeding shift is reflected within the data as a decrease (8.4 vs. 7.3 kg; $P =$

0.05) in DM ruminal contents for the main effect of glycerin (Table 3.5). This is thought to support Carvalho et al. (2011), where glycerin-fed cattle eat small meals throughout the day rather than consuming larger portions immediately following feeding. The main effect of glycerin decreased DM ruminal contents as well as decreased (P = 0.04) digesta flow (kg/d), supporting the hypothesized shift in intake behavior when glycerin is fed (Table 3.5). A change in eating behavior could also explain the decrease (P = 0.04) in total digesta flow (kg/d) for the main effect of glycerin (Table 3.5).

There was no change in true digesta flow (kg/d) for main effect of DGS. A decrease (P = 0.05) in flow rate with respect to volume was observed for diets with DGS inclusion compared with those without (Table 3.5). Greater (P = 0.04) DMI paired with decreased flow rate (%/h) for cattle fed diets with DGS inclusion compared to those without would indicate an increase in ruminal feed retention. No difference (P = 0.11) in retention time was observed, but a high standard error of the mean may have contributed to the lack of increase in retention time for cattle fed DGS (Table 3.5).

Fermentation parameters and rumen environment

No effects were observed for the interaction of DGS and GLY for VFA data, except isobutyrate molar proportion (P = 0.02; Figure 3.1). Despite the main effect of DGS reducing flow rate, DGS did not affect total mM VFA concentration (P = 0.46; Table 3.1) or ruminal pH (Figure 3.2 and Table 3.7). These observations suggest diets with DGS are slower-fermenting. The slower %/h rumen removal (P = 0.05) and trend for longer retention time (P = 0.10) observed for the main effect of DGS should provide more time for fermentation of feed. However, the main effect of DGS showed no

increase in total VFA concentration, nor in time spent below 5.7 pH. Based on the lack of effects, reduced fermentation rate can be assumed for the main effect of DGS.

A decrease ($P = 0.01$) in acetate as a percentage of total VFA was observed for the main effect of DGS. This decrease paired with no change in total VFA concentration suggests a shift in the VFA pathways when DGS replace SFC in the diet. Firkins et al. (1984) and Leupp et al. (2009) observed a linear decrease in acetate production when dietary DGS concentrations increased. A decrease in acetate is not consistent across all DGS fermentation studies, because Walter et al. (2012) observed no change acetate concentration. This variation may be due to differences in type and nutrient content of DGS.

No differences in flow rate %/h ($P = 0.33$), ruminal pH (Figure 3.3 and Table 3.7 $P = 0.53$), or total mM VFA concentration ($P = 0.49$) were observed for main effect of GLY. Despite concerns that glycerin may decrease microbial digestion within the rumen, it does not appear that total fermentation is affected. The main effect of GLY showed a trend for decreased ($P = 0.09$) acetate as percent of total VFA produced (Table 3.6). Johns (1953) determined that glycerin in the rumen is fermented directly to propionate. Although the current study was unable to detect an increase in propionate, the decrease in acetate with no change in total VFA concentration suggests a shift in the VFA pathways as a result of including glycerin.

Nitrogen Flow

Diets were not isonitrogenous, therefore differences in ruminal N metabolism are challenging to interpret. Intake appears to be the predominant driver of differences in N flow, as observed differences in total N flow mimic those seen in N intake. The

interaction of DGS and GLY did not have a significant effect on any of the N flow variables, so only main effects will be discussed. As expected because of dietary N content, the main effect of DGS increased ($P = 0.02$) total N flow, while the main effect of GLY tended to decrease ($P = 0.10$) total N flow. However, the interest in N flow lies not in the total flow differences but in the partitioning of N by source (Table 3.8). Inclusion of GLY in the diet had no effect on ammonia-N flow ($P = 0.26$), bacterial-N ($P = 0.36$), or non-ammonia non-microbial-N (NANMN; $P = 0.55$). Increases in ammonia concentration might be explained by inefficient microbial N use, suggesting that slower fermentation rates may not accurately supply C chains at an adequate time for deamination of dietary CP. A second explanation is simply that dietary CP concentrations for DGS (Table 3.2) far exceed ruminal N requirements, therefore there was increased available N for ammonia production as well as increased RUP leading to increased NANMN.

Fatty acid profile transformation

Intake of unsaturated fat ranged from 70.4 – 80% of the total fat within the diets fed in this experiment (Table 3.2). Interaction of DGS and GLY inclusion in the diet affected intake of stearic ($P < 0.01$; Figure 3.4), linoleic ($P < 0.01$; Figure 3.5), and linolenic acid ($P < 0.01$; Figure 3.6). Linoleic and Linolenic acid intake were greatest for diet including DGS and no GLY (Figures 3.5 and 3.6). When only GLY replaced a portion of SFC the lowest linoleic and linolenic acid intakes were observed. Because GLY is a poor source of PUFA compared to other dietary ingredients, it dilutes the linoleic and linolenic acid content. Stearic acid intake was greatest for diets with a portion of SFC replaced by both DGS and GLY (Figure 3.4). Although DGS contributes

more stearic acid to total dietary stearic content (Table 3.4), GLY also appears to increase stearic acid intake as well, with the combination of the two having additive effect on total stearic acid intake.

Through biohydrogenation a significant portion of these dietary fatty acids are transformed into PUFA isomers, monounsaturated fatty acids, or saturated fatty acids. Increased numeric values for stearic acid in true digesta compared to values of intake (Table 3.10) are expected as a result of the biohydrogenation process. The interaction of DGS and GLY affected ($P < 0.01$) the g of stearic acid flow with greatest g stearic acid flow coming from cattle fed DGS and GLY. The diet with DGS and GLY was also the greatest for stearic acid intake so this is most likely a diet effect. Unlike stearic acid intake relationship (figure 3.4), when no DGS was included GLY inclusion did not impact ($P = 0.30$) g of stearic acid in true digesta flow. Because the diet without DGS but with GLY had more stearic acid intake than the diet without DGS and GLY but no difference was seen in total digesta flow a change in fermentation with GLY is concluded. This interaction between GLY and DGS did not affect g of flow of Linoleic ($P = 0.87$) or linolenic acid ($P = 0.88$). For the main effect of DGS, 93.5% of linolenic acid and 84.5% of linoleic acid were transformed to isomers or saturated forms of the original dietary fatty acid. This is a 6.9% unit increase ($P < 0.01$) for linoleic acid and 6.2% unit increase ($P < 0.01$) for linolenic acid transformation compared with diets without DGS. Conjugated linoleic acid (CLA; C18:2 cis-9, trans-11) also increased ($P < 0.01$) with the main effect of DGS in the diet. This increase in CLA appears to be related to the increase in dietary intake of linoleic acid, which serves as the precursor to CLA formation in the rumen.

The interaction between DGS and GLY did not impact the transformation (g FA flow/ g FA intake) of stearic acid ($P = 0.17$), linoleic acid ($P = 0.18$), or linolenic acid ($P = 0.66$; Table 3.10). The main effect of GLY inclusion had no impact on g of linolenic ($P = 0.16$) or linoleic ($P = 0.32$) acid transformed (Table 3.10). A trend was identified for an increase ($P = 0.07$) in CLA for the main effect of GLY. Glycerin inclusion in the diet had no impact on the percent of saturated fat ($P = 0.44$) or unsaturated fat ($P = 0.43$) in omasal flow. Based on previous published literature, glycerin has been found to interfere with *butyvibrio fibrosolvens*, which has a role in the transformation of CLA to vaccenic acid (*trans*-C18:1; Harfoot and Hazelwood, 1997). The current study appears to agree with those conclusions as an observed increase in CLA along with no differences in percentage of stearic acid or linoleic transformed indicate that the biohydrogenation pathway was inhibited at the point of CLA transformation.

CONCLUSIONS

Distillers grains or crude glycerin could replace a portion of SFC in diets without causing large changes to basic fermentation processes such as ruminal pH patterns or total mM VFA concentration. Observed feeding behavior could explain changes in flow rate and DM retention within the rumen for byproduct-fed cattle, but further research is needed to conclude this. The fatty acid composition of digesta when cattle are fed either corn or soy byproducts is different from a SFC diet without byproducts. This difference in fatty acid profile may imply differences in meat shelf life stability when these byproducts are fed. Total unsaturated fatty acids in digesta were not different for the main effect of GLY, which may indicate GLY is an effective alternative to corn when provided at the dietary concentrations evaluated in this study. The main effect of DGS

decreased unsaturated fatty acids which may be beneficial for shelf life stability of meat. In order to further evaluate the effect of crude glycerin on shelf life of meat products, more research is needed to examine if microbial-created isomers of linoleic acid, such as CLA, cause shelf life stability issues to the same extent as untransformed linoleic acid.

Table 3.1 Actual fed ingredient composition of treatment diets with and without modified distillers grains and crude glycerin

Inclusion, % of DM	**DGS-N GLY-N[1]**	**DGS-N GLY-Y[2]**	**DGS-Y GLY-N[3]**	**DGS-Y GLY-Y[4]**
Grass hay	11	11	10	9
Steam flaked corn	72	63	42	36
Dry rolled corn + soybean meal mix	12	11	-	-
Glycerin	-	11	-	10
Modified Distillers Grains	-	-	42	40
Liquid supplement[a]	-	-	5	5
Liquid supplement[b]	4	4	-	-

[1]DGS-N GLY-N = No dietary distillers grains inclusion, no dietary glycerin inclusion
[2]DGS-N GLY-Y = No dietary distillers grains inclusion, Yes dietary glycerin inclusion
[3]DGS-Y GLY-N = Yes dietary distillers grains inclusion, no dietary glycerin inclusion
[4]DGS-Y GLY-Y = Yes dietary distillers grains inclusion, Yes dietary glycerin inclusion
[ab]Liquid supplement - provides increased RDP in form of Urea and formulated to supply 300 mg/hd/d monensin

Liquid supplement[a] = low protein

Liquid supplement[b] = high protein

Table 3.2 Nutrient composition of treatment diets with and without modified distillers grains and crude glycerin

	DGS-N GLY-N[1]	DGS-N GLY-Y[2]	DGS-Y GLY-N[3]	DGS-Y GLY-Y[4]
DM, %	85.4	85.6	66.8	68.0
CP, % DM	10.8	9.8	17.4	16.0
NDF, % DM	14.3	13.3	22.7	20.9
ADF, % DM	6.3	6.0	8.6	7.9
FA, % DM	2.6	2.3	5.6	5.2
TDN, % DM	83.0	83.0	83.7	83.7
NEm, Mcal/kg	2.12	2.12	2.14	2.14
NEg, Mcal/kg	1.40	1.40	1.42	1.42

[1]DGS-N GLY-N = No dietary distillers grains inclusion, no dietary glycerin inclusion
[2]DGS-N GLY-Y = No dietary distillers grains inclusion, Yes dietary glycerin inclusion
[3]DGS-Y GLY-N = Yes dietary distillers grains inclusion, no dietary glycerin inclusion
[4]DGS-Y GLY-Y = Yes dietary distillers grains inclusion, Yes dietary glycerin inclusion

Table 3.3 Nutrient composition of Distillers Grains (DGS) and Glycerin (GLY) fed

	DGS	Crude Glycerin
DM, %	44.4	88.0
CP, % DM	28.3	0
NDF, % DM	30.5	0
ADF, % DM	9.4	0
FA, % DM	10.2	0.05
C14:0	0.1	1.7
C15:0	0.0	18.5
C16:0	14.6	12.5
C16:1c9	0.1	0.0
C17:0	0.1	0.0
C18:0	2.0	26.8
C18:1c9	23.8	12.0
C18:1c11	0.8	0.0
C18:2c9c12	55.8	15.1
C18:3c6c9c12	0.0	0.0
C20:0	0.5	0.0
C18:3c9c12c15	1.7	1.8
C20:1c11	0.3	0.0
C24:0	0.3	0.0
C24:1n9	0.0	0.0
unsaturated	82.4	28.9
Omega 3	1.7	1.8
Omega 6	55.8	15.1

Table 3.4 Fatty acid composition (% of total FA) of treatment diets with and without modified distillers grains and crude glycerin

	DGS-N GLY-N [1]	DGS-N GLY-Y [2]	DGS-Y GLY-N [3]	DGS-Y GLY-Y [4]
Total FA, % DM	2.6	2.3	5.6	5.2
C14:0	0.2	0.4	0.1	0.3
C15:0	0.1	2.1	0.1	1.8
C16:0	15.6	15.5	15.7	15.5
C16:1c9	0.3	0.2	0.3	0.2
C17:0	0.1	0.1	0.1	0.1
C18:0	1.9	3.2	2.2	3.3
C18:1c9	23.7	22.2	22.3	21.2
C18:1c11	0.7	0.6	0.7	0.7
C18:2c9c12	50.3	45.9	51.9	48.2
C18:3c6c9c12	0.1	0.1	0.1	0.1
C20:0	0.7	0.6	0.6	0.6
C18:3c9c12c15	4.9	4.9	4.6	4.4
C20:1c11	0.3	0.3	0.3	0.3
C24:0	0.9	0.9	0.8	0.7
C24:1n9	0.3	0.3	0.2	0.2
% unsaturated	80.5	74.4	80.4	75.3
% omega-6	50.4	46.0	52.0	48.3
% omega-3	4.9	4.9	4.6	4.4

[1]DGS-N GLY-N = No dietary distillers grains inclusion, no dietary glycerin inclusion
[2]DGS-N GLY-Y = No dietary distillers grains inclusion, Yes dietary glycerin inclusion
[3]DGS-Y GLY-N = Yes dietary distillers grains inclusion, no dietary glycerin inclusion
[4]DGS-Y GLY-Y = Yes dietary distillers grains inclusion, Yes dietary glycerin inclusion

Table 3.5 Dry matter intake, flow rate, and rumen volume for cattle fed diets with and without modified distillers grains and crude glycerin

	DGS		GLY			*P*-value		
	No	Yes	No	Yes	SEM [1]	DGS	GLY	DGS*GLY
DMI, kg/d	8.7	9.3	9.0	8.9	0.5	0.04	0.64	0.28
True digesta flow, kg/d	9.7	9.0	10.2	8.4	1.3	0.34	0.04	0.40
Flow rate, % rumen volume/h	5.4	4.5	5.2	4.8	0.8	0.05	0.33	0.47
Retention time, h	20.5	23.5	21.9	22.1	3.6	0.11	0.91	0.64
Ruminal contents DM, kg	7.5	8.2	8.4	7.3	0.5	0.18	0.05	0.93
Ruminal contents DM, % SBW	1.9	2.0	2.1	1.8	0.1	0.35	0.11	0.96
Ruminal contents as-is, kg	45.8	46.8	46.4	46.2	1.3	0.59	0.92	0.48
Ruminal contents as-is, % SBW	11.4	11.5	11.4	11.5	0.7	0.86	0.79	0.48

[1]SEM = Standard error of the mean
[2]SBM = shrunk body weight

Table 3.6 Main effects of dietary inclusion of distillers grains (DGS) or glycerin (GLY) on ruminal VFA concentration (mM) and composition (%)

	DGS		GLY			*P* - value		
	No	**Yes**	**No**	**Yes**	**SEM[1]**	**DGS**	**GLY**	**DGS*GLY**
Total, mM	167.2	142.2	165.3	144.1	46.8	0.42	0.49	0.13
Total Mol in rumen volume	7,621	6,720	7,575	6,766	2,151	0.60	0.64	0.17
acetate, %	46.0	40.1	44.6	41.5	3.1	0.01	0.09	0.12
propionate, %	38.4	42.8	40.1	41.0	2.0	0.16	0.74	0.23
butyrate, %	11.0	11.1	10.8	11.3	2.6	0.98	0.80	0.66
valerate, %	2.06	2.83	1.80	3.08	0.71	0.35	0.14	0.86
isobutyrate, %	0.79	1.19	1.14	0.85	0.29	0.16	0.29	0.02
isovalerate, %	1.54	1.35	1.30	1.59	1.13	0.60	0.44	0.22
2-MB[2], %	0.25	0.66	0.29	0.62	0.26	0.03	0.07	0.32
Branched-chain VFA, mM	2.20	2.57	2.58	2.19	0.82	0.65	0.64	0.16
Acetate : propionate	1.23	0.96	1.13	1.05	0.10	0.06	0.52	0.18

Figure 3.1 Interaction of dietary inclusion of distillers grains (DGS) and Glycerin (GLY) (P = 0.02) on isobutyrate as percent of total VFA.

