INFLUENCE OF PLASMA NUCLEAR MAGNETIC RESONANCE PROFILE, UREA-

NITROGEN, GLUCOSE AND NON-ESTERIFIED FATTY ACIDS ON DRY MATTER

INTAKE IN DEVELOPING HEIFERS AND MATURE COWS

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Samuel Oluwale Olorunkoya

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The Supervisory Committee certifies that this *disquisition* complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Lauren Hanna

Chair

Dr. Kendall Swanson

Dr. Ronald Deggs

Dr. Zachary Carlson

Approved:

July 15, 2022	Dr. Guillermo Scaglia
Date	Department Chair

ABSTRACT

The objective of this experiment was to determine if nuclear magnetic resonance (NMR) profiles or blood metabolites could explain dry matter intake (DMI) in developing heifers and mature cows in the weeks leading to breeding. A total of 335 heifers and 60 cows were fed a forage-based diet. A general linear model (GLM) was fit for DMI using fixed effects; breed, frame size and birth year. Non-esterified fatty acids (NEFA) had the greatest association with DMI (R^2 ranged 54.4% to 58.1% and 64.4% to 70.6%) in heifers and cows, respectively. The NMR profiles accounted for the smallest variation (51.9% and 55.6%) for rumen metabolism and (52.0% and 55.8%) for cellular metabolism in heifers and cows, respectively. Additional exploration to profile NMR data is needed. The models containing NEFA accounted for high levels of variation, fit plots indicated these predictions could be used to manage animals in distinct groups for DMI.

ACKNOWLEDGMENTS

DEDICATION

To my parents, who were more than generous with their prayers and advice. Seeing the potential

in me even when I did not.

A special thanks to my Pastor; Sunday Dare Iyunade for his continuous encouragement, advice,

and help.

To my siblings; John and Victor, for their support and believing in me.

Gbayi (KJBL)

To Adebimpe Omotayo, you are a gem, thank you for always being there.

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LIST OF ABBREVIATIONS

3-HDB..3-Hydroxybutyrate

3-HDI..3-Hydroxyisobutyrate

3-HDV...3-Hydroxyisovalerate

ACE..Acetate

ACT..Acetone

AD..American Aberdeen

ADF ....Acid detergent fiber

ADG ....Average daily gain

ALA ....Alanine

ALL...Allantoin

AN...Angus

ANR ....Angus and Red Angus

AR..Red Angus

ASP ....Aspartate

BET..Betaine

BUT..Butyrate

BW...Body weight

CAR..Carnitine

CHL..Choline

CHO..Cholate

CIT..Citrate

CM...Cellular metabolism

CRE...Creatine

CRT...Creatinine

1. INTRODUCTION

In contrast to the monogastric stomach, the ruminant stomach is capable of converting inedible feed like grasses and forages into edible food such as meat and milk; this shows that ruminants possess the ability to use these types of feed efficiently (input to desired output) given their digestive system. Notwithstanding, this feat is accompanied by a low conversion efficiency of feed to gain, particularly in cattle (Eisler et al., 2014). Increased cattle prices have spurred the maximization of production outputs such as gain and weaning weight (Schulz, 2015). Efficiency in beef cattle is determined by feed and other input factors of all physiological stages in animal production as well as the desired output of the production system (weaned calves, slaughter animals, etc.). An animal's physiological state, maintenance requirement, body composition, sizes of visceral organs, digestion efficiency and level of physical activity are possible sources that can be explored to improve feed efficiency (Archer et al., 1999). Even so, improving feed efficiency is challenging because it is both a complex and polygenic trait. This might be achieved by calculating feed intake in growing animals coupled with genetic correlations that may exist between efficiency of growing animals and mature animals (Archer et al., 1999). Research has focused mostly on identifying superior cattle during the finishing phase when cattle are fed grain-based, high-energy diets before harvest (Shike, 2013). However, physical fill from low-energy, forage-based diets may limit intake (Mertens, 1994) because physiological energy demand and chemical factors regulates intake. Feed provision constitutes the largest direct expenditure incurred by beef producers and is a major economic factor determining profitability of beef production (Finneran et al., 2010), where the majority of total energy expenditure for beef production goes towards cow herd maintenance alone (Johnson, 1984; Ferrell and Jenkins, 1985).

The future of the beef industry is dependent on our ability to keep producing high-quality beef for the global market through the use of new technologies, genetics and economic management strategies (Corah, 2008). Some studies on blood metabolites considered the association between plasma urea-Nitrogen (PUN; Kelly et al., 2010), glucose (GLC; Kelly et al., 2010) and non-esterified fatty acids (NEFA; Wood et al., 2014) with feed efficiency. Such studies were conducted in growing heifers and cows using low-energy diets. It is known that some blood metabolites are associated with feed efficiency (Kelly et al., 2010), which might be able to predict dry mater intake. Even so, little research has been conducted on cattle fed low-energy diets and how blood metabolites relate to feed efficiency measures at different physiological stages of production in developing heifers and mature cows, including how it may be used to predict breeding season dry matter intake needs. Targeting phenotypes that improve efficiencies in female animals, for example fertility, pregnancy rate, gestation length, feed conversion ratio, average daily gain and weaning weight (Crowley et al., 2011) will ultimately improve beef and livestock agriculture on a global scale (Clemmons et al, 2019). It is important to collect dry matter intake (DMI) because it provides information about the amount of digestible nutrients, microbial yield, as well as nutrient and energy supply (Khampa and Wanapat, 2006). Therefore, the purpose of this study was to consider blood metabolites as potential predictors for explaining dry matter intake levels in developing heifers and mature cows leading up to the breeding season.

2. LITERATURE REVIEW

2.1. Overview of feed efficiency in beef cattle

Feed efficiency can be defined as the relationship between an animal's dry matter intake (DMI) and productive output. Adopting only one measure of feed efficiency in the beef industry as a universal selection criterion is inadequate because feed intake is dependent upon the complexity of traits like growth rate, reproduction, basal metabolic rate, activity, body composition, health, and climate (Arthur et al., 2004). While feed efficiency has been studied for many years and feedlot profitability is directly impacted by feed efficiency, the beef industry is lagging behind when compared to the swine, fish and poultry industries. Feedlot cattle typically have a feed conversion ratio (FCR), which is the mass of feed per mass of meat, carcass, and fat deposition (Cusack et al., 2021), of about 6 to 1. Even so, beef cattle are not as efficient as monogastric animals in FCR due to the higher fiber diets fed to ruminants that lose energy partly as carbon dioxide and methane through rumen fermentation (Shike, 2013), among other reasons. Snelling et al. (2011) stated that feed efficiency may become increasingly important due to environmental concerns and competition from alternative uses of traditional livestock feedstuffs (e.g., corn and soybean biofuels). In beef production systems, the cow-calf herd accounts for an average of 75% of overall feed costs (Montaño-Bermudez and Nielsen,1990). Feed efficiency becomes very important because of the increased cost incurred in feeding and maintaining animals (Nkrumah et al., 2006). Therefore, improvements in feed efficiency of beef cattle may potentially increase producers' profitability and also lower the environmental impact of beef production (Kenny et al., 2018).

Generally, cattle tend to have a high maintenance requirement because of their large frame (Ferrell et al., 1986) and the lag in improvement in growth rate is partially because we have not

genetically selected for feed efficiency. Before now, more efficient cattle were identified when fed individually, however this process is expensive, labor-intensive and does not allow for social interaction compared to when fed as a group (Shike, 2013). There are several factors that can be responsible for impacting feed efficiency, including, age, sex, type of diet, breed, production level, environmental temperature, the use of growth enhancers, physical activity, and many other management variables (National Academies of Science, Engineering, and Medicine [NASEM], 2016). Although dietary modification and genetic selection have resulted in superior beef cattle populations (Arthur and Herd, 2005), the feed efficiency status of beef cattle can be improved by other factors including diets and feed additives (Song and Choi, 2001), environmental conditions (Grandin, 2016), and management practices.

2.2. Feed types and feed efficiency

Some cattle can effectively convert feed to products more easily than others, thus selection of those more efficient cattle will improve the efficiency and likely the profitability of beef production (Basarab et al., 2013; Berry and Crowley, 2013). Around the world, beef cattle breeding values for feed intake and feed efficient care mostly derived from cattle fed high concentrate diets more than those fed forages because of faster growth (Kenny et al., 2018). Even so, only 7% to 13% of the world's beef production is raised on grain-feeding systems (Mottet et al., 2017). Grass often is the cheapest source of feed available to ruminants when compared to concentrates and grass silage (Finneran et al., 2011). Feed consumption for ruminant animals is more complex, especially under grazing conditions, as ruminants consume pasture with varying supply and nutritive value to meet requirements for growth and maintenance (Gregorini et al., 2008). Maximizing profit in beef production in the near future will generally depend on selecting and breeding cattle that convert forage-based feed efficiently, especially across different diets. Thus,

there is a need to explore feed efficiency in beef cattle further, especially for the majority of beef cattle whose dietary intake is mostly from forage (O'Donovan et al., 2011).

2.3. Types of feed efficiency measures in beef cattle based on animal stage

Feed efficiency is not a new concept as researchers have been studying this for over 40 years and different methods in agriculture have shed more light on feed efficiency research (Shike, 2013). Further understanding of feed efficiency might show correlated responses to selection, identification of less expensive traits to measure feed efficiency, and a non-genetic method, which might be used to understand metabolism in beef cattle (Montanholi, 2007; Swanson and Miller, 2008). Altogether, there isn't any feed efficiency measure that is all-encompassing to all cattle industry sectors.