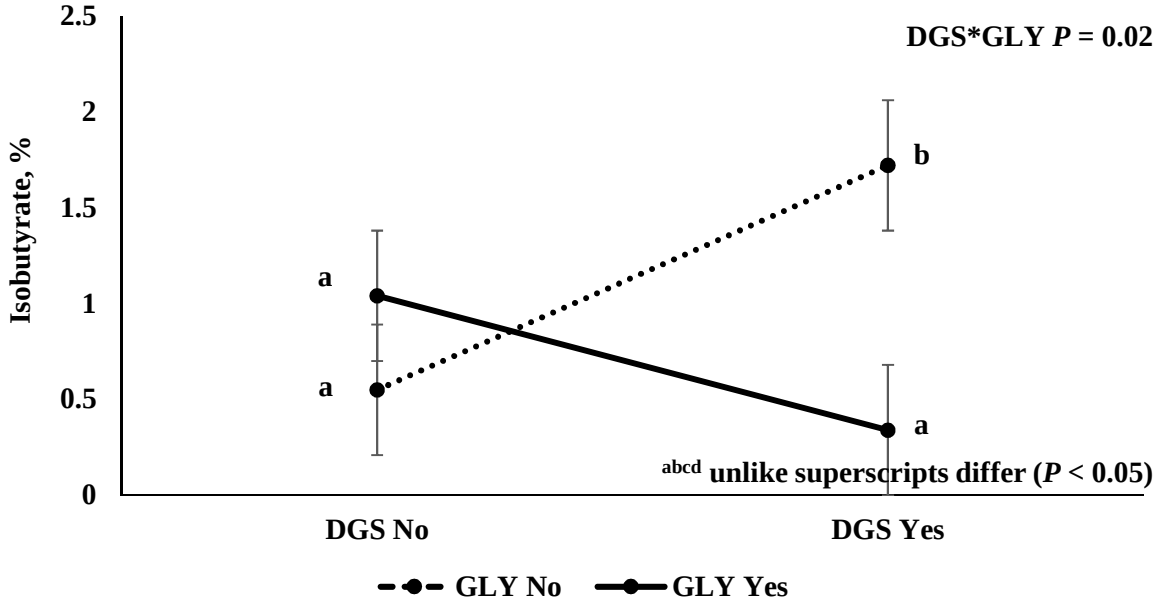


Figure 3.2 Main effect of dietary distillers grains (DGS) inclusion on ruminal pH 24 h post-feeding (DGS*hour $P < 0.01$)

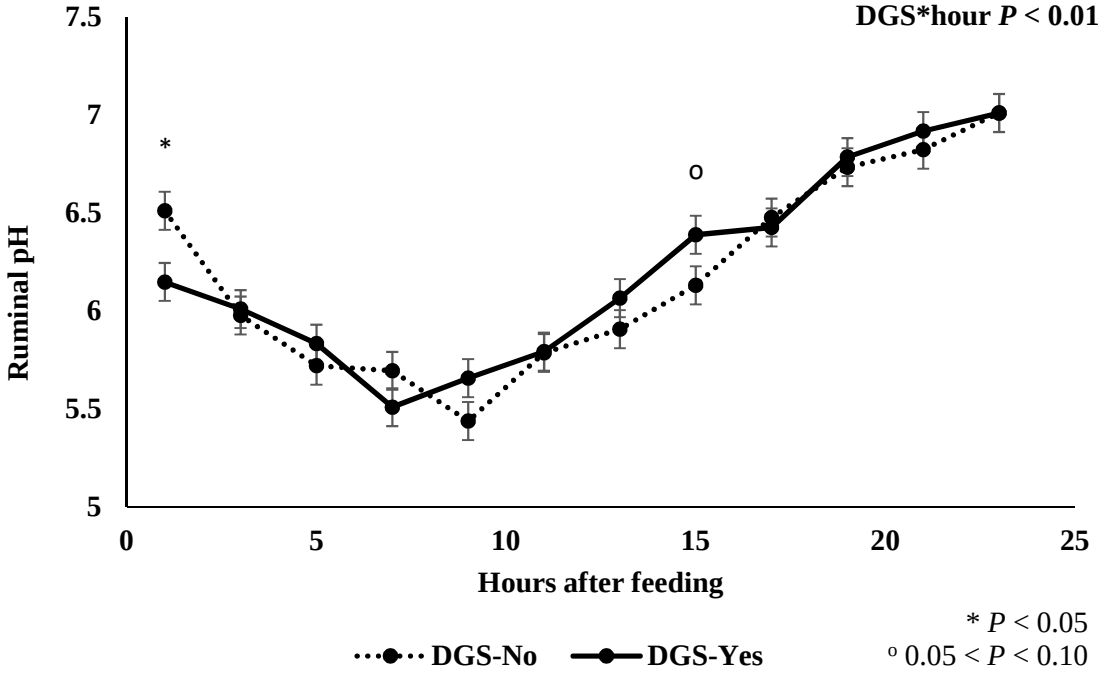


Figure 3.3 Main effect of dietary crude glycerin (GLY) inclusion on ruminal pH 24 h post-feeding (GLY*hour $P = 0.39$)

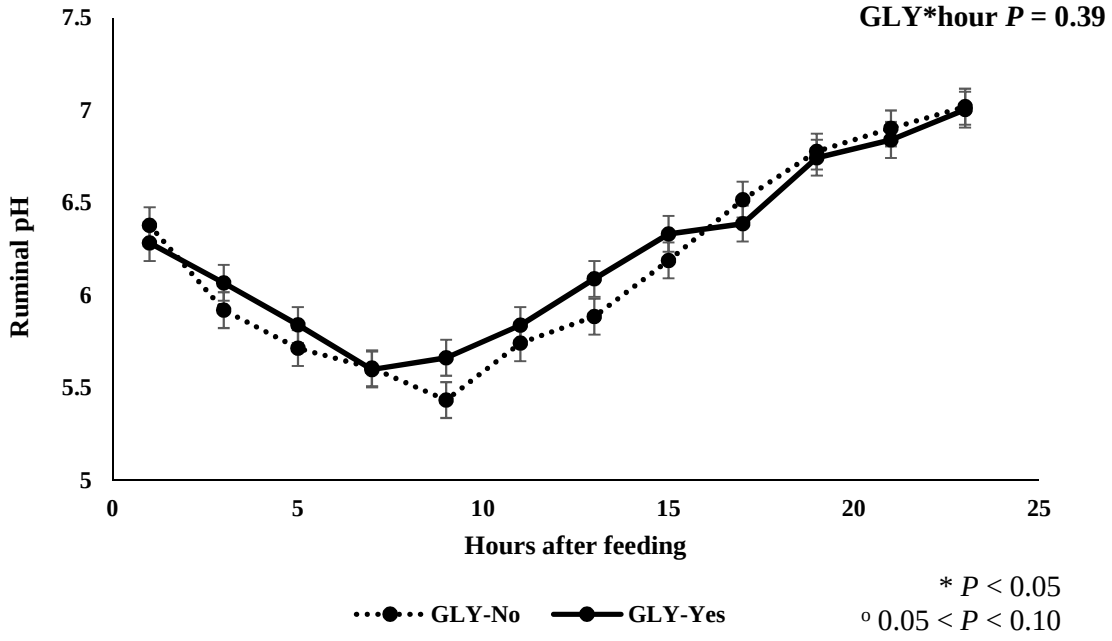


Table 3.7 Main effects of dietary inclusion of distillers grains (DGS) or crude glycerin (GLY) on ruminal pH and time below pH 5.7

	DGS		GLY			*P*-value		
	No	**Yes**	**No**	**Yes**	**SEM[1]**	**DGS**	**GLY**	**DGS*GLY**
Average ruminal pH	6.18	6.21	6.17	6.22	0.09	0.72	0.53	0.47
Time below pH 5.7, min	365	307	402	270	79	0.50	0.16	0.89

[1]SEM = Standard error of the mean

Table 3.8 Partitioning of N sources in omasal true digesta flow for cattle fed diets including distillers grains (DGS) or glycerin (GLY) to replace a portion of steam flaked corn

	DGS		GLY			*P*-value		
	No	**Yes**	**No**	**Yes**	**SEM[1]**	**DGS**	**GLY**	**DGS*GLY**
N Intake, g/d	141.7	247.0	204.6	184.1	5.32	< 0.01	< 0.01	0.06
NH_3-N[3], mg/dl	2.47	3.42	2.70	3.19	0.65	0.09	0.34	0.42
Total N Flow, g/d	265.9	372.3	350.5	287.7	43.0	0.02	0.10	0.34
NH_3-N[2] flow, g/d	0.41	0.91	0.71	0.61	0.15	< 0.01	0.26	0.66
Bacterial N flow, g/d	180.1	166.9	191.5	155.4	25.7	0.73	0.36	0.49
NANMN[3] flow, g/d	85.5	204.7	158.4	131.8	39.9	0.03	0.55	0.88

[1]SEM = standard error of the mean
[2]NH_3-N = Ammonia N
[3]NANMN = Non-Ammonia Non-microbial Nitrogen

Table 3.9 Fatty acid (FA) composition of true omasal digesta flow for cattle fed diets with distillers grains (DGS) or crude glycerin (GLY) to replace a portion of dietary steam flaked corn

	DGS		GLY			*P*-value		
	No	**Yes**	**No**	**Yes**	**SEM[1]**	**DGS**	**GLY**	**DGS*GLY**
FA concentration, % of DM	2.13	4.59	3.13	3.59	0.28	< 0.01	0.03	0.05
saturated, % of FA	43.9	59.5	51.1	52.2	3.3	< 0.01	0.44	0.31
unsaturated, % of FA	56.1	40.5	48.9	47.7	3.3	< 0.01	0.43	0.33
Composition of FA, %								
iC14:0	0.28	0.13	0.21	0.20	0.06	0.01	0.96	0.36
C14:0	1.66	1.40	1.56	1.49	0.07	< 0.01	0.21	0.04
iC15:0	0.41	0.28	0.35	0.35	0.08	0.10	0.96	0.88
aC15:0	2.57	1.89	2.33	2.13	0.55	0.19	0.67	0.62
C14:1c9	0.00	0.00	0.00	0.00	.	.	.	.
C15:0	0.88	0.73	0.71	0.90	0.07	0.07	0.04	0.08
C16:0	19.5	19.5	19.68	19.36	0.53	0.93	0.52	0.52
iC17:0	0.32	0.28	0.21	0.40	0.04	0.46	0.01	0.33
C16:1c9	0.06	0.10	0.09	0.07	0.02	0.07	0.11	0.13
aC17:0	0.79	0.45	0.66	0.58	0.06	< 0.01	0.31	0.70
C17:0	0.57	0.38	0.43	0.52	0.02	< 0.01	0.02	0.02
C18:0	15.52	33.08	23.61	24.99	3.27	< 0.01	0.26	0.07
C18:1t5	0.00	0.00	0.00	0.00	.	.	.	.
C18:1t4	0.06	0.06	0.07	0.06	0.01	0.82	0.43	0.81
C18:1t6-8	1.53	1.00	1.29	1.24	0.13	0.02	0.82	0.59
C18:1t9	2.23	1.08	1.46	1.86	0.39	0.04	0.40	0.08
C18:1t10	16.68	7.20	11.49	12.39	1.39	< 0.01	0.54	0.34
C18:1t11	3.22	3.00	2.90	3.33	0.50	0.70	0.45	0.74
C18:1c9	16.23	13.05	15.28	14.00	1.11	0.01	0.14	0.62
C18:1c11	1.7	1.48	1.68	1.51	0.08	0.05	0.11	0.45
C18:1c12	0.24	0.60	0.48	0.36	0.08	< 0.01	0.15	0.45
C18:2t11c15	0.58	0.20	0.37	0.40	0.05	< 0.01	0.63	0.71
C18:2c9c12	11.74	9.64	11.50	9.87	1.41	< 0.01	0.01	0.08
C20:0	0.63	0.64	0.64	0.63	0.02	0.51	0.30	0.70
C18:3c9t11c15	0.68	0.37	0.54	0.50	0.06	< 0.01	0.43	0.98
C20:1c11	0.3	0.24	0.28	0.26	0.02	< 0.01	0.19	0.60
CLAc9t11	0.58	2.15	1.17	1.55	0.17	< 0.01	0.16	0.64
CLAt10c12	0.24	0.36	0.27	0.33	0.07	0.20	0.49	0.51
C24:0	0.73	0.69	0.74	0.68	0.07	0.34	0.18	0.91

[1]SEM = standard error of mean

Table 3.10 Transformation of fatty acids from intake to omasal true digesta for cattle fed diets including distillers grains or glycerin in place of a portion of dietary steam flaked corn

	DGS		GLY			*P*- value		
	No	Yes	No	Yes	SEM[1]	DGS	GLY	DGS*GLY
Intake, g/d								
C18:0	5.4	13.3	7.9	10.8	0.4	< 0.01	< 0.01	< 0.01
C18:2	101.8	250.8	194.1	158.5	8.2	< 0.01	< 0.01	< 0.01
C18:3	10.3	22.4	17.5	15.2	0.8	< 0.01	< 0.01	0.01
True digesta flow, g/d								
C18:0	29.5	126.8	75.6	80.6	4.3	< 0.01	0.21	0.02
C18:2	23.5	39.4	35.5	27.4	6.2	< 0.01	0.1	0.87
C18:2c9t11	1.1	8.6	4.5	5.2	0.01	< 0.01	0.51	0.60
C18:3	1.3	1.5	1.6	1.2	0.2	0.41	0.16	0.88
g flow/g intake								
C18:0	6.00	9.60	9.10	6.6	0.9	< 0.01	< 0.01	0.17
C18:2	0.22	0.15	0.20	0.18	0.03	< 0.01	0.32	0.18
C18:2c9t11 / C18:2	0.01	0.03	0.02	0.03	0.003	< 0.01	0.07	0.34
C18:3	0.13	0.06	0.10	0.09	1	< 0.01	0.47	0.66

[1]SEM = Standard error of the mean

Figure 3.4 Interaction of dietary inclusion of distillers grains (DGS) and glycerin (GLY) ($P < 0.01$) on stearic acid C18:0 intake, g/d

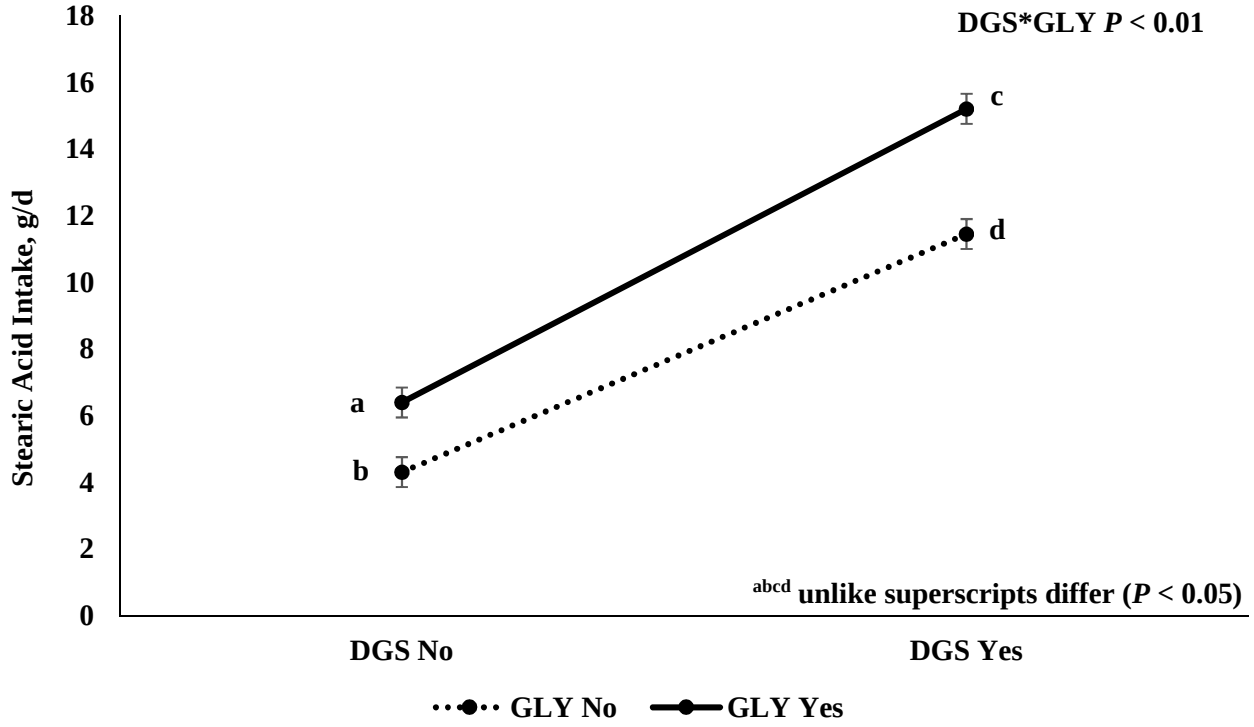


Figure 3.5 Interaction of dietary inclusion of distillers grains (DGS) and glycerin (GLY) ($P < 0.01$) on linoleic acid C18:2 intake, g/d

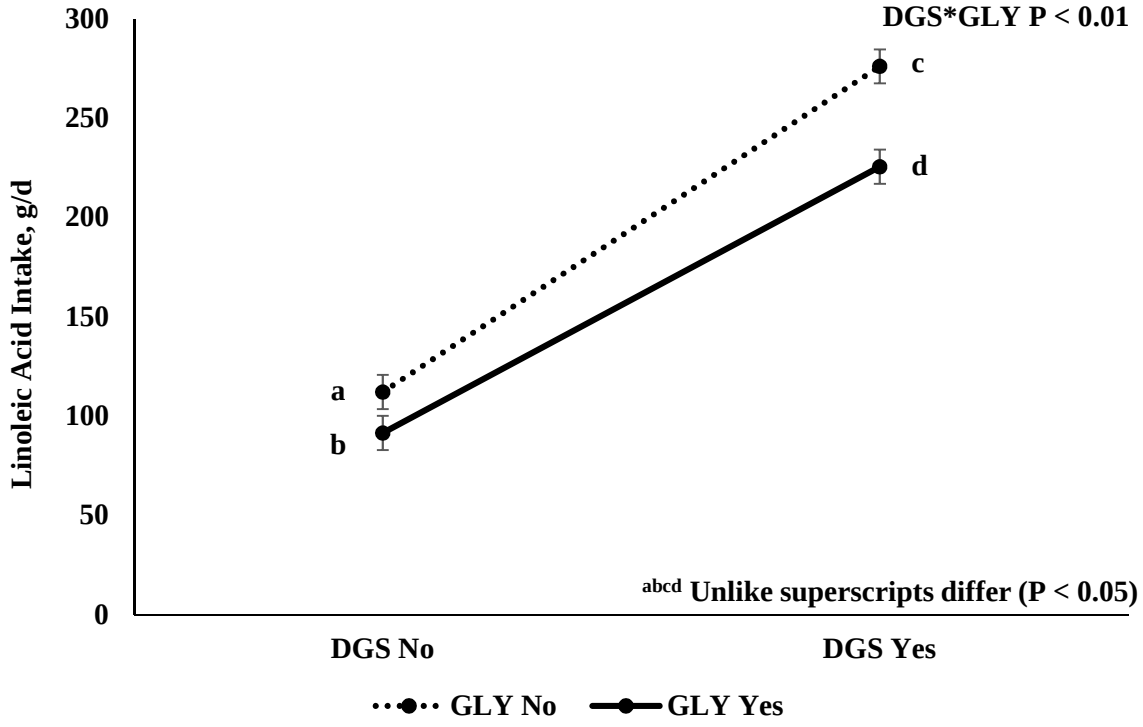


Figure 3.6 Interaction of dietary inclusion of distillers grains (DGS) and glycerin (GLY) (P = 0.01) on linolenic acid C18:3 intake, g/d

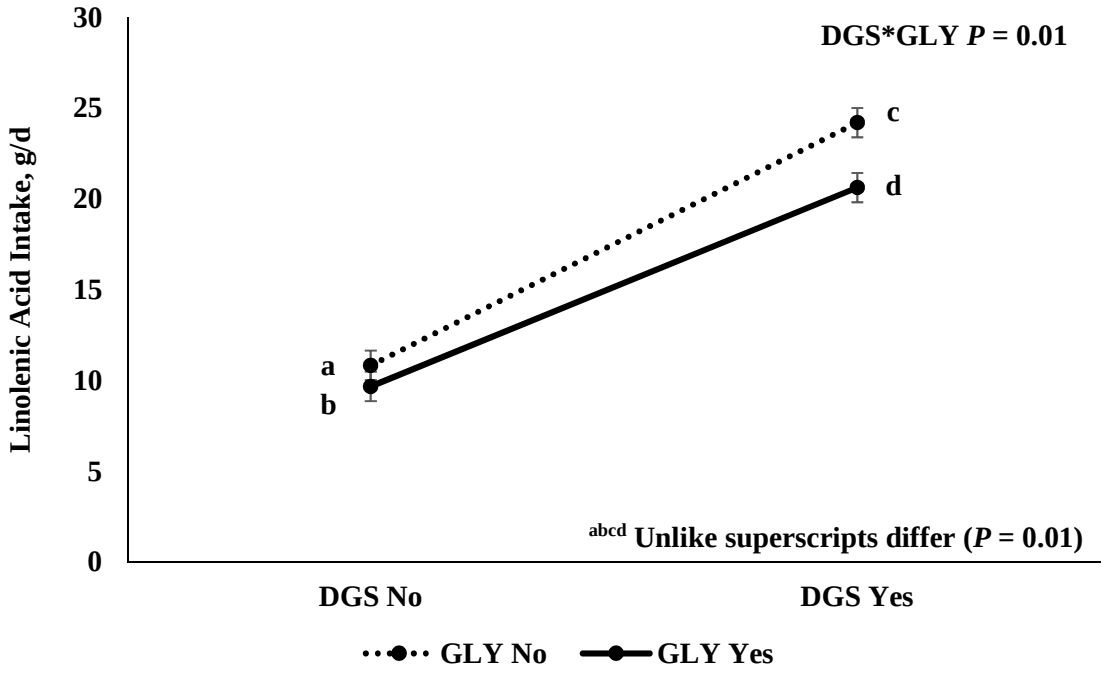
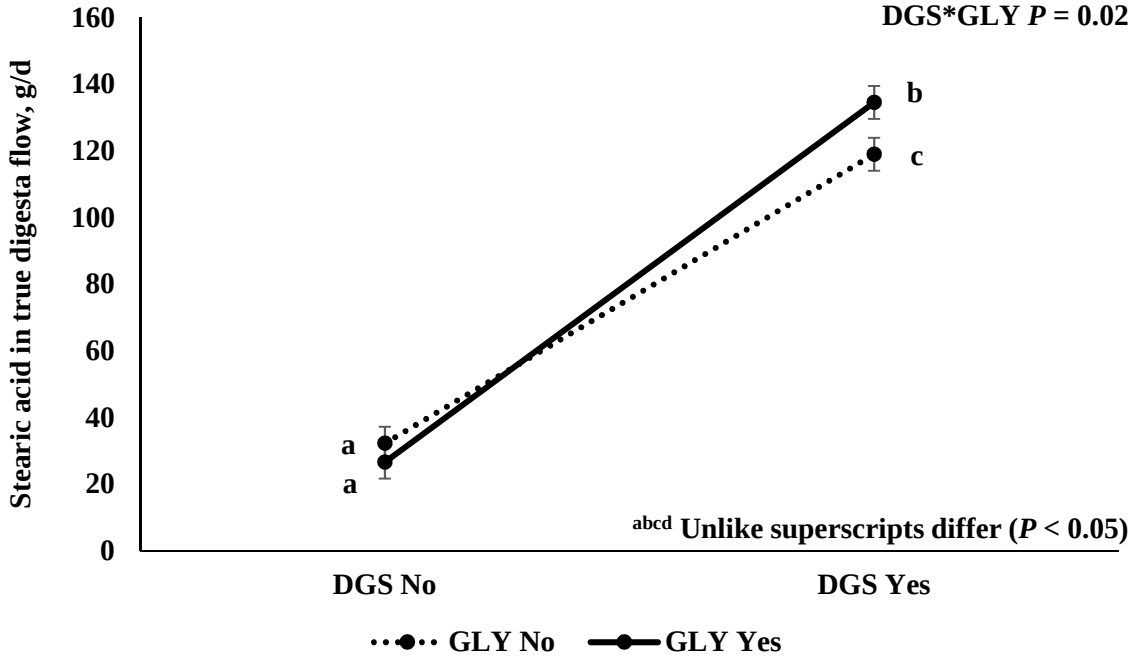


Figure 3.7 Interaction of dietary inclusion of distillers grains (DGS) and glycerin (GLY) (P = 0.02) on stearic acid C18:0 in true digesta flow, g/d



Chapter IV.

A META-ANALYSIS ON THE EFFECTS OF GROWING STRATEGY ON FEEDLOT AND CARCASS PERFORMANCE

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INTRODUCTION

Interest in incorporating a growing phase stems from the understanding that rate of gain and feed conversion are enhanced following a period of energy intake restriction. This effect, referred to as compensatory gain, is driven by a series of physiological endocrine changes in growth hormone and insulin concentrations (Hornick et al., 2000). Restricting caloric intake during a growing period causes an animal to partition nutrients towards bone and muscle development, shifting the growth curve of the animal to delay maturity (i.e. fat deposition). This "framing out" period results in an animal with greater potential to carry more carcass mass, thus lean growing cattle appearing to have undergone a period of nutrient restriction are often more desirable for placement in the feedlot.

The ability to predict precisely how differences in growing strategies impact finishing and carcass performance is imperative to maximize the benefits of relying on a growing phase post-weaning. However, specific effects of caloric intake, length of growing phase, and resulting rate of gain during growing phases on finishing

performance are not well elucidated. This leads to making specific recommendations about a growing phase impossible. Likely, differences in the duration of growing phase, severity of caloric restriction, maturity of animal at harvest, sex, genetic background, and diet composition all play a role in the observed inconsistencies between studies. Carstens et al. (1991) determined that much of the compensatory gain response was related to changes in gut fill and composition of gain. Hogg (1991) proposed that variability in finishing DMI following a growing phase is partially explained by dietary energy content fed during the growing phase. Concurrently, Sainz et al. (1995) determined that compensating growth of cattle previously subjected to restricted caloric intake cattle is due to increased capacity for DMI, and not a reduction in metabolic rate resulting from lower caloric intake

Growing phase strategies vary from feeding low energy dry lot rations to relying on grazing systems on native range pasture. Days in the growing phase interact with caloric intake to determine performance response during the growing and finishing phases. Previous meta-analyses (Galyean et al., 2011; Lancaster et al., 2014) suggested growing phase ADG, BW entering the finishing period, and DMI for days 8 to 28 of the finishing period were primary predictors of finishing performance and carcass characteristics. Yet, interactions of length of growing phase and rate of gain have not been studied using a meta-analysis approach. In this study, a meta-analysis approach was used to determine effects of growing phase days and rate of gain on performance of cattle at theoretically similar maturity.

It was hypothesized that the response by cattle to growth manipulation during a growing phase results in predictable finishing and carcass performance. The objective

was to model how feedlot and carcass performance is affected by growing phase strategies including high-energy feeding (placed directly on a finishing phase; HG), ad-libitum feeding silage (grass, corn, and sorghum) diets (DL-Forg), moderate energy (0.88 to 1.23 Mcal NE_g/kg DM) diets (DL), grazing dormant native pastures (G), or winter wheat pastures (WW).

MATERIALS AND METHODS

This study was a conducted by pooling pertinent information from previously published research studies. No animals were used in the execution of this study as all data were obtained from previously published works. Therefore, there was no requirement for an animal care and use protocol.

Data selection criteria

A dataset derived from 53 manuscripts previously published in peer-reviewed journals based on background and stocker phase strategy studies was constructed to determine how growing phase nutritional decisions, particularly days on feed and resulting ADG, affect finishing phase performance using a meta-analysis approach. English-language search of peer-reviewed journals was conducted using MNCAT Discovery, Science Direct and Google Scholar search engines with key words such as “background”, “stocker”, “growing cattle”, “growing”, “strategy” and “performance”. In addition, to ensure the search engines did not overlook certain publications, Journal of Animal Science, the Professional Animal Scientist, Canadian Journal of Animal Science, Nebraska Beef Reports, and South Dakota Beef Research Reports were searched directly.

Strategies for feeding growing cattle evaluated were dry-lot feeding either silage (DL-forg; n = 32), a moderate energy diet (DL; n = 78), stocking cattle on winter wheat (WW; n = 16), or pasture/native range (G; n = 43). Control treatments were comprised of observations where cattle were fed a high-energy finishing diet immediately post-weaning. These observations were grouped under the high-energy label (HG; n = 16). Observations derived from treatments where intake was restricted were not included. Based on reported experimental units, this data set encompasses performance data from 8,730 head of cattle from growing to finishing phases.

Selection criteria for the study required that performance data from post-weaning growing and finishing phase be presented as well as carcass performance. Only data from treatments where a single growing phase strategy were selected; sequential application of two or more growing phase strategies was not considered. Experiments were excluded if no interim weight was taken at end of the growing phase, prior to the start of the finishing phase. Within each manuscript, data were recorded to identify manuscript (n = 53), separate experiments within manuscript (n = 72), and separate treatments within each study (n = 185). Based on these three identifiers, one data ID value was created for each observation within the dataset (n = 185).