2.3.1. Measures of growing animal efficiency

2.3.1.1. Gross efficiency

Gross efficiency, commonly known as gain to feed (G:F) ratio, is a generally accepted measure of feed efficiency defined as a ratio of outputs to inputs. Precisely put, G:F is an individual animal's body weight (BW) gains relative to feed consumption over a specific period of growth (Swanson and Miller, 2008).Gross efficiency has been widely used in the beef industry and is a great tool to monitor feedlot cattle performance (Schenkel et al., 2004). Although selection for improved gross efficiency may improve animal performance during the growing and finishing phases of beef cattle production, it may not necessarily improve the profitability of the entire production system (Arthur et al., 2001a). Care must be taken during selection because retaining heifers with improved gross efficiency in the herd could yield increased average cow BW, cow size, feed cost, and greater maintenance requirements (Swanson and Miller, 2008).

2.3.1.2. Partial efficiency of growth

Partial efficiency of growth (PEG) is defined as the ratio of weight gain to feed intake, after taking into account the energy for maintenance (Berry and Crowley, 2013). Maintenance requirements are obtained using the National Research Council (NRC) prediction equations for a group of cattle. This can be challenging to achieve, so it is advisable to singly determine individual animal maintenance requirements even though this procedure may not be economically achievable (Retallick, 2012). In a study by Nkrumah et al. (2004) on growing cattle, it was discovered that PEG is correlated to the relative growth rate (r = 0.36). Predicting individual maintenance requirements and effectively utilizing PEG as a selection criterion may positively impact overall beef production efficiency.

2.3.1.3. Residual average daily gain

Residual average daily gain (RG) represents the difference between actual gain (i.e., average daily gain (ADG)) and the predicted gain, which is performed through regression analysis among the test population, accounting for the animals' body weight, feed intake (Koch et al., 1963) and body composition (Freetly, 2014). Animals are considered more efficient when they exceed the predicted body weight gain (Crowley et al., 2010), therefore a greater RG value connotes a more efficient animal. A feed test can serve as a metric to determine individual RG values, as RG can represent the residuals of a multiple regression model regressing ADG on DMI and metabolic BW (North et al., 2010).

On average, an animal has a RG value of zero and those that have increased daily gains per unit of feed consumption are considered more efficient animals (Iowa Beef Center, 2010). Given that RG is moderately heritable, it can serve as an effective selection tool for feedlot producers, especially considering its strong correlations with accelerated growth rates and FCR (Berry and

Crowley, 2012; Retallick, 2012). Even so, RG may not be the most efficient feed efficiency tool because its calculations omit animals' growing periods (North et al., 2010).

2.3.1.4. Residual feed intake

Residual feed intake (RFI) is a measure of feed efficiency that has gained more interest in recent years. The idea of RFI in cattle was proposed many years ago by Koch et al. (1963), suggesting that one of the factors affecting feed requirements were weight maintained and weight gained. Residual feed intake is the difference between actual feed intake and predicted feed intake over a specific time period. Cattle having a negative RFI are considered more efficient given that they produce more on a given consumption level compared to other cattle. Selection for RFI has been studied in both growing and mature animals. In a study by Basarab et al. (2003) on growing crossbred steers, it was discovered that efficient cattle had a 6.4 and 10.4% reduction infeed intake when compared to medium and high-RFI cattle, respectively. This difference can easily be used to manage groups of cattle better to improve use of feedstuff, thereby cutting costs for the producer. Advantages of RFI are that it adjusts feed intake based on production traits used for its calculation, is relatively repeatable, and is moderately heritable (Swanson and Miller, 2008). These characteristics altogether show that RFI is an efficiency trait that should be considered in selection programs (Crowley et al., 2011).

2.3.2. Measures of mature cow efficiency

2.3.2.1. Cow-calf efficiency

Defining feed efficiency in the mature cow is quite different and more challenging than in growing animals. Different production systems and environments make it challenging to generate a one method fits all feed efficiency plan within the cow-calf sector. Dinkel and Brown (1978) defined cow efficiency as a ratio of calf weaning BW pertinent to both calf and cow total digestible

nutrient (TDN) intake. This is a better measure of production system efficiency than other measures of biological efficiency and is a very instrumental indicator to verify the phenotypic variation in the beef herd (Archer et al., 1999). According to Jenkins and Ferrell (1994), cow-calf efficiency involves measuring the total feed intake of both the dam and the progeny, over an entire production cycle starting from weaning of one calf to the weaning of another calf. Since cow efficiency can affect long-term profitability, an efficient cow will be one that weans off a heavy calf yearly, returns to estrus appropriately before the breeding season, and has moderate intake. Unfortunately, there is no universally accepted measurement of cow efficiency. This is critical because about half of all feed resources are required to maintain the cowherd (Ferrell and Jenkins, 1985) and the majority of a cow's feed consumption occurs between conception and weaning. Considering most producers wean their calves all at once in a bid to achieve heat synchronization in cows, calves born earlier in the calving season are able to nurse their dams for a longer period of time pre-weaning, typically resulting in a heavier weaned calf. The downside of this measure is that the intake of the progeny isn't measured until weaning. This leaves an information gap regarding the performance from birth until weaning. Also, the cost and labor to evaluate these parameters for an extended time period can be a major limiting factor.

2.4. Sources of variation in feed efficiency

Many factors constitute variations in feed efficiency. The type and the amount of feed consumed by an animal, the sex, breed of animal and the environmental condition in which an animal is kept are capable of causing variations on how nutrients are used for maintenance and growth (Corbett et al., 1990). There are at least five major processes by which variation in efficiency is influenced by feed intake, feed digestion, body composition and metabolism, activity, and thermoregulation.

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2.4.1. Feed intake

Variation in feed intake is related to variation in maintenance requirements of ruminants because as feed intake increases, the quantity of energy released to digest the feed increases, partially due to a change in the size of the digestive organs. The amount of energy used by the tissues increases per unit weight of the animal. Heat increment of feeding (HIF) is described as all the heat production caused by the act of eating, chewing, regurgitating, digesting and absorbing the nutrients from the gut. This HIF has been known for a considerable time, whereas in ruminants, it is ~9% of metabolizable energy intake (Corbett et al., 1990). Weeks and Webster (1975) measured the amount of energy used within the gut of sheep as a consequence of eating. It was estimated that energy could account for ~40% of the entire HIF. Weeks and Webster (1975) hypothesized the rest was due to increased metabolism in peripheral tissues. Weather variables can also affect DMI in beef cattle (Yusuf et al., 2020). As long as selection for RFI is related to variation in intake, animals that eat less with similar performance can be expected to have less energy expended as HIF (Nkrumah et al., 2006).

2.4.2. Feed digestion

It has been established that an increase in the level of feed intake relative to maintenance usually results in a decrease in feed digestion (Corbett et al., 1990). Richardson et al. (1996) examined beef cattle for net feed conversion efficiency (FCE) for possible physiological indicators of net FCE. It was found that young heifers and bulls ranked low or high for RFI differed in their ability to digest dry matter by a small margin and a dry matter digestibility of 68%. This difference in dry matter digestibility accounted for 14% of the difference in intake between the 2 groups of cattle studied, regardless of sex. In dairy cows, selection for high milk yield in animals is accompanied by improvements in digestion and absorption of dietary energy and protein (Adams

and Belyea, 1987). Kahn et al. (2000) suggests that differences in the substrate availability and processes of draining blood from the gastro-intestinal tract (GIT) to liver; (creating a circulation of nutrient-rich blood between the GIT and the liver) do occur. Also, increased microbial protein presence in the rumen derived from the proteolysis of dietary protein increased digestion and wool growth in sheep. This provides a feasible mechanism to explain variation in the efficiency of feed utilization.

2.4.3. Body composition and metabolism

There is more variation associated with the efficiency of depositing lean gain than fat gain, and their energy costs also vary. Efficiencies of nutrient used for lean gain are about 40 to 50%, showing more variation in efficiency of lean (protein) gain, and more turnover in organs than in fat gain. Efficiency of fat deposition is dependent on dietary type. Dulloo et al. (1995) stated that the differences in the efficiency of fat deposition are obvious during a rapid phase of tissue deposition. Fat gain will be highly dependent on the genetic background of the animal. This should be considered when creating diet-based strategies for fatty acid composition of beef cattle tissues (Da Costa et al., 2013). Protein synthesis is more efficient than fat synthesis as indicated by predictions of energy retained to energy expended ratio of 0.88 for protein and 0.81 for fat (McDonald et al., 1988). Once produced, protein is continually degraded and reproduced which results in a reduced efficiency of maintaining protein than fat with 0.4 for protein versus 0.70 to 0.75 for fat (McDonald et al., 1988). Protein turnover in living animals is not a cost-effective process and variation in protein metabolism has been associated with genetic selection for growth and other traits in domestic animals (Oddy, 1999). Further evidence proving an association between protein turnover and RFI was reported by Tatham et al. (2000). They discovered a positive relationship between RFI and plasma creatinine to urea ratio, which translates to a higher turnover

of creatine phosphate in the muscle of high-RFI (low-efficiency) bulls. Changes in the conversion of feed to gain and rate of protein degradation, in response to selection for growth and leanness, have been noted in many species such as chickens (Tomas et al., 1991) and rainbow trout (McCarthy et al., 1994). Oddy et al. (1993) found that the sensitivity of muscle to insulin varied among sheep selected for high and low growth rates; also, the sensitivity of insulin towards feed intake varied.