Data Collection

Classes were established for the growing strategy selected (HG, DL, DL-Forg, G, WW), use of technologies such as ionophores (y = yes, n = no), implants (y = yes, n = no), Tylan (y = yes, n = no), and sex (steer, heifer, or mixed group). Continuous variables of interest recorded from each study were initial growing phase BW, $ADG_{growing}$, days-on-feed ($DOF_{growing}$), and final growing phase BW (Final $BW_{growing}$). Yield grade (YG)

was also recorded and utilized to determine degree of maturity at harvest. Values for finishing and carcass performance served as dependent variables. Finishing performance variables included DMI, ADG, DOF, and final BW. Carcass performance variables included HCW, dressing percent (DP), longissimus muscle area (LMA), 12^{th} rib fat thickness (FT), and marbling score (MARB). A value for revenue was generated based on total gain during both growing and finishing phases. Total gain was calculated by multiplying $ADG_{growing}$ x $DOF_{growing}$ and $ADG_{finishing}$ x $DOF_{finishing}$. This value was then multiplied by the market price for weight class of the animal based on values reported in weekly USDA stocker cattle price report for week of November 3, 2017. A summary of growing data utilized is presented in Table 4.1, while performance and carcass data summaries can be found in Tables 4.2 and 4.3, respectively.

Degree of maturity calculations

In an effort to correct for variability resulting from various end points (weight, days on feed, fat cover, visual or ultrasound appraisal of Choice grade) selected for each study, a variable describing degree of maturity was generated.

Maturity in beef cattle is described as the point at which protein accretion plateaus. Comparisons based on final weight are not equivalent, within or across studies, due to differences in composition of gain, particularly when considering the impact of various growing phase strategies on body composition. Predictive models developed since publication of NRC (1984) correct differences in body composition by scaling observed weight to that of a moderate-frame, British calf reaching Choice grade. This adjustment is based on 28% empty body fat (EBF) for the reference animal.

To correct for differences in maturity of cattle at slaughter, within the meta-analysis data set, an adjustment for percent of mature weight was added to the model using relationship between EBF and empty body weight (EBW). From the equation derived by Tedeschi et al. (2004), reported YGvalues were utilized to calculate EBF:

$$YG + 0.604 / 0.127 = \% EBF$$

Empty body fat derived by this equation and HCW were used to predict EBW:

$$EBW_{predicted} = 106.56 * (EBF)^2 + 1771.9 * (EBF) – 52.364$$

This equation was derived from NRC (2016) where EBF (kg) is predicted from EBW (kg). Predicted EBW represents a size-scaled weight, which reflects percentage EBF at harvest for a given data point. Actual EBW (National Research Council, 1984) was calculated as:

$$EBW = SBW * 0.891$$

Degree of maturity was then determined by dividing actual EBW by predicted empty body weight:

$$\text{Degree of maturity} = EBW_{actual} / EBW_{predicted}$$

It follows that if data derived from a study in the meta-analysis data set have a value of 1 for degree of maturity, then cattle represented by that data point were harvested at the appropriate EBW given their degree of fatness. Similarly, cattle in data points with a value higher or lower than 1.0 would reflect cattle that were harvested heavier or lighter, respectively, than their scaled weight given empty body fat content at harvest.

Statistical Analyses

Data were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC), with treatment means weighted by experimental units (EU) per treatment. Standard error (SE) values were not available for all variables of interest, therefore EU was utilized instead. The inverse of SE was calculated for observations that contained it, but little difference was observed when comparing model fit of EU vs. 1/SE weighing for this subset. Treatment within study was incorporated as the subject of the random statement to model the within-subject variation.

Linear regression was used to model prediction values for finishing and carcass performance. Statistical significance for the effect of growing phase covariates and class variables on finishing and carcass performance was declared at $P \leq 0.05$ with trends established at $0.05 \geq P \leq 0.10$. No differences in Aikaike information criterion were observed between random and no intercept models. A random intercept model would allow for the response function to remain explained by predictive variables not accounted for in the model. While a no-intercept model forces all regressions through (0, 0) implying when all predictive variables in the model are set to 0, the response function is also 0. Because the objective was to model the impact of growing strategies on finishing responses, a no-intercept model was selected and the discrete variable of growing strategy assumed to explain any variation not accounted for in the model. Regressions models were selected by backward elimination; discrete variables or covariates were removed from model if *P*-value was greater than 0.10. Discrete variables from growing phase including strategy (HG, DL, DL-Forg, G, WW), ionophore use (y, n), liver abscess antibiotic use (y,n), impant use (y,n) and sex (steer, heifer, mixed) were all treated as random effects. Fixed and random effects of covariates, which included $ADG_{growing}$,

$DOF_{growing}$, Initial $BW_{growing}$, and Final $BW_{growing}$ as well as the interactions of $ADG_{growing}$ by $DOF_{growing}$ and $DOF_{growing}$ by Initial $BW_{growing}$, were tested. In order to extend conclusions to the larger population and not confine the results to the parameters tested within meta-analysis, it is necessary to treat all covariates as random variables (SAS/STAT ® 9.2 User's Guide Introduction to Regression Procedures, 2008). By testing random effects, there is not one assumed population effect but rather the population effect is distributed amongst variables (Gloudemans et al., 2012). Based on the described parameters, the model for this meta-analysis can be described as a within-subject, no intercept, random effects model.

Calculating equivalent effects of two continuous variables

A two variable multiple regression is represented by the following equation:

$$Y = b_0 + b_1X + b_2Z$$

The response variable is represented by Y, while X and Z are continuous predictor variables with their coefficients (b_1 and b_2). Equivalent effects of two continuous variables within a regression equation were calculated by setting the two variables with their coefficient equal to each other and solving for the variable of interest.

$$b_1 X = b_2 Z$$

$$X = b_2 Z / b_1$$

For variables with interaction effect, the coefficient from the interaction was added to the covariate coefficient prior to division, yielding the following equation:

$$X = (b_2 + b_3) Z / b_1$$

Calculating intersect points for two continuous variables

The intersect point for regression equations containing two interactive continuous covariates was solved using methods outlined by Aiken and West (1991). Intersecting variables (X and Z) and their coefficients as well as the regression intercept were incorporated into the following equation:

$$Y = b_1 X + b_2 Z + b_3 XZ + b_0$$

In order to solve for the intersect, two levels (h and l) of Z variable were selected to create two equations to solve for Y:

$$Y_h = (b_1 + b_3 Z_h) X + (b_2 Z_h + b_{0)}$$

$$Y_l = (b_1 + b_3 Z_l) X + (b_2 Z_l + b_{0)}$$

These two equations were then set equal to each other to determine their point of intersection. When simplified the following equation was derived to solve for X crossing point:

$$X_{cross} = -b_2 / b_3$$

This methodology was repeated with two levels of variable X to yield the following equation for the crossing value for covariate Z:

$$Z_{cross} = -b_1 / b_3$$

Calculations for Gross Profit from Growing Strategy

Gross profit was calculated using historical data from USDA, National Agricultural Statistics Service for 2017. Profit for cattle at the end of the growing phase was calculated by generating a regression of feeder cattle prices for 227 kg to 318 kg cattle and applying the equation to each of the BW at end of the growing phase (Final $BW_{growing}$). Separate regression equations were generated for each mo of the yr to ensure any market volatility was captured within final profit values. Profit at the end of the

finishing period was generated from monthly market price for fed cattle marketed in Nebraska (USDA).

RESULTS AND DISCUSSION

Differences in performance by growing strategy

The decision to use a growing strategy is dictated by a number of factors specific to the operation. Of particular importance are pen space and feed type and availability. It is challenging to pinpoint which strategy is most economically favorable because cattle response during the finishing phase will vary based on the growing strategy selected. Thus, it is import to understand the performance differences that exist between nutritional growing strategies (Table 4.5).

Observations described by Owens et al. (1993) provide support that finishing DMI is affected by growing nutritional strategy selection. Cattle grazing were observed by Owens et al. (1993) to have greater DMI when entering the feedlot compared to cattle placed in the feedlot immediately post-weaning. The current meta-analysis review demonstrated finishing DMI was greatest ($P < 0.01$) for cattle stocked on winter wheat (WW) and cattle fed a forage-based growing diet (DL-Forg) and lowest ($P < 0.01$) for cattle fed rations with high energy density in place of a growing phase (HG) (Table 4.5). The weight and volume consumed by forage feeding would explain an increase in capacity to consume due to an increase in gut fill (Allen, 2000). Therefore, greater finishing DMI was expected for cattle fed either of the forage or grazing growing-phase strategies (Table 4.5). This likely reflects a larger rumen capacity. This larger rumen mass was apparent in the lower dressing percentages of cattle fed corn silage or grazing native range compared to other strategies ($P < 0.01$; Table 4.5). Cattle that grazed WW

had the greatest ($P < 0.01$) dressing percentage of all strategies. This increased dressing percentage could indicate that visceral mass lost at slaughter was matched by carcass weight gain or that ruminal mass contracted once grazing WW ended. Carstens et al. (1991) determined that much of the compensatory gain response was correlated to changes in gut fill and energy density of ADG. Finishing ADG was greater ($P < 0.05$) for cattle grazing pasture (G) or WW, compared to dry-lot cattle fed either silage (DL-Forg), or a less energy dense dry-lot ration (DL) (Table 4.5). This greater finishing ADG value confirms the value of a larger rumen capacity of cattle from a forage-fed growing phase. This response was not observed with silage-fed cattle (DL-Forg); the extent of this response may be limited in silage-fed cattle for other reasons.

When ADG increases, carcass composition of gain must also be considered. Choice of growing phase strategy impacted LMA and BF, but not MARB; and therefore was not included in the model for MARB. Longissimus muscle area was found to be greatest ($P < 0.01$) for cattle that grazed WW and G. Cattle fed moderate energy or silage were intermediate while those placed on a finishing diet after weaning had the smallest LMA. An increase in muscling is often cited as a reason to add a growing phase (Owens et al., 1993); these data confirm that assertion of increased muscle hypertrophy. Cattle grazing winter wheat had greater ($P < 0.01$) BF compared with all other strategies. The significance of degree of maturity as a covariate in BF analysis demonstrates that cattle grazing WW were harvested at weights beyond expected maturity given their EBF. Previous meta-analyses (Galyean et al., 2011; Lancaster et al., 2014) conducted to predict finishing performance from growing phase characteristics have failed to utilize a maturity correction factor. Therefore, their findings were that BW at start of finishing was most

effective for predicting finishing performance. This would give an advantage to cattle finished at greater weights without considering body fat composition.

Final BW differences are similar to those observed for dressing percentage; with greatest BW achieved by WW or G and lowest final BW attained by HG ($P < 0.01$; Table 4.5). The addition of a growing phase knowingly delays the onset of maturity allowing animals to increase frame growth with the intention of increasing final BW (Owens et al., 1993; Hornick et al., 2000). The final BW means presented provide convincing evidence to support beneficial compensatory gain effects for all growing strategies evaluated.

Interpretation of interacting covariates within regression models

When examining feedlot and carcass performance attributes by growing strategy it becomes clear that factors, like $ADG_{growing}$, $DOF_{growing}$, initial $BW_{finishing}$, etc., interact to create the optimal compensatory gain response. Two interacting growing phase variables, DOF and initial BW, affected carcass performance. Previously, a meta-analysis conducted by Lancaster et al. (2014) also determined the importance of BW as BW at the end of the growing phase.

Optimal combinations of $DOF_{growing}$ and initial $BW_{growing}$ differed between performance characteristics (Table 4.6) but as an average across dressing percent, LMA, Final $BW_{finishing}$, and HCW it was identified that maximized performance occurred when cattle were fed at or less than 80 $DOF_{growing}$ and began the growing phase at approximately 240 kg. Lighter-weight cattle are more responsive to benefits of growing systems, and there is a limit to how long these animals should have restricted caloric intake to maximize the benefits of compensatory gain (Droulliard et al., 1991a,b). These data are confounded within standardized values for other attributes of growing

performance (ie- $ADG_{growing}$). Taylor et al. (2013) reported no differences in feed efficiency for steers in growing phases of either 79 or 93 d, although $DMI_{finishing}$ and $ADG_{finishing}$ were greater for steers with longer $DOF_{growing}$ and slower $ADG_{growing}$. They also found no differences in LMA or dressing percentage. Therefore, supporting that 80 d may be optimum for a growing strategy.

Utilizing interactions to determine critical points of interest

Final BW was predicted by the interacting effects of $ADG_{growing}$ and $DOF_{growing}$. Thus, to predict optimum relationships between $ADG_{growing}$ and $DOF_{growing}$, critical points of interest were determined. Figure 4.1 depicts the relationship between Initial $BW_{growing}$ and a 0.1 kg increase in final $BW_{finishing}$. Because $ADG_{growing}$ directly relates to final $BW_{finishing}$ increases without impact of Initial $BW_{growing}$ its effect is modeled as static across all values of Initial $BW_{growing}$. However, the linear relationship between the impact of $DOF_{growing}$ and Initial $BW_{growing}$ characterizes their interactive relationship. The point where the line crosses the X-axis represents the critical point of interest for this interaction. Figure 4.1 depicts the impact of $DOF_{growing}$ on final $BW_{finishing}$ to match the impact of an increase of 0.1 kg $ADG_{growing}$ as Initial $BW_{growing}$ shifts. The critical point (X = 230 kg) is represented by the asymptote. This critical point represents the point at which beyond it, additional $DOF_{growing}$ positively benefit final $BW_{finishing}$. Positive final $BW_{finishing}$ performance will be observed for cattle with Initial $BW_{growing}$ below this critical point; however performance of cattle in these scenarios would benefit from $ADG_{growing}$, rather than $DOF_{growing}$. In practical application, this means for lighter weight cattle (lighter than 230 kg) entering the growing phase, $ADG_{growing}$ carries great

importance for improving final $BW_{finishing}$. Table 4.6 lists the critical point for Initial $BW_{growing}$ in relation to $DOF_{growing}$ for REA, DP, Final $BW_{finishing}$, and HCW.

Evaluating economic impacts of adding a growing phase

Due to the intentional energy restriction that occurs with growing strategies, economic benefits are not seen prior to finishing phase when caloric intake is restored and compensatory gain occurs. Figure 4.3 demonstrates this idea as gross profit from cattle without a growing phase (i.e.- HG) demonstrate much greater revenue than counterparts with a growing phase. As cattle shift from growing to finishing phase and compensatory gain occurs there is an obvious increase in the economic value of these same cattle. Figure 4.4 shows the roles reversed with HG cattle grossing the least profit relative to cattle raised with growing phase. Of the growing phase groups, WW cattle grossed the largest profit – most likely due to greater final $BW_{finishing}$ achieved. However, neither of these figures account for the differences in feeding program duration. Figure 4.5 depicts gross profit/head by DOF to better demonstrate the differences in program length to achieve similar profit. The slopes of each of these line segments (Table 4.7) depicts the 'potential to gain' for that animal, with greater slopes indicating more aggressive gains. Slopes for the first line segment (0, 0) to mid-point rank themselves according to $ADG_{growing}$, with cattle without a growing phase gaining the most per $DOF_{growing}$ and grazing cattle gaining the least. What perhaps is of more interest is the slope of the second line segment. This value for 'potential to gain' serves as a reference for animals on a traditional growth curve. Because restricting intake early in the growing phase delays the onset of maturity, shifting the growth curve so more aggressive growth occurs later, a greater slope value for the second line segment is expected. As expected,

HG cattle appear to have undergone more aggressive growth early on; their growth potential has begun to plateau by end of feeding period ($0.35/d). The higher potential to gain values for cattle fed through a growing phase within finishing period are what allowed for increased final profit relative to cattle not subjected to a growing phase. Cattle grazing WW had the greatest potential to gain in finishing phase ($0.76/d) followed by those fed moderate energy diets in dry lot cattle (DL) ($0.65/d), cattle grazing (G) native range/pasture ($0.61/d), and those fed silage in a dry lot (DL-Forg) ($0.58/d). For the majority of simulated cattle, the profit/d exceeded traditional yardage costs ($0.37/d) indicating they retained reasonable profit margins between input and output costs. Cattle finished without a growing phase did not maintain these same profit margins (relative to traditional yardage costs), which may have influenced why in certain months when prices were low, these cattle yielded net profit loss in the finishing phase.