There are notable signs of variation in metabolism regulation at the endocrine, autocrine and paracrine levels, which could contribute to variation in efficiency of nutrient utilization (Hill and Herd, 2003). Overall, the evidence supports different possible mechanisms of variation in metabolism but suggests that these mechanisms are principally regulated at the tissue level (Müller et al.,2009). Clearly, there is a large scope for sources of variation in efficiency to arise at the tissue level. Observing the variation in intake relative to animal size and growth performance will be difficult because without specific selection of target attributes, the emergence of a desired feed efficient type of cattle seems unlikely. This will make it difficult to identify major genes that account for substantial variation in the efficiency of nutrient use (Herd et al., 2004).

2.4.4. Activity

Animal activity can greatly contribute to the variation in heat production as a result of varying energy expenditure. Luiting et al. (1991) concluded that the difference in physical activity accounts for about 79% of the genetic difference in RFI between lines of chickens divergent in RFI. For example, in mice selected for and against RFI after weaning (Hughes et al., 1997), more efficient mice were not as active as less efficient mice. Changes in activity have been associated with variation in RFI in cattle. Richardson and Herd (2004) reported a phenotypic correlation of 0.32 for RFI with daily distance covered, which equates to about 10% of the observed variation in

RFI. Techniques associated with variation in activity include work involved in eating, ruminating and movements from place to place at various speeds. Estimates of the energy cost of these activities exist (Corbett et al., 1990). Considering the high-efficiency and low-efficiency selection line of heifers and bulls tested by Arthur et al. (2001b), the possible variation from eating and walking activity to the difference in RFI can be calculated. Measured by a pedometer, Richardson and Herd (2004) reported that heifers and bulls took about 4,000 steps per day with high-RFI bulls walking 6% more than low-RFI bulls. These cattle were observed for about two days in their feeding stall and both high- and low-RFI animals were assumed to spend similar time ruminating. Since both bulls and heifers were grouped based on high- and low-RFI, the high-RFI animals were assumed to spend 13% longer in the feeding stall and ruminating due to their greater daily intake. The increase in distance walked and time spent standing and ruminating accounted for more than5% of the increased feed energy intake by the high-RFI low-efficiency cattle, compared to the low-RFI (high-efficiency) cattle.

2.4.5. Thermoregulation

The primary means of energy loss in ruminants is evaporative heat loss, which normally occurs through heat exchange in the lungs and nasal conchae (Blaxter, 1962). Primarily, energy loss is controlled by the rate of respiration, although we are not aware of any study affirming the relationship between respiration rate and RFI. Postural position changes and other adaptation measures such as seeking shelter and huddling do not individually constitute a large proportion of variation in heat loss, except in extreme situations. Luiting et al. (1994) has shown that chickens with lower RFI have a smaller body area, having a fuller and better feather coverage, are less active, and conserve energy better. As suggested earlier, each of these factors impact thermoregulation because they contribute to the variation in RFI of chickens (Luiting et al., 1994).

The large variation in body size between poultry and cattle suggests that the contribution of thermoregulation to variation in energy expenditure could differ remarkably (Luiting et al., 1991).

2.5. Metabolic markers of efficiency

Recently, the spotlight has been on biological markers of efficiency as an alternative due to measuring feed efficiency. It has become important to explore potential biological markers of efficiency in animals fed a forage diet to seek a better option for measuring feed efficiency (Meale et al., 2017).There are many factors responsible for controlling metabolic processes that influence the efficiency of feed utilization, resulting in numerous options for potential biological markers. For example, blood metabolites and hormones (Wood et al., 2014), tissue morphology (Montanholi et al., 2013), tissue functional proteins (Mader et al., 2009), enzymes and metabolites, as well as inhibitors of tissue catabolism associated with energetic body demands could all be a pointer to the dissimilarity in the efficiency of feed utilization (Blaxter, 1962). Often times, GLC, PUN and NEFA are indicators of animal's carbohydrate, protein and lipid metabolism, respectively, showing variations in feed efficiency since these metabolites' concentration can be dependent on levels of DMI (Yambayamba et al., 1996).

2.5.1. Plasma urea-nitrogen

Urea-Nitrogen (Urea-N) from Nitrogen (N) metabolism in ruminant animals can be associated with some variations in feed efficiency. Since the 1950s, researchers have explored urea as a cheap N source capable of replacing a part of the protein component in ruminant diets (Reid, 1953). Many studies have proven that when urea is fed at proper levels (for example, up to 40 mg dL^{-1}), it has no adverse effect on the DMI (Almora et al., 2012; Sweeny et al., 2014), rumen fermentation (Currier et al., 2004), nutrition digestibility (Benedeti et al., 2014), or growth performance (Spanghero et al., 2017) in ruminants. However, Coleman et al. (2017) stated that

blood urea-N can be affected by time of sampling and age of animals even though their effects are very small.

Animals possess a certain level of protein metabolism, varying from negative to positive protein balances, which is influenced by the availability of urea-N in the diet and efficiency of N utilization in animals. Urea-N kinetics can be considered as a mechanism where liver synthesis is similar to digested N, with one-third lost through urine via the kidney, and the remaining two-third returning to the GIT at some fixed levels of urea-N. Upon reaching the digestive tract, about 10% or more of N is lost via feces with 40% being reabsorbed as ammonia. Half of the ammonia (50%), which is mainly amino acids, is then reconverted to anabolic N, which can be absorbed again and used for productive purposes. Most of the remaining half of the gut entry rate (GER) is reabsorbed as ammonia that is reconverted to urea and can be further re-partitioned between urinary loss and GER. The process then allows the conversion of urea-N into anabolic forms, which stays longer within the body, and provides the animal with increased opportunities to utilize products derived from dietary N (Archibeque et al., 2001; Sarraseca et al., 1998; Lapierre and Lobley, 2001). Both ruminants and non-ruminants have a mechanism that makes it possible for urea produced by the liver to get into the GIT where it is then used for microbial protein production. It is reasonable to think that urea recycled in ruminants is much larger than those in non-ruminants, which stresses the importance of urea recycling in ruminants (Lapierre and Lobley, 2001). Data from a variety of studies has shown that hepatic urea-N synthesis may sometimes equal digestible N intake (33 to 99%) and even exceed digestible N intake (Kiran and Mutsvangwa, 2010). Due to the low quantity requirement of dietary N contents needed in ruminant feed and how cheap these are, feed costs are reduced. Below are three reasons to achieve an efficient urea recycling in ruminants (Huntington and Archibeque, 2000): 1) Maximization of the microbial functioning in the rumen, 2)

Optimization of the amino acid supply to the host ruminant and 3) Minimization of the negative effects of N excretion into the environment. Numerous research studies have investigated the relationship between PUN and feed efficiency, but have reported varying outcomes. Gonano et al. (2014), when studying the circadian profile of various plasma analytes in beef heifers at different physiological stages, discovered that more efficient pregnant heifers had lower PUN levels in late gestation. Kelly et al. (2010) while observing the effect of divergence in RFI on blood metabolic variables, found positive correlations between serum urea-N and DMI in growing heifers. Considering the relationship between RFI measures during mid- to late-gestation in mature beef cows with circulating serum metabolites, Wood et al. (2014) found that urea-N concentration was positively correlated with DMI, ADG and G:F. This result shows that protein metabolism may help regulate feed efficiency in the pregnant beef cow. Therefore, additional research is needed to further investigate the associations between PUN concentrations and feed efficiency in ruminant animals.

2.5.2. Glucose

In livestock, dietary carbohydrates provide more than half of the energy required for maintenance (Hristov and Ro, 2003). A required energy source for animals is glucose. The major sources of carbohydrates in ruminants are fibrous feeds containing cellulose, hemicellulose and grains rich in starch. Most of the dietary carbohydrates are fermented in the rumen but a small fraction of consumed carbohydrates is digested in the small intestine (Huntington, 1997). Gluconeogenesis explains the synthesis of new glucose from non-carbohydrate precursors; it provides glucose when dietary intake is not enough or absent. Gluconeogenesis also regulates acid-base balance, amino acid metabolism, and synthesis of carbohydrate derived structural components (Ha andBhagavan,2011). Gluconeogenesis occurs in the liver and kidneys. The pointers of

gluconeogenesis are lactate, propionate, glycerol and amino acids. The gluconeogenesis pathway consumes ATP, which is derived primarily from the oxidation of fatty acids. The process of gluconeogenesis in ruminant animals is continual; therefore, they have little need to store glycogen in their liver cells (Ha andBhagavan, 2011). This indicates that ruminants rely mostly on gluconeogenesis in the liver and lesser on kidneys for their tissue glucose requirements (Weeks, 1979).

Gluconeogenesis is very important in young and adult ruminant animals because it provides 75% (Donkin et al., 2005) and 90% of the total glucose needs (Young, 1977) in these animals, respectively. Early research on ruminant gluconeogenesis (Armstrong, 1965; Bergman et al., 1974; Leng, 1970) showed that glucose generated from propionate, amino acids, valerate, glycerol and lactate were high in ruminants overall and important in lactating ruminants. The authors also showed that after ruminants consumed feed, the rate of gluconeogenesis increases. Due to dietary carbohydrates being fermented to short chain volatile fatty acid (VFA) production in the rumen, less than 10% of the animal glucose requirement is absorbed from the digestive tract. This was shown in classic glucose kinetic studies by Bergman et al. (1974) and Young et al. (1974). Other products before rumen fermentation such as acetate and 3-hydroxybutyrate, are substantial substrates for oxidation in the heart, kidneys and skeletal muscle (Weeks, 1979). Overall, these factors support that most ruminant tissues are unable to considerably substitute VFA and their derivative ketoacids for glucose as respiratory fuel or lipogenic substrate (Bell and Bauman, 1997).