CONCLUSIONS

Overall growing strategies can be a valuable tool for increasing pounds of beef /carcass, but understanding how management decisions, especially changes in targeted $ADG_{growing}$, $DOF_{growing}$ and initial $BW_{growing}$, affect finishing and carcass performance is the key to a successful growing program. Differences do exist between strategies, however, often times changing growing strategy is not an option for producers due to feed or space limitations. Instead, understanding the physiology behind the differences in performance responses and how equivalent changes in $DOF_{growing}$ and $ADG_{growing}$ can be used to mitigate management challenges provides a way for producers to benefit from this data regardless of the strategy implemented.

Table 4.1 Summary of growing data utilized to analyze the impact of growing strategy and performance on finishing and carcass performance

Variable	n	Mean	SD	Minimum	Maximum
High Grain (HG)					
Initial BW, kg	14	223	371	142	391
DOF	13	84	100	56	154
ADG, kg	13	1.44	1.04	1.06	2.32
Final BW, kg	15	308	379	238	469
Dry Lot (DL)					
Initial BW, kg	79	279	233	76	391
DOF	79	90	125	55	196
ADG, kg	79	1.09	1.12	0.58	2.40
Final BW, kg	80	330	398	81	482
Dry Lot Forage (DL-Forg)					
Initial BW, kg	44	227	202	136	391
DOF	44	97	74	56	141
ADG, kg	43	0.86	1.06	0.39	1.38
Final BW, kg	43	301	218	238	458
Grazing (G)					
Initial BW, kg	66	238	113	149	276
DOF	66	198	448	70	443
ADG, kg	66	0.58	0.85	0.15	0.89
Final BW, kg	66	358	188	255	455
Winter Wheat (WW)					
Initial BW, kg	28	223	129	184	261
DOF	28	125	189	74	201
ADG, kg	28	0.93	1.34	0.37	1.37
Final BW, kg	28	344	286	229	432

Table 4.2 Summary of finishing performance data utilized to analyze the impact of growing strategy and performance on finishing and carcass performance

Variable	n	Mean	SD	Minimum	Maximum
High Grain (HG)					
Final BW, kg	16	519	259	458	597
ADG, kg	16	1.44	1.51	1.02	1.91
DOF	16	159	222	37	196
DMI, kg/d	15	8.87	5.27	6.58	12.20
Dry Lot (DL)					
Final BW, kg	80	529	246	325	714
ADG, kg	80	1.37	1.14	0.67	1.95
DOF	80	112	130	44	258
DMI, kg/d	80	9.43	5.58	5.64	12.10
Dry Lot Forage (DL-Forg)					
Final BW, kg	44	510	237	421	619
ADG, kg	44	1.42	1.14	0.67	2.02
DOF	34	132	124	72	173
DMI, kg/d	43	10.02	4.01	7.19	11.40
Grazing (G)					
Final BW, kg	66	526	225	452	632
ADG, kg	66	1.42	1.53	0.79	2.12
DOF	66	117	122	81	222
DMI, kg/d	66	10.83	5.16	7.90	12.50
Winter Wheat (WW)					
Final BW, kg	28	528	310	372	628
ADG, kg	28	1.56	1.13	1.15	1.90
DOF	28	118	109	70	156
DMI, kg/d	25	10.90	2.76	9.70	11.80

Table 4.3 Summary of carcass performance data utilized to analyze the impact of growing strategy and performance on finishing and carcass performance

Variable	n	Mean	Std Dev	Minimum	Maximum
High Grain (HG)					
HCW, kg	16	325	163	294	371
Dressing Percent, %	16	62.6	12.0	60.0	66.4
BF, cm	14	1.3	1.1	0.9	1.6
LMA, sq cm	14	78	30	71	94
Marbling[1]	16	575	413	448	719
Yield Grade	16	2.7	4.2	1.0	3.5
Dry Lot (DL)					
HCW, kg	76	318	150	179	426
Dressing Percent, %	76	60.18	9.92	53.16	64.88
BF, cm	79	1.2	1.2	0.1	1.7
LMA, sq cm	77	82	32	71	104
Marbling[1]	64	540	420	57	713
Yield Grade	78	2.7	3.3	1.0	4.3
Dry Lot Forage (DL-Forg)					
HCW, kg	44	306	187	241	379
Dressing Percent, %	44	59.7	14.1	52.6	66.8
BF, cm	35	1.2	0.7	0.7	1.6
LMA, sq cm	43	77	40	65	99
Marbling[1]	33	527	205	400	635
Yield Grade	32	2.8	3.3	1.0	3.7
Grazing (G)					
HCW, kg	66	316	181	38	397
Dressing Percent, %	66	60.3	11.0	56.0	64.4
BF, cm	56	1.2	1.3	0.9	2.4
LMA, sq cm	49	77	41	67	99
Marbling[1]	59	499	203	437	680
Yield Grade	43	2.6	2.6	1.4	3.5
Winter Wheat (WW)					
HCW, kg	28	336	204	247	424
Dressing Percent, %	28	63.7	8.6	60.3	67.5
BF, cm	16	1.5	1.1	1.1	1.9
LMA, sq cm	16	78	48	66	101
Marbling[1]	25	555	440	487	710
Yield Grade	16	3.1	3.1	1.6	3.5

[1]Marbling Score: 400 – slight, 500 – small, 600 – modest

Table 4.4 Regression Coefficient and Fit Statistics for Covariates for all dependent variable models

Dependent Variable	Growing Phase	R^2	Intercept	Regression Coeffiecients for Covariates within Model
$DMI_{finishing}$, kg	HG	0.60	6.59	1.2362(Per_Mat) + 0.0096(DOF_PF) + 0.1766(ADG_PF) - 0.0093(DOF_PF)(ADG_PF) + out_BW_PF(0.0036)
	DL		7.15	1.2362(Per_Mat) + 0.0096(DOF_PF) + 0.1766(ADG_PF) - 0.0093(DOF_PF)(ADG_PF) + out_BW_PF(0.0036)
	DL_Forg		7.72	1.2362(Per_Mat) + 0.0096(DOF_PF) + 0.1766(ADG_PF) - 0.0093(DOF_PF)(ADG_PF) + out_BW_PF(0.0036)
	G		7.21	1.2362(Per_Mat) + 0.0096(DOF_PF) + 0.1766(ADG_PF) - 0.0093(DOF_PF)(ADG_PF) + out_BW_PF(0.0036)
	WW		7.98	1.2362(Per_Mat) + 0.0096(DOF_PF) + 0.1766(ADG_PF) - 0.0093(DOF_PF)(ADG_PF) + out_BW_PF(0.0036)
$ADG_{finishing}$, kg	HG	0.28	1.48	0.1849(Per_Mat) - 0.0015 (DOF_PF)
	DL		1.58	0.1849(Per_Mat) - 0.0015 (DOF_PF)
	DL_Forg		1.56	0.1849(Per_Mat) - 0.0015 (DOF_PF)
	G		1.92	0.1849(Per_Mat) - 0.0015 (DOF_PF)
	WW		1.87	0.1849(Per_Mat) - 0.0015 (DOF_PF)

Dependent Variable	Growing Phase	R^2	Intercept	Regression Coeffiecients for Covariates within Model
Dressing %	HG	0.39	0.67	0.000075(Out_BW_PF) - 0.00086(DOF_PF) + 0.01294(ADG_PF) - 0.00038(In_BW) + 0.00000337(DOF_PF)(In_BW)
	DL		0.67	0.000075(Out_BW_PF) - 0.00086(DOF_PF) + 0.01294(ADG_PF) - 0.00038(In_BW) + 0.00000337(DOF_PF)(In_BW)
	DL_Forg		0.66	0.000075(Out_BW_PF) - 0.00086(DOF_PF) + 0.01294(ADG_PF) - 0.00038(In_BW) + 0.00000337(DOF_PF)(In_BW)
	G		0.68	0.000075(Out_BW_PF) - 0.00086(DOF_PF) + 0.01294(ADG_PF) - 0.00038(In_BW) + 0.00000337(DOF_PF)(In_BW)
	WW		0.70	0.000075(Out_BW_PF) - 0.00086(DOF_PF) + 0.01294(ADG_PF) - 0.00038(In_BW) + 0.00000337(DOF_PF)(In_BW)
LMA, sq cm	HG	0.55	74.33	10.6674(Per_Mat) + 9.95(ADG_PF) - 0.2619(DOF_PF) - 0.08689(In_BW) + 0.001084 (DOF_PF)(In_BW)
	DL		79.01	10.6674(Per_Mat) + 9.95(ADG_PF) - 0.2619(DOF_PF) - 0.08689(In_BW) + 0.001084 (DOF_PF)(In_BW)
	DL_Forg		77.46	10.6674(Per_Mat) + 9.95(ADG_PF) - 0.2619(DOF_PF) - 0.08689(In_BW) + 0.001084 (DOF_PF)(In_BW)
	G		82.12	10.6674(Per_Mat) + 9.95(ADG_PF) - 0.2619(DOF_PF) - 0.08689(In_BW) + 0.001084 (DOF_PF)(In_BW)
	WW		81.40	10.6674(Per_Mat) + 9.95(ADG_PF) - 0.2619(DOF_PF) - 0.08689(In_BW) + 0.001084 (DOF_PF)(In_BW)

Dependent Variable	Growing Phase	R^2	Intercept	Regression Coeffiecients for Covariates within Model
BF, cm	HG	0.20	1.48	(-0.00086)(DOF_PF)-0.00059(Out_BW_PF)
	DL		1.49	(-0.00086)(DOF_PF)-0.00059(Out_BW_PF)
	DL_Forg		1.43	(-0.00086)(DOF_PF)-0.00059(Out_BW_PF)
	G		1.55	(-0.00086)(DOF_PF)-0.00059(Out_BW_PF)
	WW		1.79	(-0.00086)(DOF_PF)-0.00059(Out_BW_PF)
Marbling[1]		0.14	592.08	73.3999(ADG_PF) - 0.2984(Out_BW_PF) - 41.7885 (Per_Mat)
Final $BW_{finishing}$, kg	HG	0.58	434.80	67.35(Per_Mat) + 85.5164(ADG_PF) - 0.08907(Out_BW_PF) - 1.6039(DOF_PF) - 0.3546(In_BW) + 0.006955(DOF_PF)(In_BW)
	DL		464.65	67.35(Per_Mat) + 85.5164(ADG_PF) - 0.08907(Out_BW_PF) - 1.6039(DOF_PF) - 0.3546(In_BW) + 0.006955(DOF_PF)(In_BW)
	DL_Forg		465.54	67.35(Per_Mat) + 85.5164(ADG_PF) - 0.08907(Out_BW_PF) - 1.6039(DOF_PF) - 0.3546(In_BW) + 0.006955(DOF_PF)(In_BW)
	G		512.74	67.35(Per_Mat) + 85.5164(ADG_PF) - 0.08907(Out_BW_PF) - 1.6039(DOF_PF) - 0.3546(In_BW) + 0.006955(DOF_PF)(In_BW)
	WW		501.05	67.35(Per_Mat) + 85.5164(ADG_PF) - 0.08907(Out_BW_PF) - 1.6039(DOF_PF) - 0.3546(In_BW) + 0.006955(DOF_PF)(In_BW)

Dependent Variable	Growing Phase	R^2	Intercept	Regression Coefficients for Covariates within Model
HCW, kg	DL	0.54	35.11	37.6798(Per_Mat) + 62.1982(ADG_PF) -1.633(DOF_PF) - 0.4911(In_BW) + 0.007022(DOF_PF)(In_BW)
	DL_Forg		35.55	37.6798(Per_Mat) + 62.1982(ADG_PF) -1.633(DOF_PF) - 0.4911(In_BW) + 0.007022(DOF_PF)(In_BW)
	G		35.59	37.6798(Per_Mat) + 62.1982(ADG_PF) -1.633(DOF_PF) - 0.4911(In_BW) + 0.007022(DOF_PF)(In_BW)
	HG		37.38	37.6798(Per_Mat) + 62.1982(ADG_PF) -1.633(DOF_PF) - 0.4911(In_BW) + 0.007022(DOF_PF)(In_BW)
	WW		25.75	37.6798(Per_Mat) + 62.1982(ADG_PF) -1.633(DOF_PF) - 0.4911(In_BW) + 0.007022(DOF_PF)(In_BW)

[1]*Marbling Score: 400 – slight, 500 – small, 600 – modest*

Table 4.5 Least squares means for differences in finishing and carcass performance as affected by growing strategy

	HG	DL	DL-Forage	G	WW	SEM[1]	*P*-value[2]
$DMI_{finishing}$, kg/d	9.6 [c]	10.2 [a]	10.8 [b]	10.3 [ac]	11.0 [b]	0.3	< 0.01
$ADG_{finishing}$, kg/d	1.28 [a]	1.39 [a]	1.36 [a]	1.72 [b]	1.68 [b]	0.10	< 0.01
Dressing Percent, %	59.6% [ab]	59.6% [a]	58.8% [a]	60.9% [b]	62.7% [c]	0.4%	< 0.01
LMA, sq cm	74.7 [d]	79.4 [ab]	77.8 [ad]	82.5 [c]	81.7 [bc]	1.2	< 0.01
BF, cm	1.1 [a]	1.1 [a]	1.0 [a]	1.1 [a]	1.4 [b]	0.1	< 0.01
Final $BW_{finishing}$, kg	482 [c]	512 [a]	512 [a]	560 [b]	548 [b]	10	< 0.01
HCW, kg	287 [a]	306 [a]	306 [a]	343 [b]	346 [b]	7	< 0.01

HG = No growing phase
DL = Drylot
DL-Forg = Drylot silage only
G = Pasture/native range
WW= Winter wheat
[1]SEM = standard error of the mean
[2]*P*-value for effect of growing strategy on dependent finishing variable prediction
[abcd]Unlike superscripts differ ($P < 0.05$)

Figure 4.1 Critical point (x = 230.4 kg) for the effect of initial body weight at the start of growing on equivalent $DOF_{growing}$ needed to impact final body weight to same extent as 0.1 kg ADG change

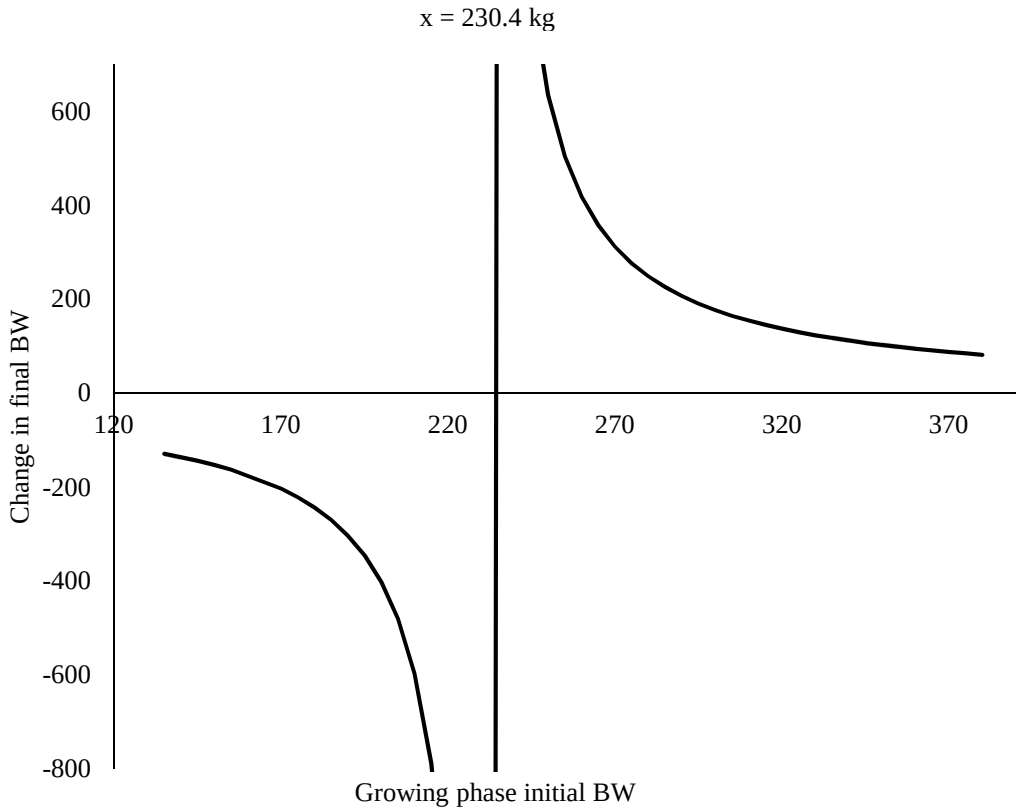


Table 4.6. Critical point for Inital BW $_{growing}$ to positively affect the magnitude of impact of DOF$_{growing}$ on finishing variables of interest

Finishing variable	Initial BW, Kg
LMA	255
DP	242
Final BW	231
HCW	233

Figure 4.2 Relationship between body weight at start of growing (Initial BW) and the increase in final body weight for each 0.1 kg increase in growing ADG or each 10 d increase in growing DOF

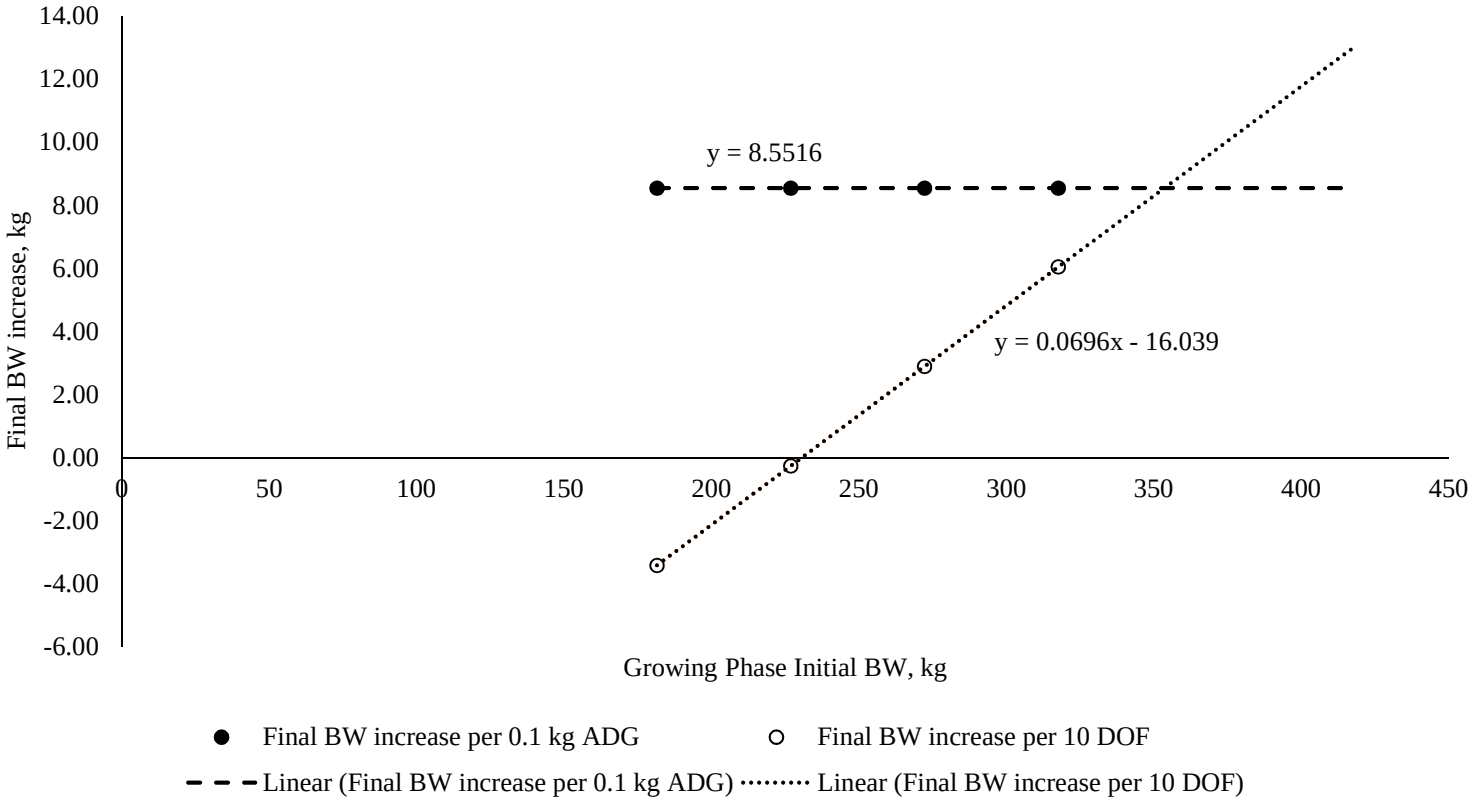


Table 4.7 Value for slopes derived from gross profit by DOF to examine differences in potential to gain profit

	Growing Phase, $/d	**Finishing Phase, $/d**
No growing (HG)	2.92	0.35
Dry lot (DL)	2.44	0.65
Dry lot silage fed (DL_Forg)	2.25	0.58
Grazing (G)	1.18	0.61
Winter Wheat (WW)	1.82	0.77

Figure 4.3 Changes in gross profit per head at end of growing phase from January to October 2017

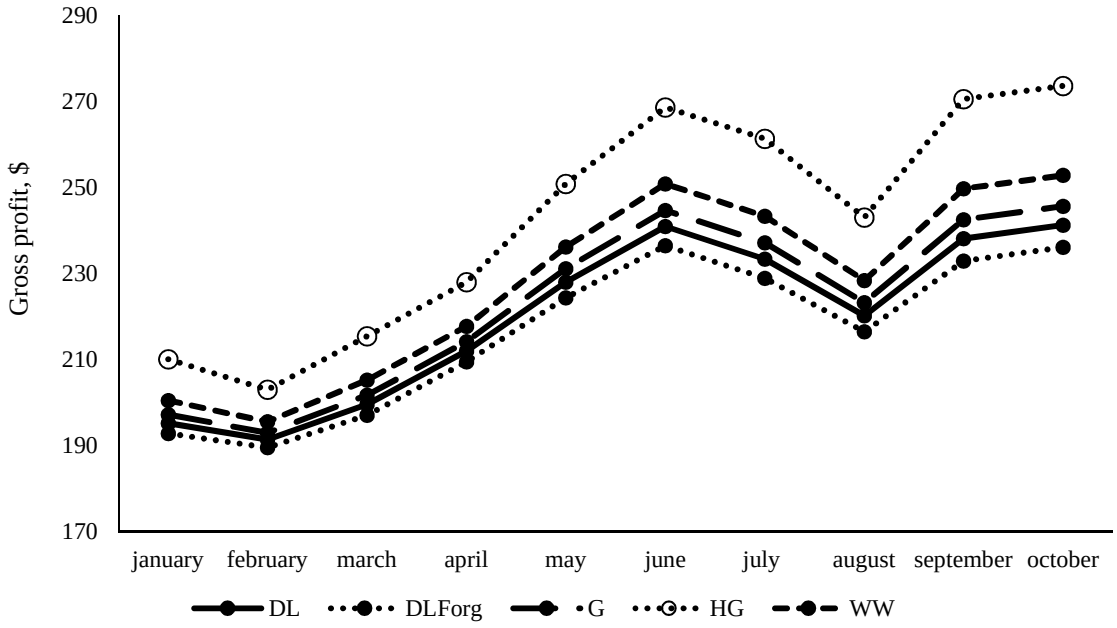


Figure 4.4 Changes in net profit per head (gross profit at end of finishing – cost of to purchase animal at end of growing period) during finishing phase from January to October 2017

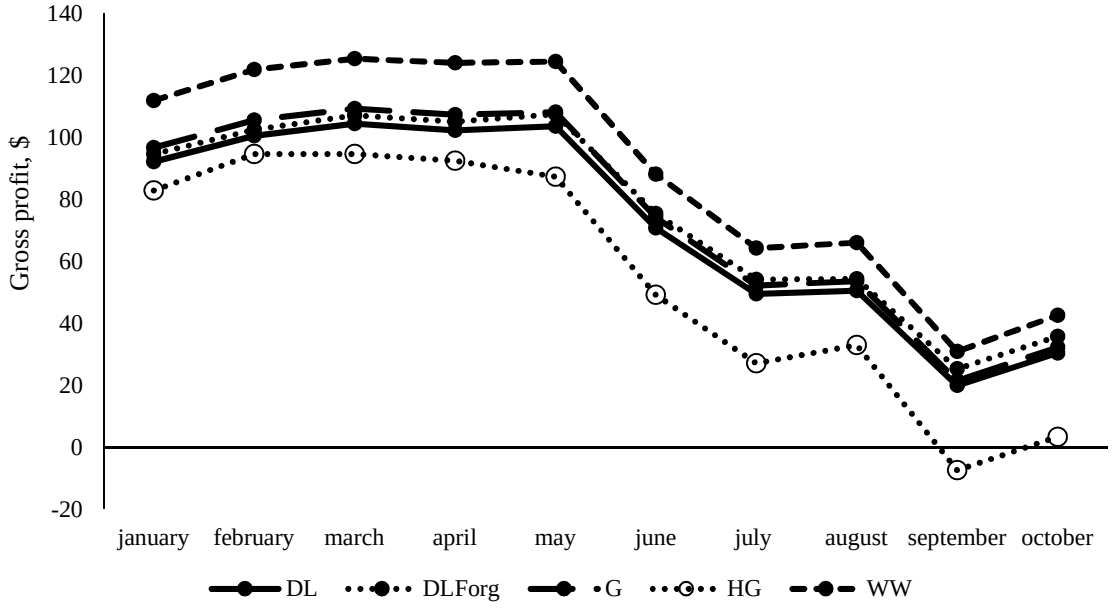
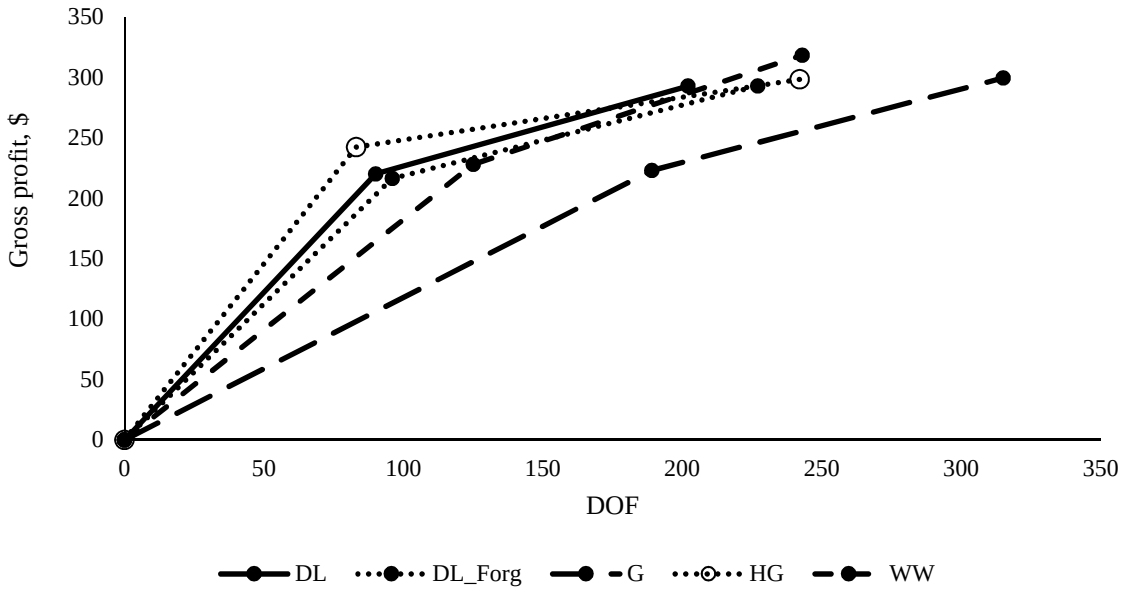


Figure 4.5 Changes in gross profit per head by days on feed (DOF) over entire feeding period from January to October 2017 (center dot represents gross profit at end of growing phase, right most dot represents gross profit at end of finishing phase)



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APPENDEX

Figure A1.11 Diagram bacterial groups involved in the primary and alternative biohydrogenation pathways for linolenic (C18:2) and linoleic acid (C18:3) in the rumen (adapted from Harfoot and Hazelwood, 1997 and Kramer et al., 2004) (Chapter I: part I)

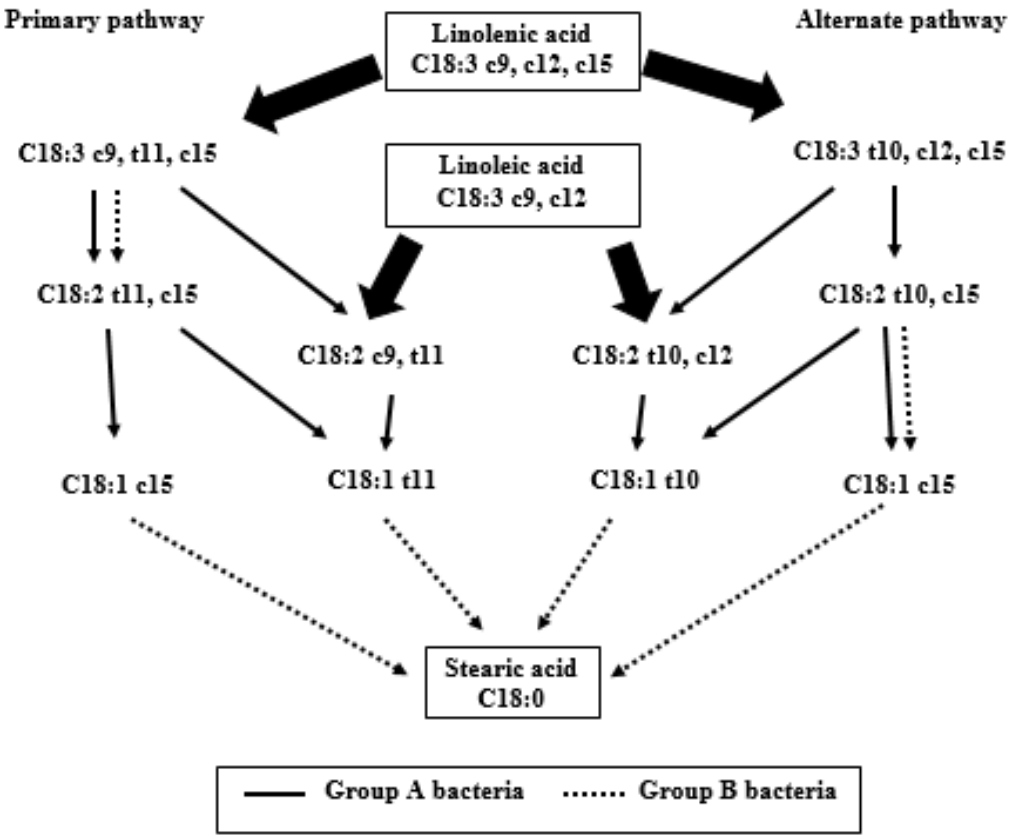

Figure A1.12 Diagram of volatile fatty acid production pathways of rumen microbes (Chapter I: part I)