Although ruminants normally do not take a great deal of glucose from dietary intake, glucose supply is still important for production and maintenance functions, because it serves as a metabolic fuel for tissues such as the brain and liver (Weeks, 1979; Bell, 1981). Studies that attempted to investigate the relationship between RFI and blood metabolites suggested that glucose

metabolism might be associated with high or low RFI in forage and grain diets (Kelly et al., 2010; Richardson and Herd,2004). The study further showed that blood glucose to insulin concentrations, an indicator of glucose metabolism, did not differ between high- or low-RFI animals. These results show that greater insulin concentrations in high-RFI animals may be linked to the greater visceral fat deposition observed because insulin reduces lipolysis and stimulates lipogenesis in adipose tissue (Brown et al., 2004). Glucose concentration in the blood of beef cows fed a mixed ration with crude protein (CP) of 14%were higher than those on balanced mixed ration with CP of 7.9%. This suggests that glucose concentration was greater in cows fed high CP diet. The surplus amino acids from the high CP diet were possibly channeled towards gluconeogenesis. Amino acid catabolism converts their carbon backbone into citric acid cycle or their precursors and they can be subsequently metabolized to CO_2 and H_2O releasing ATP or used to produce glucose (gluconeogenesis). With these findings, it is evident there are still contrasting views on the results.

2.5.3. Non-esterified fatty acids

Research on lipid metabolism in livestock over the years has partly focused on studying the anatomical sites and main hydrogen and carbon sources for lipogenesis in different animal species (Regert, 2010). Using radioactively labeled precursors with in vivo and in vitro assays, it was shown that adipose tissue is the key anatomical site for fatty acid synthesis in non-lactating ruminants (Vernon, 1981). Smith and Crouse (1984) refuted the ideology of Ballard and Hanson (1967) studies which established that glucose and acetate were the primary sources of carbon for fatty acid synthesis in ruminant adipose tissue. Smith and Crouse (1984) showed that substrate specificity can change depending on storage site, as seen in intramuscular adipose tissue of beef cattle, where glucose was preferentially used instead of acetate as the primary substrate for fatty acid synthesis.

Lipids from dietary supply such as triglycerides are converted into non-esterified fatty acids (NEFA) and long-chain fatty acids (LCFA) in the rumen, and are primarily absorbed in the small intestine. The major metabolic pathway in the small intestine will repackage LCFA into triglycerides, in order to supply energy to accessary tissues, which consist of adipose tissue, heart and skeletal muscle. The lipoprotein enzyme in the accessary tissues converts the triglycerides into NEFA to store energy in the growing ruminant (Drackley, 2005). According to Wathes et al. (2007), the circulating NEFA concentrations present in the plasma of mature cows can be associated with the catabolism of body fat to provide the metabolic needs of the animal rather than dietary supply of lipids. The concentrations of NEFA in the plasma could be of great importance as an indicator of energy delivery to peripheral tissues. A negative correlation was found in growing beef heifers between NEFA concentrations and RFI while investigating the relationship between feed efficiency and blood metabolic variables (Kelly et al., 2010). Also, negative associations have been recorded between NEFA and RFI in mature pregnant cows (Wood et al., 2014). Considering that greater NEFA concentrations may be associated with increased energy delivery to accessary tissues in growing animals, and that fat mobilization by mature cows may play an important role in feed efficiency, the above findings appear to be physiologically relevant.

2.6. Metabolomics

Metabolomics is the study of metabolite profiles for a detailed description of biological mechanisms at the molecular and cellular levels (Sun et al., 2015). Metabolites are molecular compounds in an animal that have undergone enzyme activity that can be measured in an animal's blood, milk, and ruminal fluid (Kühn et al., 2014). Nuclear Magnetic Resonance (NMR) spectroscopy has been used for more than three decades to identify many metabolites because the process is non-biased, preserves samples, and also is easily quantified (Wishart, 2008). The NMR

measures spectral patterns and compares them to databases of pure compounds to identify metabolites, with an internal standard (usually 4,4-dimethyl-4-silapentane-1-sulfonic acid) used to quantify the metabolites (Wishart, 2008). Metabolites can be described as important indicators or pointers to problems in the genome. They are the outcome that emerges from complicated interactions happening inside the genome and the phenotype. This enabled researchers to detect the relationship with genes and the environment; metabolomics has helped researchers obtain a better and more complete description of the human phenotype (Bouatra et al., 2013; Monteiro et al., 2012). Metabolomics have been incorporated into other areas of agriculture like crop evaluation and crop trait selection (Mahdavi et al., 2015; Summer et al., 2015; Simo et al., 2014), but its application to livestock has been sparsely used. The ability of metabolomics to detect difficult to understand phenotypic changes, innate phenotypic impulse and dietary responses without the use of invasive experimental approaches makes it a useful tool for livestock exploration in research and breeding (Duggan et al., 2011; Jones et al., 2012; Gilany et al., 2013). There has been some livestock research involving metabolomics, which has provided convincing results (Goldansaz et al., 2017). This study show how metabolomics and metabolite-based phenotyping can help researchers, farmers, and the livestock industry. Also, there have been researches showing how metabolomics can be useful in predicting feed efficiency and RFI (Karisa et al., 2014), evaluating dietary responses to different feeds (Abarghuei et al., 2014), and other important economic traits in livestock. Conventional phenotypic measures pose some challenges because they require measurement of individual animal feed intake for a minimum of 70 days. This procedure is expensive, time consuming, labor intensive and limited by specific recording equipment (Williams, 2010). When carcass trait evaluations are done, animals are slaughtered in order to get their data. This eliminates the use of such animals for potential breeding. However,

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with metabolomics many of these traits can be measured earlier in the animal's life and it is often more cost-effective than current techniques (Fontanesi et al., 2016; Zhang et al., 2012). Metabolomics approaches are a newer technology and only available in a handful of livestock research facilities, which translates to limited available data for interpreting livestock animals' metabolic data. Goldansaz et al. (2017) applied metabolomics to study changes in rumen metabolites of cattle fed three different diet types. Cattles fed a high concentrate diet showed increases in some amino acids, glucose, valerate and proline. The results provided a new look into the relationship between rumen metabolites, variations in methane production, and diets. Karisa et al. (2014) conducted a study using the NMR technique to study the concentration of metabolites in plasma and discovered metabolites associated with RFI, ADG, and DMI in beef cattle. Metabolites associated with RFI were used to analyze metabolic interactions that suggest biological pathways related to these interactions. Furthermore, Foroutan et al. (2020) used three metabolomics platforms, including NMR, to identify serum biomarkers for RFI that could be translated into an RFI blood test. These results show that serum metabolites could be used to categorically predict RFI early on in the animal's life and inexpensively distinguish high-RFI cattle from low-RFI cattle for management and nutrition purposes (Foroutan et al., 2021).

2.7. Research hypothesis and objectives

The hypothesis is that animal size and blood metabolites related to protein, glucose and lipid metabolism may be associated with various measures of feed efficiency because of the energy and protein metabolism linkage between these factors. This may provide new insights and reveal basic aspects of efficiency of feed utilization that could be later used as indirect assessments of feed efficiency. The objectives of this study were to (i) use blood metabolite models as potential biological markers to identify the best predictor for DMI at different time points leading to

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breeding in beef heifers or mature cows and (ii) use NMR analyte quartile scores to predict DMI

at the week leading to breeding season in beef heifers and mature cows.

3. MATERIALS AND METHODS

3.1. Animals

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of North Dakota State University (NDSU; Protocols A15062, A18065, and A20028). Animals used in this study were from the NDSU Dickinson Research Extension Center (DREC) ranch near Manning, ND. The original base herd at DREC (dams producing females used in this project) consisted of two distinct groups, referred to as the "beef herd" and "range herd". The beef herd was comprised of moderate to large-framed Angus-based cows bred to sires of the same type (rotated based on dam's breed), with breeds influencing current project females consisting of Angus (AN), Red Angus (AR), Gelbvieh (GV), Shorthorn (SH), and Simmental (SM). The range herd was comprised of small to moderate-framed American Aberdeen (AD) F_1 cows, which were produced by breeding AD bulls to AN or AR-based heifers (typically produced from the beef herd). An individual was considered as "MIX" when the difference between the two greatest breed fractions was ≤ 0.05 (Bhowmik et al., 2021)

Heifers used in this study, called Generation 1, are daughters of the base (beef and range) herd produced at DREC. From 2015 to 2018, yearling age Generation 1 heifers (n = 287; excludes freemartins) were transferred to the NDSU Beef Cattle Research Complex (BCRC) in Fargo, ND for a 15-week feeding trial based on year born (2014, 2015, 2016 or 2017). Prior to their arrival at the BCRC, heifers would have been weaned in late November and housed in a dry-lot over winter until they were transferred to BCRC. Prior to their arrival to the BCRC, heifers were given salts and mineral and were on a hay ration of 12% crude protein and were sustained at 1.5 to 2.0 pounds ADG.

For the purpose of this project, only heifers that completed the feed trial were used. This consisted of Generation 1 heifers (n = 254 completed the feed trial) and also daughters of Generation 1 heifers (i.e., Generation 2). Of the Generation 1 heifers that completed the feed trial, only those that successfully weaned their first calf (n = 213) stayed in the DREC breeding herd for a long-term project related to cow longevity. These breeding Generation 1 heifers supplemented sample sizes in 2017 to 2018 by providing 89 Generation 2 heifers with the 107 Generation 1 heifers (including in previous Generation 1 counts). The Generation 2 heifers were specifically from AR and AD bulls based on the long-term breeding plans of Generation 1 females. Only 81 of the Generation 2 heifers finished the feed trial, leaving a total of 335 heifers (Generation 1 and 2) available for this study over the 4-year period.

Prior to the feeding trial, heifers were assigned to a frame score (FS) group based on their respective weaning hip height and age using Beef Improvement Federation equations for heifers (Beef Improvement Federation, 2018). Frame score groups consisted of small (SM; FS ≤ 4.00), moderately small (MS; 4.00 < FS ≤ 5.50), moderately large (ML; 5.50 < FS ≤ 6.50) and large (LG; FS ≥ 6.50). Heifers (Generation 1 and 2) were grouped using these categories into two larger groups based on frame size of small (FS ≤ 5.50) or large (FS > 5.50) for allocation into pens for the feeding trial. A subset of Generation 1 females born in 2014 to 2015 were brought back as 4- and 5-year-olds (n = 60) for a similar 8-week feeding trial in 2020.Prior to coming to the BCRC, mature cows were on calving pastures, fed with available grass and given salts and minerals. They were selected by balancing previously assigned size and type with age as best as possible based on available cows. Cows with current weights over 816 kg were avoided due to limitations in the handling facilities at the BCRC.

3.2. Data collection

The BCRC consists of 6 adjacent pens that are each 15.24 m x 56.39 m. Each pen has a covered feeding area equipped with 8 feeding stations on concrete and access to an uncovered dirt yard. A shared water source (Ritchie Omni 3 18270 Automatic Heated Cattle Waterer, Ritchie Industries, Inc., Iowa, USA) was available between every two pens (n = 3 groups of two pens). The Ritchie Omni 3 has a fast refill valve and can serve up to 100 beef cattle, which is well above the 40 to 44 heifers it served in the two adjoining pens for the 4-year period. Feedstuffs were distributed to animals using the Insentec Roughage Intake Control (RIC) feeding system (Hokofarm Group B. V., Marknesse, The Netherlands) and intake was monitored using RFID tags in each animal's ear.

Based on size group (small vs large), heifers were randomly allocated to one of two pens at arrival to BCRC (n = 4 pens total). Prior to the start of the feeding study, heifers were trained to the BCRC facilities and feeding equipment for approximately two weeks. This occurred around June 1st of each year. At the beginning and end of each feeding experiment, a 2-d body weight (BW) and measurements of heifer size were collected (e.g., linear measurement of body length, hip height, hip width, heart girth, middle girth and flank girth as well as reproductive tract measures). Additionally, BW and blood samples were collected on the first day and every 2 weeks until the breeding season (the Monday closest to August 1st each year). Bulls were brought to the BCRC to train at least 2 weeks prior to breeding season. Each heifer pen had a single bull assigned to it so that one pen from each size group was assigned an AD bull and one pen from each size group was assigned an AR bull. In later years, Generation 2 heifers were always bred to AR bulls and a fifth pen was used to maintain the appropriate number of heifers per pen.

Heifers were not sampled again during the 45-d breeding season until the bulls were removed. This ended the feeding trial with a 2-d collection (weight, blood draw and body parameters) around September 15th of each year. When a subset returned as mature cows, the same collection design was used except the cows started their feeding study around July 1st, resulting in a shorter period of time at the BCRC (n = 8 weeks for cows vs. 15 weeks for heifers), and all cows were bred to Hereford bulls. In all cases, pregnancy status was obtained during the end 2-d collection using transrectal ultrasonography. Females pregnant with fetus at least 30 d of age were transported back to DREC within a few days after the end of the feeding study. The remaining females were placed on pasture for an additional 30 d before pregnancy status was checked again and remaining heifers were transported back to DREC.

At the end of each year, Insentec data was used to calculate the average daily feed intake (DMI; kg/d) over the entire study period. Average daily gain (ADG; kg/d) was computed as the coefficient of linear regression of body weight at time of measurement. The gain to feed ratio (G:F; ratio) was calculated as a ratio of ADG:DMI during the study's duration .For this project, analyses will focus on using DMI, ADG, and G:F ratio as variables of interest from data collected. Specifically, values for each variable available during the month leading up to breeding season (approximately July 1 to August 1 each year) were used to calculate an average value for each animal (heifer or cow). These average values were used in statistical modeling.

3.2.1. Diet and feed analysis

Heifers were fed for *ad libitum* intake on a forage-based diet containing grass hay, corn silage, dry rolled corn, fined ground corn, and dried distiller's grains with soluble for the entire study. Daily, feed intake data was downloaded from the Insentec Roughage Intake Control computer and summarized.

Diet samples were collected weekly following Swanson et al. (2014). Briefly, samples were dried in a 55°C oven and ground to pass through a 1-mm screen. The samples were analyzed for dry matter (DM), ash, nitrogen (N; Kjehldahl method), calcium, and phosphorus by standard procedures (AOAC, 1990) and for neutral detergent fibre (NDF) (assayed with heat stable amylase and sodium sulfite and expressed inclusive of residual ash) and acid detergent fibre (ADF) (expressed inclusive of residual ash) concentration by the method of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology Corp., Fairport, USA). Percent crude protein (CP) was calculated by multiplying N concentration × 6.25. The ingredient and nutrient composition available to heifers over the 4-yr period are present in Table 3.1 and 3.2, respectively. Ingredient and nutrient composition for mature cows are presented in Table 3.3.

Table 3.1. Ingredient and feed composition for growing heifers during study.

	2015[1]		2016- 2018
Item (%DM)	Diet 1	Diet 2	Diet 1
Ingredient			
Grass hay	78.50	88.40	16.50
Corn silage	16.50	0.00	0.00
Corn, dry rolled	0.00	6.60	6.60
Corn fine ground	1.92	1.92	1.92
DDGS[2]	1.93	1.93	1.93
Supplement[3]	2.00	2.00	2.00

[1]Diet had to be reformulated because of lack of silage supply. Diet 1 was fed from arrival to August 2, 2015. Diet 2 was fed August 3, 2015 (start of breeding season) to September 15, 2015.
[2]Dried distiller's grains with solubles.
[3]Supplement contained urea, salt, monensin (176.4 g/kg premix, Elanco, Greenfield, IN), Vitamin premix, and a trace mineral premix.

Table 3.2. Nutrient average composition over weeks of diet in growing heifers.[1]

Item[2](% DM)	2015	2016	2017	2018	Average
OM	90.0	88.5	89.0	90.1	89.4
CP	11.8	7.7	12.4	12.0	11.0
NDF	63.6	64.3	62.2	62.9	63.3
ADF	36.7	38.8	35.7	36.9	37.0
Ca	0.43	0.40	0.59	0.62	0.51
P	0.31	0.28	0.21	0.16	0.24

[1]Nutrient sample compositions were averaged over 15 weeks for each year.
[2]Chemical composition included organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), phosphorus (P).

Table 3.3. Ingredient and nutrient composition of TMR for multiparous cows.

Item	% DM
Ingredient[1]	
Hay	68.5
Corn Silage	15.0
DDGS[2]	11.5
Fine Ground Corn	4.72
Salt	0.20
Vitamin Premix	0.01
Trace Mineral Premix	0.05
Monensin Premix	0.02
Chemical Composition[3]	
OM	71.43
CP	11.29
NDF	63.18
ADF	36.34
Ca	0.56
P	0.29

[1]Diet components as percent of total diet in a dry matter (DM) basis.
[2]Dried distiller's grains with solubles.
[3]OM = Organic Matter; CP =Crude protein; NDF = Neutral detergent fiber; ADF = Acid detergent fiber Ca = Calcium and P = Phosphorus.

3.2.2. Plasma

Blood was collected through the jugular venipuncture while each animal was restrained in a chute. Blood was collected with 1.1 x 25 mm blood collection needles (BD Vacutainer® Precision Glide, BD Inc., Franklin Lakes, USA) and 10 mL sodium heparin blood collection tubes (BDz Vacutainer®, BD Inc., Franklin Lakes, USA). After collection, samples were inverted multiple times to ensure mixture with anticoagulant and then refrigerated at 4°C until centrifugation. A total of 6 samples (heifers) and 3 samples (cows) were collected per animal. The samples were centrifuged at 4 °C at 3000×g for 20 minutes. Afterwards, plasma was transferred into three 2-mL micro centrifuge tubes and stored at -20°C until analysis. Plasma was analyzed for PUN, GLC, and NEFA. Plasma urea-N concentration was measured by the QuantiChrom™ Urea Assay Kit (BioAssay Systems, Hayward, USA) and was quantified using the urease/Berthelot procedure (Chaney and Marbach, 1962; Fawcett and Scott, 1960). Plasma GLC concentration was analyzed using the hexokinase/gluxose-6-phosphate dehydrogenase method (Farrance, 1987) and quantified using a 96-well microplate reader (Synergy, HI Microplate Reader, BioTek Instruments, Winooski, VT). Plasma NEFA concentration was analyzed using the acyl-CoA synthetase-acyl-CoA oxidase method using a kit from Wako Pure Chemical Industries (Dallas, TX).