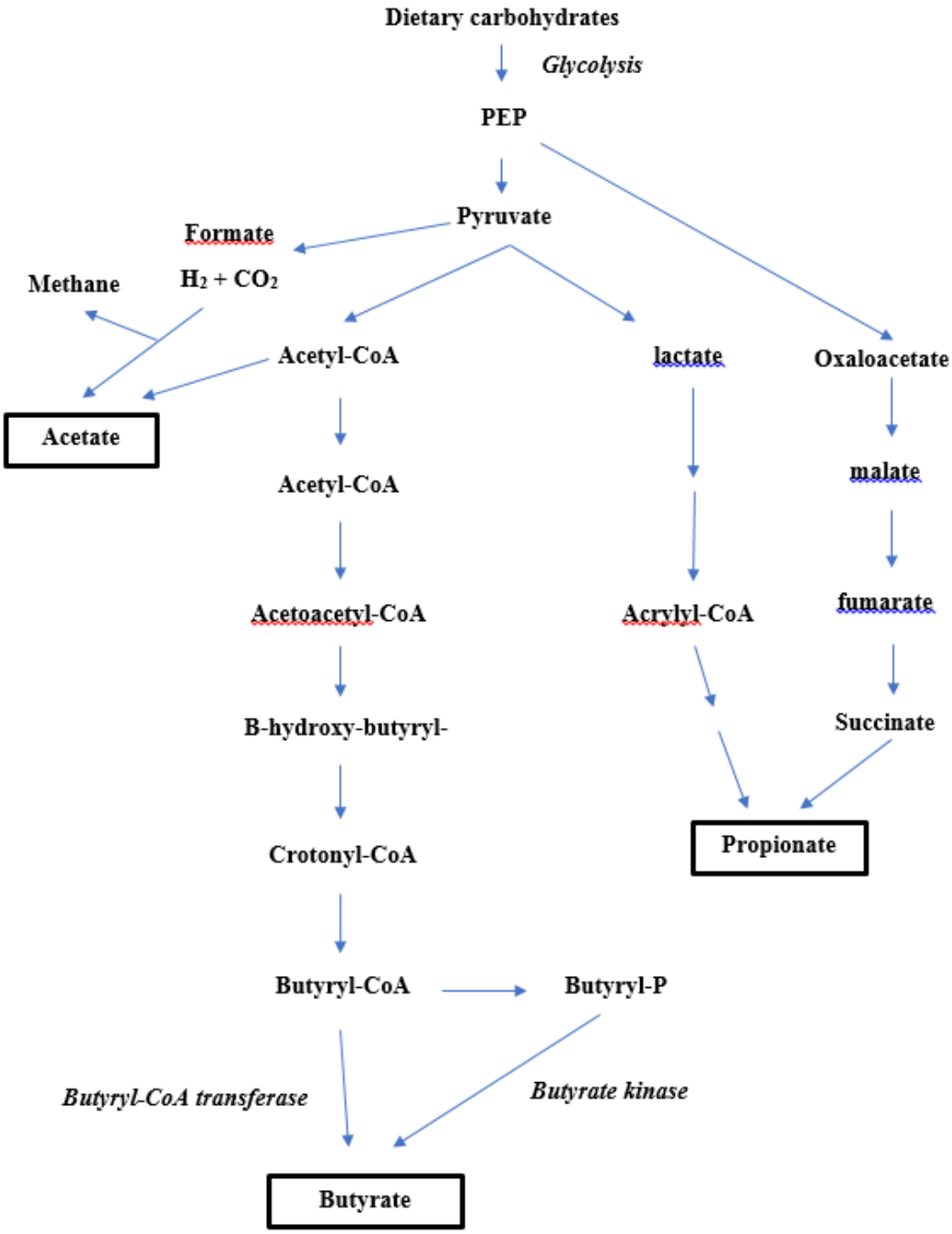

Table A1.11 Influence of pH on lipolysis and biohydrogenation of linoleic and linolenic acid in vitro (Van Nevel and Demeyer, 1996) (Chapter I: part I)

	Freed Fatty Acids (mg)		Disappearance (%)	
pH	**18:2**	**18:3**	**18:2**	**18:3**
6.78 ± 0.04	16.53[a]	2.16[a]	94.6[a]	100.0[a]
6.34 ± 0.07	15.97[a]	2.04[a]	97.8[a]	100.0[a]
5.98 ± 0.06	15.16[a]	2.01[a]	95.2[a]	100.0[a]
5.56 ± 0.06	9.61[b]	1.21[b]	80.5[ab]	89.9[b]
5.22 ± 0.06	4.36[c]	0.48[c]	59.5[b]	68.8[b]

[xyz]linoleic acid = 18:2; linolenic acid (ALA) = 18:3; Freed fatty acids = FA liberated from TAG as representative of lipolysis; disappearance (%) of FA as consequence of biohydrogenation

[abc]differing superscripts indicate significance $p < 0.05$

Table A1.12 Effect of antimicrobial additives on inhibition of lipolysis and volatile fatty acid (VFA) production (Van Nevel and Demeyer, 1995) (Chapter I: part I)

Additive	Bacteria affected	Inhibition of	
		Lipolysis (%)	VFA Production (%)
Amoxicillin	Broad Spectrum	18.2	6.4
Avoparcin	Gram positive	10.4	3.9
Salinomycin sodium	Gram positive	20.1	12.2
Lincomycin hydrochloride	Anaerobic gram positive	14.5	14.8
Lasalocid sodium (Bovatec)	Gram positive	19.3	6.3
Monensin (Rumensin)	Gram positive	16.7	8.7
Terramycin	Broad spectrum	15.7	17.4
Virginiamycin	Gram positive	16.2	14.5
Mentronidazole	Gram negative	9.1	10.4

Table A1.13 Effect of three diets fed to sheep on ruminal biohydrogenation and absoption of linolenic acid (C18:3) (Bauchart D. and Poncet C., unpublished data published in Chilliard et al., 2000) (Chapter I: part I)

		Hay : Concentrate	
	Fresh Grass	**75:25**	**30:70**
FA intake (g/100g DM)	2.38	0.88	0.88
C18:3 intake (g/d)	14.00	0.85	0.46
C18:3 % total FA	56.20	8.80	4.50
C18:3 hydrogenation (%)	96.0	93.0	87.0
C18:3 Presented at Sm. Intestine (g)	0.57	0.06	0.06
C18:3 absorbed (g/d)	0.49	0.04	0.05
Biohydrogenation escape[x], %	4.10	7.05	10.87
Absorption Efficiency[x], %	85.9	66.6	83.3
Efficiency of Utilization[x], %	3.50	4.71	9.05

[x] biohydrogenation escape = C18:3 presented at small intestine / C18:3 in diet; absorption efficiency = C18:3 absorbed / C18:3 presented at small intestine; Efficiency of utilization = biohydrogenation escape x absorption efficiency

Figure A1.13 Efficiency of transferring C18:3 infused into the abomasum or small intestine of dairy cow to milk (Chilliard et al., 1991; Drackley et al., 1992; Christensen et al., 1994; LaCount et al., 1994; Ottou et al., 1995; Litherland et al., 2005) (Chapter I: part I)

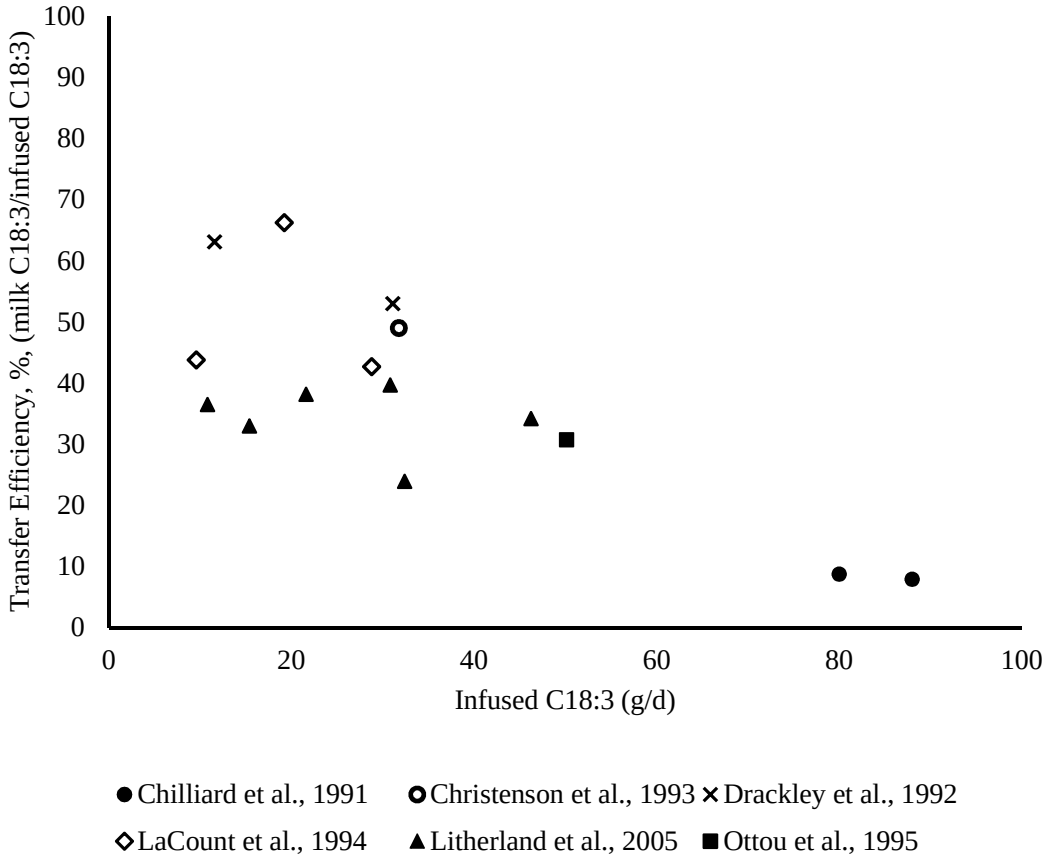

Figure A1.14 Relationship between duodenal flow of fatty acids and the quantity of fatty acids absorbed (dotted line y=x; regression line y=0.66x + 57.8 (R^2 = 0.87)) (Lock et al., 2006) (Chapter I: part I)

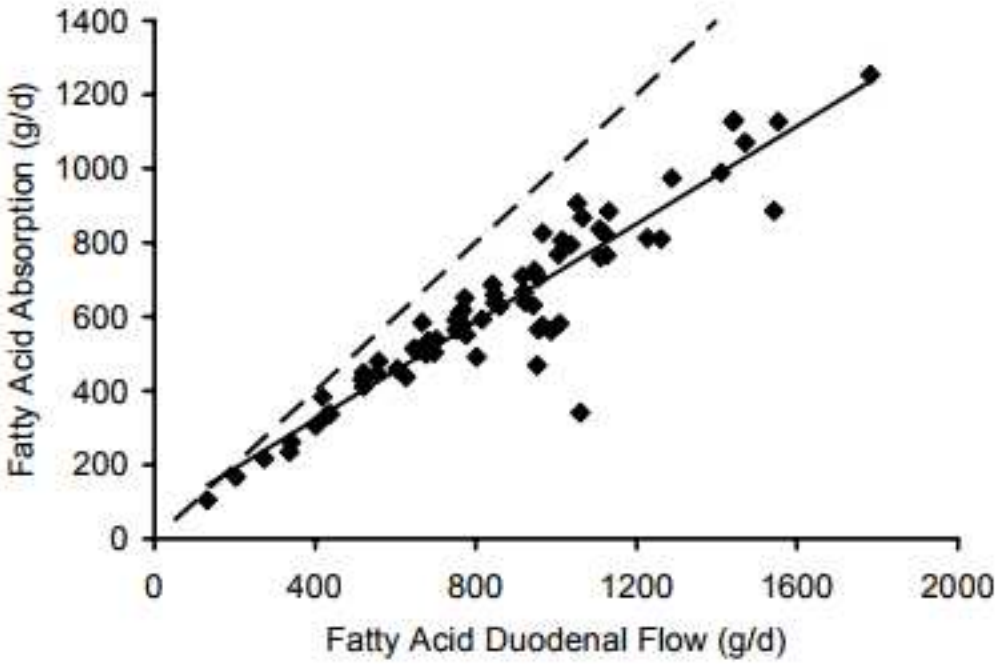

Table A1.14 Cost per bushel of flaxseed to break even with cost of 8% dietary inclusion in finishing diet when paid premium for flax feed beef above market live price (Chapter I: part I)

	Premium above live weight market price ($1.18)				
	10%	**20%**	**30%**	**40%**	**50%**
Live wt price	$1.30	$1.42	$1.53	$1.65	$1.77
Price per bu[y]	$2.07	$4.06	$6.03	$8.10	$10.16

[x]simulated steers were fed from 750 to 1400 lb with average DMI of 23 lb with 7.0lb F:G (performance data collected from Maddock et al., 2006)

[y]bushel of flax is represented by 56 lb

Figure A1.21 Growth of steers in growing and finishing phases when calories are not restricted, restricted via limited intake, or restricted via changes in caloric density of diet (● high concentrate both growing and finishing; □ concentrate limit fed both growing and finishing ■ concentrate ad libitum limit fed in growing phase and ad libitum in finishing ▲forage ad libitum for growing phase and concentrate ad libitum in finishing) (Sainz et al., 1995) (Chapter I: part II)

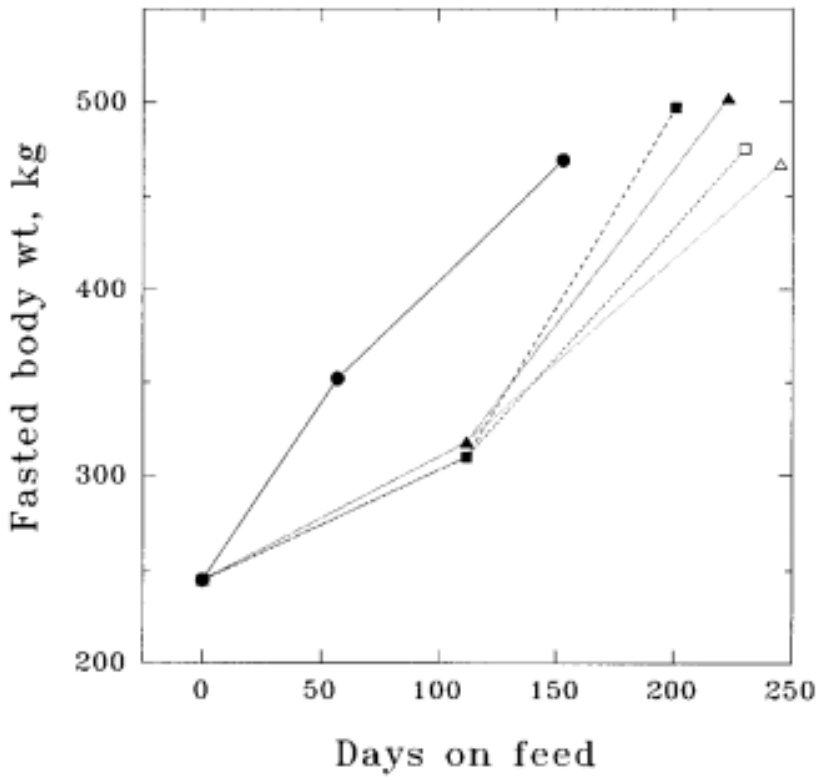

Figure A1.22 Monthly changes in live cattle price at slaughter from 2012 to 2016 (USDA, ERS) (Chapter I: part II).