3.2.3. Nuclear Magnetic Resonance Spectrometry procedure

Plasma samples were shipped to collaborators at Montana State University to be processed and analyzed using NMR. A single time point (week 8) for heifers and cows was used, which included the plasma sample collected the day the breeding season began for both heifers and cows. This timepoint was identified to determine if certain profiles existed that may help predict feed requirements and growth differences leading up to or during the breeding season. For the NMR procedure, the protocol followed Karisa et al. (2014). Briefly, macromolecules were removed from

samples including proteins and lipids. After filtration, samples less than 530 μL were diluted with

30mMKH$_2$PO$_4$ solution for enough volume for NMR to be obtained. When all the solutions needed

for metabolite quantification were added, the solution was vortexed for 30 s then it was transferred

to an NMR tube for acquisition. Furthermore, spectra were acquired on a 600 MHz VNMRS

spectrometer equipped with a 5mm HX probe (Agilent Technologies, CA, USA). Spectra was

processed with the Chenomx NMR Suite 7.5 software (Chenomx, Edmonton, Alberta, Canada)

and metabolites were identified and quantified. Concentration results were adjusted and

metabolites were reported after sample filtration.

3.3. Statistical analyses

3.3.1. Analyzing metabolites

For this experiment, summary statistics for all variables were calculated using the MEANS

procedure in SAS v.9.4 (SAS Institute Inc., Cary, NC, USA). Correlations coefficients were

generated among the dependent and independent variables using the CORR procedure for Pearson

and Spearman Rank correlation coefficients. Dependent variables of DMI, ADG, and G:F were

first assessed for base model effects using the GLM procedures that included fixed effects of

primary breed (n = 5 to 6), frame score group (n = 4), year born (n = 4 for heifers, n = 2 and for

cows) and possible interactions. After the base model was identified, PUN, GLC, and NEFA

values were used independently of each other as fixed covariates to determine predictive ability.

Models were run separately based on week the plasma metabolite was collected (week 0, 2, 4, 6

or 8 for heifers, week 6 or 8 = 0 or 2for cows); week 6 and 8 on heifers matches week 0 and 2 on

cows given these took place the same time of the month and year. The sample at the end of the

feeding trial for all study periods (week 15 for heifers, week 9 for cows) was excluded due to the

45-d time gap of the breeding season. The cow trial was shorter in duration than heifers simply

due to facility availability. Therefore, efforts were made in this study to align cow data with heifer data based on time of year for easier comparisons. For each metabolite and week, the Adjusted R^2 was reviewed to determine the week(s) that provided the best predictive ability per metabolite. Diagnostic plots were also produced to ensure modeling assumptions were being met and visualize the fit of the predictive model.

3.3.2. Metabolomics with NMR

Analytes reported from the NMR procedure were reviewed and sorted into relevant physiological categories. Summary statistics were generated for all the analytes using the MEANS procedure in SAS v.9.4 (SAS Institute Inc., Cary, NC, USA). Given the physiological categories identified (rumen metabolism, amino acids, microbial protein synthesis, vitamins, bile, cellular metabolism, protein metabolism, bacterial metabolism, sugar and urine), up to two categories were selected for further investigation in their relationship with DMI, ADG, and G:F. Linear relationships of analytes within and between the two categories were explored using the CORR procedure in SAS v.9.4 (SAS Institute Inc., Cary, NC, USA). A profile score for each category was developed by first assigning each analyte to one of four quartiles ranging from low (1) to high (4) using percentile breakdowns of each analyte (minimum, 25th percentile, median, 75th percentile, maximum). The average quartile for each individual animal across analytes in each category was then used in a similar fashion to GLC, PUN, and NEFA at a single time point to assess predictive ability of DMI, ADG, and G:F.

4. RESULTS

4.1. Summary statistics of dependent and independent variables

4.1.1. Summary statistics of dependent variables

The summary statistics of heifers for DMI, ADG, and G:F are presented in Table 4.1. All DMI values used are the average of data for the month (27.25 ± 0.96 d) leading up to breeding season (July of each year). The average number of heifers brought to the BCRC per year over the 4 years of this project was 83.75 ± 5.25. In total, there were a smaller number of LG heifers and more MS heifers. There were less GV and more Angus and Red Angus (ANR) in our heifer population. The number of animals used for the feeding trial was totaled at 335 after removing data based on plasma hemolysis, exposure of samples to extreme temperatures or inaccurate volume because these affects the integrity of the samples. Zero values on ADG was as a result of animal that do not necessarily increase in weight between the interval ADG was calculated. We observed lesser numbers (252) for calculated ADG and G:F because of few missing weight measurement. Given ADG is calculated off of the difference between weight gain between 2 weeks and divided by the number of days (14) between the period of gain, one missing weight measurement will affect both the ADG of that same week and the next. This also affect the outcome of G:F in turn.

The summary statistics of cows for DMI, ADG, and G:F are presented in Table 4.2. In total, there were more ML cows than other FS groups, but the groups were fairly similar in size. The balance of size and breeds was intentional in cows since space was limited at the BCRC and only 60 cow-calf pairs could be housed in a single year. Similar to heifers, there were more ANR in the population for cows than other breeds.

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Table 4.1. Summary statistics (number, mean and standard deviation, SD, minimum and maximum) of dry matter intake (DMI), average daily gain (ADG) and gain to feed ratio (G:F) overall and by year, size and breed of heifers.

Traits	Attributes[1]	N	Mean	SD	Minimum	Maximum
DMI; kg/day		335	8.09	1.29	3.99	12.30
Year	2014	89	8.58	1.28	5.47	12.30
	2015	73	8.14	1.10	5.60	10.43
	2016	99	7.62	1.41	3.99	11.85
	2017	74	8.07	1.10	5.68	10.46
Size	SM	63	6.77	10.98	4.28	8.67
	MS	151	7.91	1.04	3.99	10.99
	ML	96	8.74	0.84	6.10	10.40
	LG	25	10.01	0.95	8.53	12.30
Breed	AD	48	6.70	1.08	3.99	8.82
	ANR	171	8.13	1.13	4.57	12.30
	GV	3	8.30	0.65	7.86	9.04
	MIX	39	8.14	1.34	5.30	10.46
	SH	35	8.62	0.91	6.49	10.86
	SM	39	9.09	1.09	6.10	11.85
ADG; kg/day		252	0.80	0.71	-1.24	3.69
Year	2014	88	1.23	0.79	-0.71	3.69
	2015	73	0.51	0.32	-0.90	1.21
	2016	55	0.84	0.56	-0.80	2.40
	2017	36	0.30	0.67	-1.24	1.36
Size	SM	48	0.48	0.70	-1.24	1.90
	MS	107	0.72	0.65	-1.21	2.62
	ML	75	1.02	0.74	-0.80	3.69
	LG	22	1.16	0.55	0.16	2.26
Breed	AD	26	0.10	0.71	-1.24	1.33
	ANR	127	0.83	0.72	-0.71	3.69
	GV	2	0.77	0.25	0.60	0.95
	MIX	27	0.98	0.53	0.26	1.90
	SH	31	0.95	0.57	0.11	2.26
	SM	39	0.97	0.64	-0.80	2.26
G:F		252	0.10	0.09	-0.21	0.42
Year	2014	88	0.15	0.10	-0.08	0.42
	2015	73	0.06	0.04	-0.16	0.15
	2016	55	0.01	0.07	-0.13	0.33
	2017	36	0.03	0.09	-0.21	0.17
Size	SM	48	0.07	0.10	-0.19	0.29
	MS	107	0.09	0.08	-0.21	0.42
	ML	75	0.12	0.09	-0.13	0.42
	LG	22	0.11	0.05	0.02	0.21
Breed	AD	26	0.01	0.11	-0.21	0.17
	ANR	127	0.10	0.09	-0.08	0.42
	GV	2	0.10	0.03	0.08	0.12
	MIX	27	0.12	0.07	0.04	0.29
	SH	31	0.11	0.06	0.01	0.25
	SM	39	0.10	0.07	-0.13	0.25

[1]Breed: AD= American Aberdeen, ANR = Angus and Red Angus, GV = Gelbvieh, MIX = Mixed breed, SH = Shorthorn, SM = Simmental. Size: SM = Small, MS = Moderately small; ML: Moderately large, LG = Large. An individual was considered as "MIX" when the difference between the two greatest breed fractions was ≤ 0.05.

Table 4.2. Summary statistics (number, mean and standard deviation, SD, minimum and maximum) of dry matter intake (DMI), average daily gain (ADG) and gain to feed ratio (G:F) overall and by year, size and breed in mature cows.

Traits	Attributes[1]	N	Mean	SD	Minimum	Maximum
DMI; kg/day		60	22.77	3.35	14.40	31.67
Year	2014	31	22.95	3.82	15.83	31.67
	2015	29	22.58	2.82	14.40	27.60
Size	SM	16	19.48	2.40	14.40	22.63
	MS	15	21.80	2.06	16.83	25.35
	ML	19	24.17	1.89	20.03	27.81
	LG	10	26.84	2.65	22.46	31.67
Breed	AD	4	18.10	2.71	14.40	20.90
	ANR	28	22.49	3.05	18.59	31.67
	GV	-	-	-	-	-
	MIX	13	22.53	3.73	15.83	27.81
	SH	10	24.16	2.47	20.03	27.60
	SM	5	25.98	1.37	24.85	28.32
ADG; kg/day		60	0.49	0.71	-1.21	2.34
Year	2014	31	0.57	0.63	-0.39	2.27
	2015	29	0.40	0.78	-1.21	2.34
Size	SM	16	0.55	0.82	-0.90	2.34
	MS	15	0.34	0.68	-1.21	1.25
	ML	19	0.55	0.69	-0.66	2.27
	LG	10	0.48	0.64	-0.51	1.48
Breed	AD	4	0.57	1.22	-0.27	2.34
	ANR	28	0.46	0.69	-0.90	2.27
	GV	-	-	-	-	-
	MIX	13	0.75	0.56	-0.04	1.56
	SH	10	0.21	0.76	-1.21	1.21
	SM	5	0.45	0.59	-0.39	1.09
G:F		60	0.02	0.04	-0.10	0.10
Year	2014	31	0.02	0.04	0.00	0.10
	2015	29	0.01	0.04	-0.10	0.10
Size	SM	16	0.03	0.05	0.00	0.10
	MS	15	0.01	0.05	-0.10	0.10
	ML	19	0.02	0.04	0.00	0.10
	LG	10	0.01	0.03	0.00	0.10
Breed	AD	4	0.02	0.05	0.00	0.10
	ANR	28	0.01	0.03	0.00	0.10
	GV	-	-	-	-	-
	MIX	13	0.04	0.05	0.00	0.10
	SH	10	0.00	0.04	-0.10	0.10
	SM	5	0.00	0.00	0.00	0.00

[1]Breed: AD = American Aberdeen, ANR = Angus or Red Angus, GV = Gelbvieh, MIX = Mixed breed, SH = Shorthorn, SM = Simmental. Size: SM = Small, MS = Moderately small, ML: Moderately large, LG = Large. An individual was considered as "MIX" when the difference between the two greatest breed fractions was ≤ 0.05.

4.1.2. Summary statistics of PUN, GLC, and NEFA

The summary statistics for PUN, GLC and NEFA for heifers and cows are presented in Table 4.3. In some cases, heifer plasma samples were not available due to plasma hemolysis, exposure to extreme temperatures or inaccurate volume because these compromised the integrity of the samples, making them unusable for testing. Given these animals were fed a forage-based diet for the feed trial, we noticed an increase in the level of PUN across weeks for both heifers and cows. Glucose and NEFA levels decreased across weeks for heifers and cows. These trends were constant for heifers and cows even though time points for these animals were not the same.

Table 4.3. Summary statistics of plasma urea-nitrogen (PUN), glucose (GLC) and non-esterified fatty acids (NEFA) in heifers and cows fed a forage-based diet.

Variable	Week	N	Mean	SD	Minimum	Maximum
Heifers[1]						
PUN; mg/dL	0	252	9.00	4.99	0.00	22.40
	2	252	8.01	3.09	2.02	16.27
	4	295	9.31	4.26	2.82	22.83
	6	294	9.08	4.94	0.00	24.64
	8	331	10.13	5.20	2.04	26.68
GLC; mg/dL	0	250	4.36	0.84	3.02	14.82
	2	253	4.16	0.62	2.28	6.95
	4	291	4.02	0.59	2.39	6.90
	6	292	3.77	0.69	0.85	5.51
	8	333	3.94	0.53	1.94	5.88
NEFA; mg/dL	0	241	415.04	202.93	84.49	1,009.15
	2	253	452.09	204.14	92.62	1,549.13
	4	295	442.78	199.54	115.64	1,403.68
	6	297	395.65	201.89	90.39	1,175.51
	8	333	327.57	139.23	62.58	904.44
Cows[2]						
PUN; mg/dL	0	60	12.46	2.02	8.64	17.50
	2	60	13.57	2.31	8.10	19.45
GLC; mg/dL	0	60	75.37	10.53	59.66	105.42
	2	60	74.67	9.74	61.11	106.17
NEFA; mg/dL	0	60	869.37	428.66	174.4	1,805.94
	2	60	599.36	274.87	122.33	1298.58

[1]Four-year chemical composition (percentage average) in heifers included organic matter (89.4), crude protein (11.0), neutral detergent fiber (63.3), acid detergent fiber (57.0), calcium (0.51), phosphorus (0.24).
[2]Chemical composition percentage in cows included organic matter (71.4), crude protein (11.3), neutral detergent fiber (63.2), acid detergent fiber (36.3), calcium (0.5), phosphorus (0.2).

4.1.3. Summary statistics of NMR analytes

The summary statistics of NMR analytes by category for heifers and cows are presented in Table 4.4 and Table 4.5, respectively. A total of 280 heifers and 60 cows had spectral data that was analyzed. The reported NMR analytes were divided into 10 categories: rumen metabolism, amino acids, microbial protein synthesis, vitamins, bile, cellular metabolism, protein metabolism, bacterial metabolism, sugar, and urine. A total of 55 analytes were identified in both heifers and cows, respectively. All 55 metabolites were also common to heifers and cows across and within the 10 categories. However, DSS-d6 was used as a chemical shape indicator and not used in subsequent analyses.

Rumen metabolism (RM) and cellular metabolism (CM) had the same number of analytes present in both categories in heifers and cows (n = 15 and 8, respectively). The RM category shows that heifers had greater averages on all analytes except trimethylamine (TRL), which was higher in cows. Also, in the RM category, acetone (ACT), fumarate (FUM) and trimethylamine (TRL) in heifers and 3-hydroxybutyrate (3-HDV), acetone (ACT), butyrate (BUT), D-lactic acid (D-LAC), fumarate (FUM) and isobutyrate (ISB) in cow's had concentrations less than 1. The CM category showed heifers had higher averages for all analytes compared to cows except for L-lactic acid (L-LAC), which was constant between heifers and cows. The CM category showed that malonate (MAL), succinate (SUC) and sn-Glycero-3-phosphocholine (sn-GLP) had concentrations less than 1 for heifers and cows. Imidazole (IMD) in the Urine category did not vary between heifers and cows.

Table 4.4. Summary statistics of Nuclear Magnetic Resonance analytes by category in heifers.[1]

Variable	Mean	SD	Minimum	25th Pct.	Median	75th Pct.	Maximum
			Rumen metabolites				
3-HDB	0.767	0.742	0.017	0.096	0.754	1.133	4.360
3-HDI	0.147	0.168	0.004	0.025	0.143	0.211	2.017
3-HDV	0.042	0.051	0.001	0.008	0.032	0.042	0.324
ACE	3.055	2.952	0.082	0.505	2.759	4.398	16.893
ACT	0.005	0.007	0.000	0.002	0.004	0.006	0.051
BUT	0.066	0.082	0.002	0.022	0.037	0.083	0.583
D-LAC	0.018	0.021	0.001	0.009	0.014	0.020	0.201
FOR	0.292	0.289	0.002	0.053	0.279	0.397	2.011
FUM	0.007	0.007	0.000	0.001	0.005	0.011	0.042
ISB	0.052	0.051	0.002	0.016	0.035	0.073	0.354
MET	0.061	0.057	0.002	0.016	0.054	0.080	0.420
PRO	0.174	0.155	0.004	0.042	0.175	0.250	1.092
PROG	0.025	0.028	0.001	0.006	0.020	0.028	0.194
TRL	0.009	0.006	0.001	0.003	0.008	0.014	0.037
URI	0.043	0.038	0.001	0.008	0.038	0.063	0.202
			Amino acids				
ALA	1.165	1.014	0.030	0.204	1.242	1.721	5.050
ASP	0.062	0.062	0.001	0.017	0.053	0.083	0.457
GLU	0.554	0.502	0.010	0.055	0.614	0.929	2.017
GLY	0.281	0.452	0.008	0.082	0.187	0.302	5.594
ISL	0.616	0.521	0.005	0.100	0.668	0.950	2.543
LEU	0.822	0.668	0.034	0.098	1.044	1.334	3.088
METH	0.078	0.080	0.003	0.020	0.064	0.105	0.702
PHE	0.281	0.233	0.011	0.041	0.310	0.439	0.911
THR	0.638	0.581	0.029	0.119	0.618	0.968	3.849
TYR	0.311	0.263	0.012	0.064	0.314	0.464	1.511
VAL	1.275	1.031	0.058	0.185	1.425	2.145	3.756
			Microbial protein synthesis				
ALL	0.749	0.604	0.030	0.087	0.931	1.260	2.261
INO	0.011	0.013	0.000	0.003	0.007	0.013	0.104
			Vitamins				
BET	0.711	0.731	0.003	0.089	0.587	1.082	4.525
CHL	0.039	0.051	0.007	0.013	0.018	0.038	0.342
			Bile				
CHO	0.014	0.016	0.0002	0.002	0.011	0.021	0.115
			Cellular metabolism				
CIT	0.847	0.831	0.001	0.113	0.795	1.299	4.497
CAR	0.218	0.306	0.005	0.027	0.179	0.248	2.328
L-LAC	1.890	2.158	0.050	0.329	1.548	2.660	21.662
LAC	24.923	25.857	0.509	2.676	19.748	37.835	147.446
MAL	0.080	0.082	0.004	0.023	0.072	0.103	0.724
PYR	0.158	0.159	0.006	0.039	0.137	0.224	1.095
SUC	0.097	0.090	0.006	0.037	0.080	0.129	0.664
sn-GLP	0.146	0.115	0.006	0.047	0.143	0.207	0.721
			Protein metabolism				
CRE	0.905	0.898	0.018	0.104	0.801	1.332	4.946
CRT	0.617	0.559	0.015	0.105	0.634	0.895	3.280
N-ACE	0.174	0.316	0.004	0.039	0.110	0.187	3.677
O-ACE	0.052	0.083	0.001	0.011	0.018	0.073	0.751
SAR	0.023	0.018	0.001	0.008	0.019	0.035	0.103
UREA	26.383	22.096	0.111	2.281	32.921	44.186	74.666