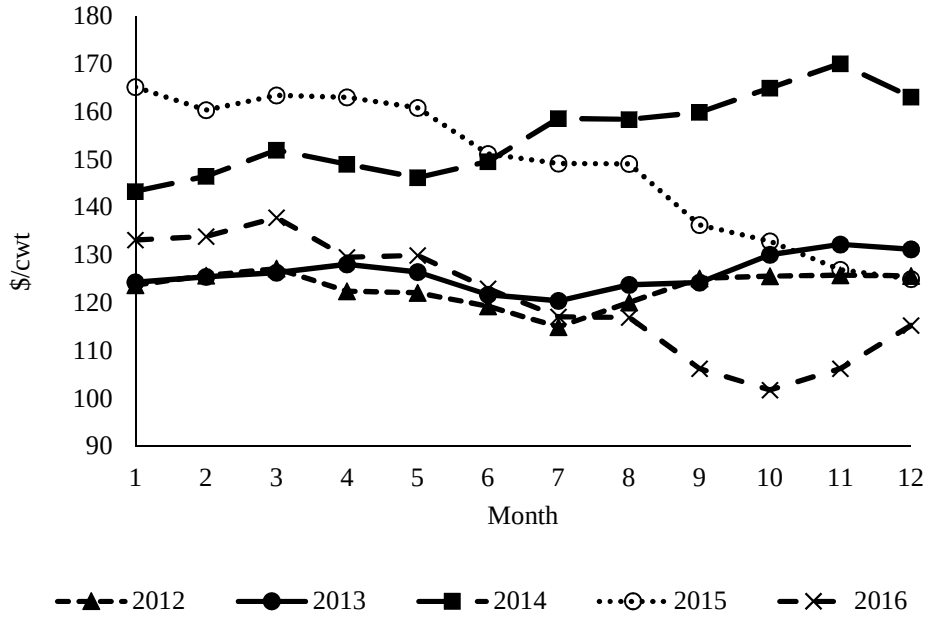

Table A1.21 Profit from cattle raised with or without a growing phase factoring in yardage costs for increased days on feed (DOF) using estimated final weights from Sainz et al. (1995) and historical fed cattle live weight prices from USDA ERS (Chapter I: part II).

Year	Month	Final wt, kg	$/kg	Yardage/d	DOF	Gross profit*	Difference in Profit
2014	July	450	$3.50	$0.37	140	$1,521.01	
2014	September	500	$3.52	$0.37	200	$1,684.46	$163.45
2016	July	450	$2.57	$0.37	140	$1,106.50	
2016	September	500	$2.33	$0.37	200	$1,092.00	-$14.50

*Gross profit = ($/kg * Final wt (kg)) - (yardage/d * DOF)

Figure A4.1 Observed DMI, kg/d, regressed on predicted DMI, kg/d with solid line representing perfect fit between predicted and observed variables from chapter IV.

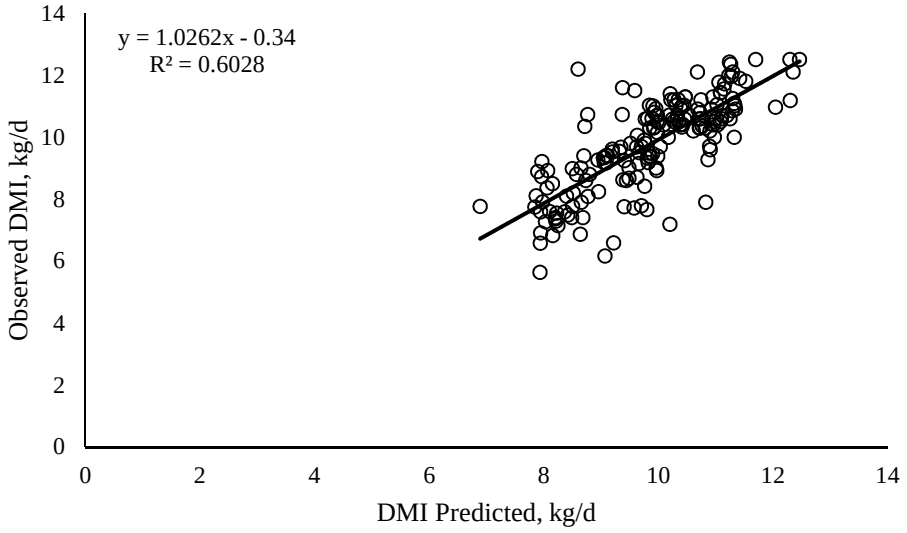

Figure A4.2 Observed ADG, kg, regressed on predicted ADG, kg with solid line representing perfect fit between predicted and observed variables from chapter IV.

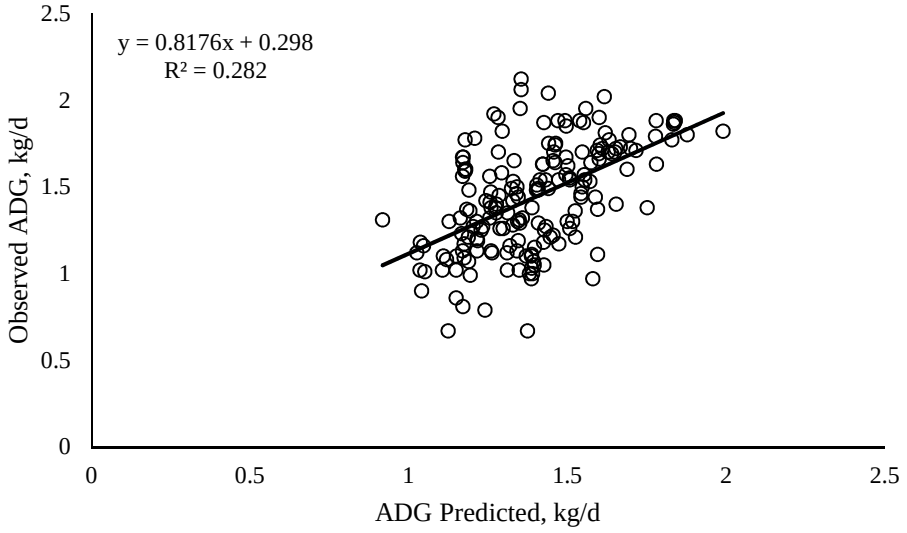

Figure A4.3 Observed dressing percent, %, regressed on predicted dressing percent, %, with solid line representing perfect fit between predicted and observed variables from chapter IV.

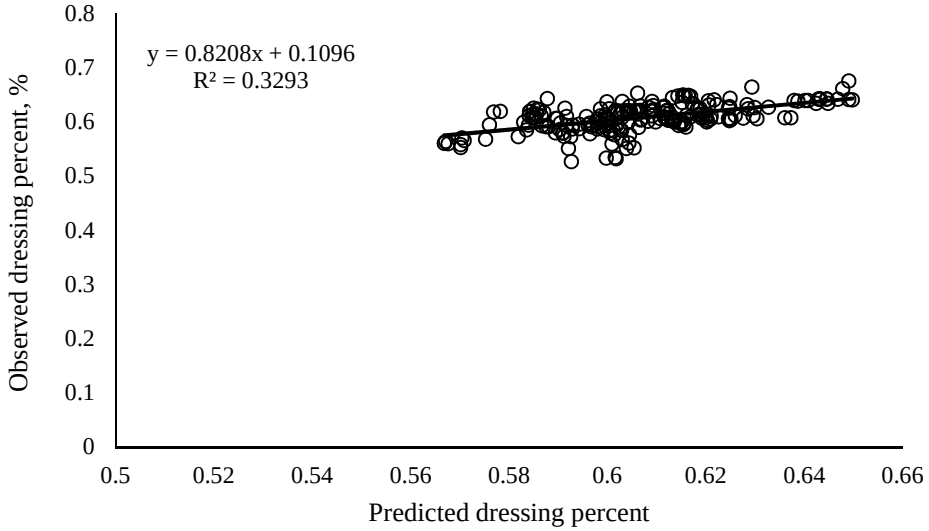

Figure A4.4 Observed LMA, sq cm, regressed on predicted LMA, sq cm, with solid line representing perfect fit between predicted and observed variables from chapter IV.

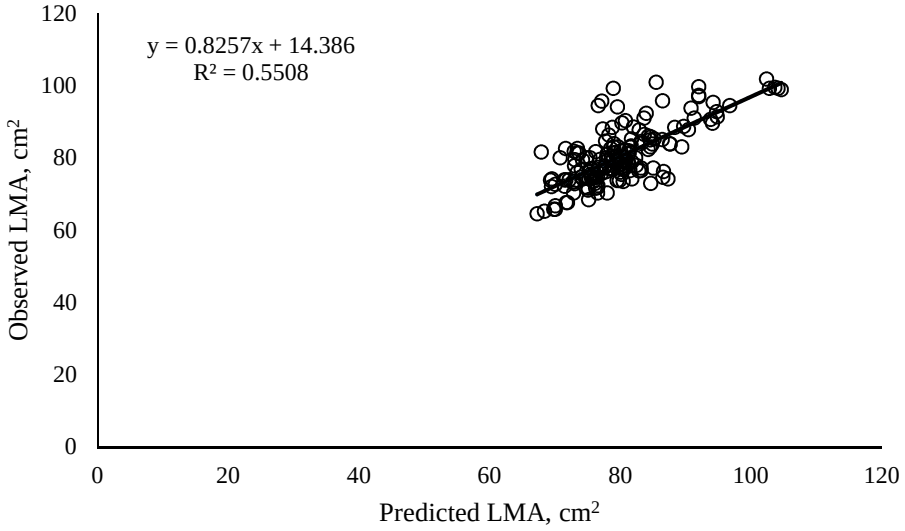

Figure A4.5 Observed 12th rib fat thickness, cm, regressed on predicted 12th rib fat thickness, cm, with solid line representing perfect fit between predicted and observed variables from chapter IV.

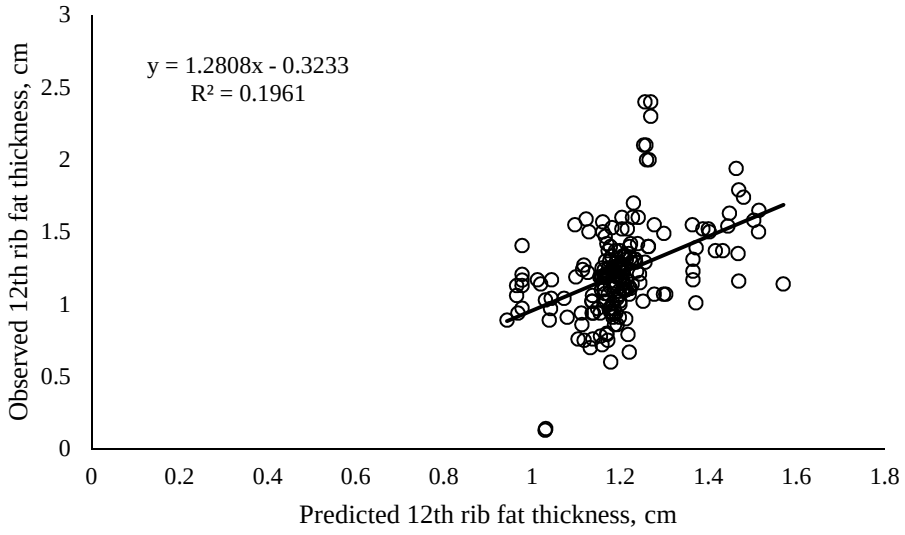

Figure A4.6 Observed marbling score regressed on predicted marbling score with solid line representing perfect fit between predicted and observed variables from chapter IV (marbling score: 400 – slight, 500 – small, 600 – modest).

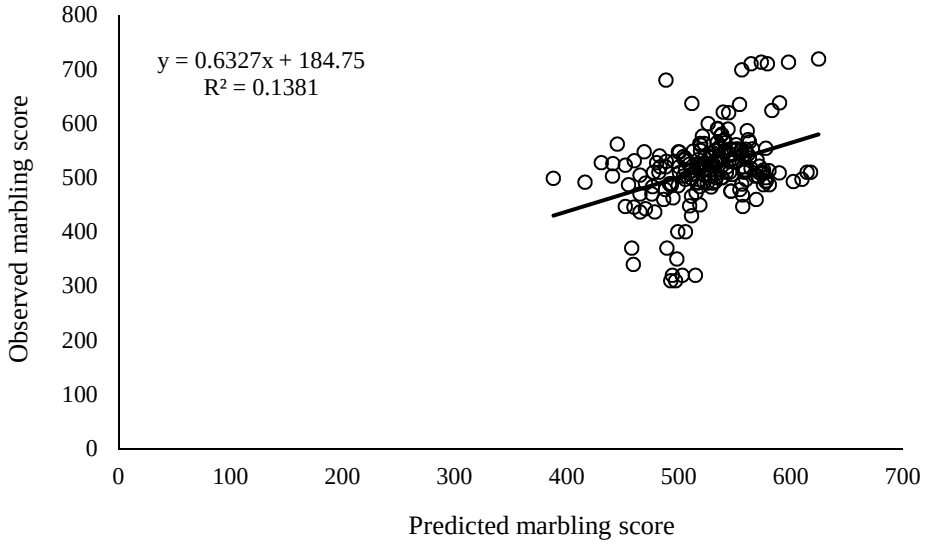

Figure A4.7 Observed Final BW, kg, regressed on predicted Final BW, kg with solid line representing perfect fit between predicted and observed variables from chapter IV.

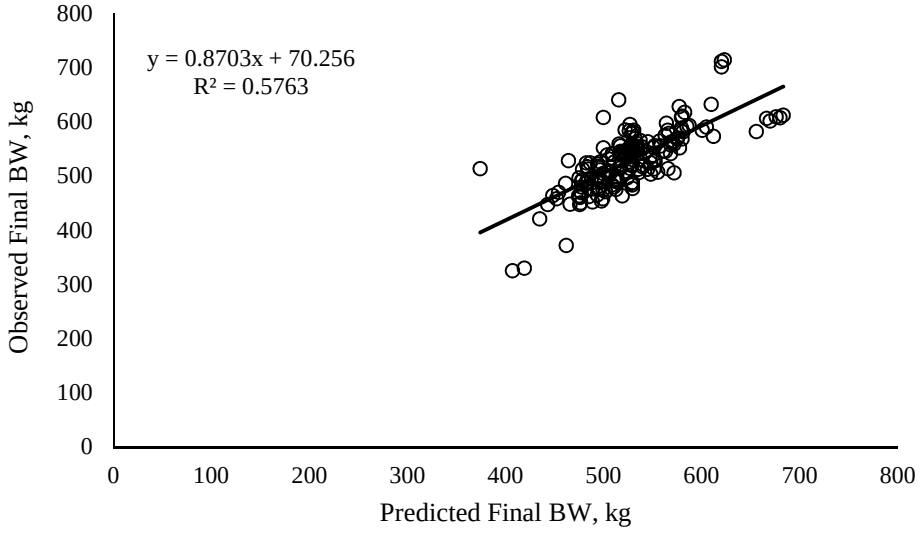

Figure A4.8 Observed HCW, kg, regressed on predicted HCW, kg, with solid line representing perfect fit between predicted and observed variables from chapter IV.