Table 4.4. Summary statistics of Nuclear Magnetic Resonance analytes by category in heifers[1] (continued)

Variable	Mean	SD	Minimum	25th Pct.	Median	75th Pct.	Maximum
τ-MEH	0.192	0.170	0.002	0.026	0.220	0.293	0.008
τ-MEH	0.024	0.023	0.001	0.005	0.020	0.034	0.136
Bacterial metabolism							
DIS	0.098	0.114	0.002	0.018	0.071	0.132	0.763
DIL	0.023	0.023	0.001	0.004	0.019	0.031	0.173
Sugar							
FRU	2.066	1.841	0.042	0.181	2.430	3.198	10.374
GLC	12.216	10.497	0.173	1.436	13.915	19.801	41.201
Urine							
GLYC	1.348	1.405	0.009	0.063	1.172	2.070	7.444
HIP	0.285	0.231	0.017	0.039	0.333	0.486	0.857
IMD	0.744	2.740	0.581	0.581	0.581	0.581	0.581

[1] 3-HDB = 3-Hydroxybutyrate; 3-HDI = 3-Hydroxyisobutyrate; 3-HDV = 3-Hydroxyisovalerate; ACE = Acetate; ACT = Acetone; ALA = Alanine; ALL = Allantoin; ASP = Aspartate; BET = Betaine; BUT = Butyrate; CAR = Carnitine; CHO = Cholate; CHL = Choline, CIT = Citrate; CRE = Creatine; CRT = Creatinine; D-LAC = D-Lactic acid; DIS = Dimethyl sulfone; DIL = Dimethylamine; FOR = Formate; FRU = Fructose; FUM = Fumarate; GLC = Glucose; GLU = Glutamine; GLY = Glycine; GLYC = Glycolate; HIP = Hippurate; IMD = Imidazole; INO = Inosine; ISB = Isobutyrate; ISL = Isoleucine; L-LAC = L-Lactic acid; LAC = Lactate; LEU = Leucine; MAL = Malonate; MET = Methanol; METH = Methionine; N-ACE = N-Acetylglycine; O-ACE = O-Acetylcarnitine; PHE = Phenylalanine; PRO = Propionate; PRO-G = Propylene glycol; PYR = Pyruvate; SAR = Sarcosine; SUC = Succinate; THR = Threonine; TRL = Trimethylamine; TRY = Tyrosine; UREA = Urea; URI = Uridine; VAL = Valine; sn-GLP = sn-Glycero-3-phosphocholine; π-MEH = π-Methylhistidine and τ-MEH = τ-Methylhistidine; Summary statistics included standard deviation (SD) and percentiles (Pct.). NOTE: There were 280 heifer data used.

Table 4.5. Summary statistics of Nuclear Magnetic Resonance analyte by category in mature cows[1]

Variable	Mean	SD	Minimum	25th Pct.	Median	75th Pct.	Maximum
Ruminal Metabolites							
3-HDB	0.679	0.758	0.041	0.074	0.103	1.404	2.401
3-HDI	0.104	0.110	0.008	0.012	0.018	0.229	0.305
3-HDV	0.017	0.018	0.002	0.002	0.003	0.034	0.065
ACE	1.946	2.153	0.143	0.221	0.325	3.864	7.070
ACT	0.002	0.002	0.001	0.001	0.001	0.003	0.009
BUT	0.048	0.053	0.003	0.007	0.009	0.097	0.160
D-LAC	0.007	0.006	0.001	0.003	0.004	0.012	0.030
FOR	0.114	0.128	0.009	0.020	0.023	0.248	0.436
FUM	0.002	0.003	0.000	0.000	0.001	0.005	0.012
ISB	0.024	0.023	0.003	0.006	0.009	0.047	0.071
MET	0.029	0.030	0.003	0.005	0.005	0.059	0.089
PRO	0.095	0.103	0.009	0.013	0.020	0.184	0.331
PROG	0.010	0.011	0.001	0.001	0.001	0.021	0.032
TRL	0.012	0.005	0.001	0.007	0.015	0.016	0.018
URI	0.022	0.025	0.002	0.002	0.003	0.047	0.070
Amino acids							
ALA	0.661	0.725	0.039	0.067	0.084	1.392	2.030
ASP	0.036	0.040	0.002	0.004	0.005	0.074	0.123
GLU	0.515	0.600	0.025	0.038	0.055	1.113	1.812
GLY	0.212	0.199	0.037	0.053	0.068	0.413	0.705
ISL	0.288	0.292	0.035	0.045	0.061	0.601	0.821
LEU	0.212	0.251	0.039	0.054	0.068	0.329	1.207
METH	0.062	0.083	0.007	0.008	0.012	0.086	0.304
PHE	0.179	0.196	0.012	0.018	0.024	0.373	0.548
THR	0.431	0.481	0.021	0.039	0.056	0.915	1.316
TYR	0.223	0.215	0.025	0.044	0.071	0.436	0.678
VAL	0.758	0.821	0.058	0.082	0.102	1.635	2.280
Microbial protein synthesis							
ALL	0.740	0.792	0.060	0.092	0.117	1.543	2.305
INO	0.005	0.006	0.000	0.001	0.001	0.010	0.027
Vitamins							
BET	0.335	0.390	0.011	0.019	0.023	0.760	1.072
CHL	0.008	0.006	0.002	0.003	0.004	0.014	0.023
Bile Acid							
CHO	0.016	0.027	0.001	0.002	0.004	0.023	0.172
Cellular metabolites							
CIT	0.630	0.708	0.031	0.073	0.095	1.356	1.932
CAR	0.050	0.025	0.007	0.030	0.038	0.074	0.114
L-LAC	1.801	2.279	0.014	0.141	0.239	3.687	8.527
LAC	19.67	27.421	0.542	1.634	2.463	35.236	128.552
MAL	0.057	0.063	0.004	0.006	0.008	0.120	0.189
PYR	0.059	0.067	0.01	0.015	0.023	0.083	0.372
Protein metabolism							
CRE	0.393	0.422	0.026	0.043	0.051	0.851	1.162
CRT	0.393	0.422	0.026	0.043	0.051	0.851	1.162
N-ACE	0.064	0.072	0.003	0.007	0.013	0.122	0.244
O-ACE	0.031	0.057	0.001	0.002	0.004	0.018	0.219
SAR	0.016	0.017	0.001	0.002	0.003	0.034	0.048
UREA	31.666	33.583	2.318	3.869	5.974	62.265	92.473
π-MEH	0.128	0.144	0.009	0.012	0.016	0.269	0.391
τ-MEH	0.014	0.015	0.001	0.002	0.003	0.026	0.053

Table 4.5. Summary statistics of Nuclear Magnetic Resonance analyte by category in mature cows[1] (continued)

Variable	Mean	SD	Minimum	25th Pct.	Median	75th Pct.	Maximum
			Bacterial metabolism				
DIS	0.088	0.099	0.004	0.011	0.015	0.168	0.306
DIL	0.009	0.011	0.001	0.001	0.001	0.018	0.060
			Sugar				
FRU	1.808	1.966	0.146	0.228	0.298	3.701	7.096
GLC	10.148	10.975	0.660	1.051	1.262	21.854	30.873
			Urine				
GLYC	0.956	1.146	0.009	0.014	0.020	2.228	3.014
HIP	0.228	0.253	0.017	0.028	0.032	0.462	0.808
IMD	0.581	0.000	0.581	0.581	0.581	0.581	0.581

[1] 3-HDB = 3-Hydroxybutyrate; 3-HDI = 3-Hydroxyisobutyrate; 3-HDV = 3-Hydroxyisovalerate; ACE = Acetate; ACT = Acetone; ALA = Alanine; ALL = Allantoin; ASP = Aspartate; BET = Betaine; BUT = Butyrate; CAR = Carnitine; CHO = Cholate; CHL = Choline, CIT = Citrate; CRE = Creatine; CRT = Creatinine; D-LAC = D-Lactic acid; DIS = Dimethyl sulfone; DIL = Dimethylamine; FOR = Formate; FRU = Fructose; FUM = Fumarate; GLC = Glucose; GLU = Glutamine; GLY = Glycine; GLYC = Glycolate; HIP = Hippurate; IMD = Imidazole; INO = Inosine; ISB = Isobutyrate; ISL = Isoleucine; L-LAC = L-Lactic acid; LAC = Lactate; LEU = Leucine; MAL = Malonate; MET = Methanol; METH = Methionine; N-ACE = N-Acetyl glycine; O-ACE = O-Acetyl carnitine; PHE = Phenylalanine; PRO = Propionate; PRO-G = Propylene glycol; PYR = Pyruvate; SAR = Sarcosine; SUC = Succinate; THR = Threonine; TRL = Trimethylamine; TRY = Tyrosine; UREA = Urea; URI = Uridine; VAL = Valine; sn-GLP = sn-Glycero-3-phosphocholine; π-MEH = π-Methylhistidine and τ-MEH = τ-Methylhistidine; Summary statistics included standard deviation (SD) and percentiles (Pct.). NOTE: There were 60 cow data used.

4.2. Correlation of dependent and independent variables

4.2.1. Correlations between dependent variables

A low to moderate positive linear relationship existed between DMI with ADG or G:F (Table 4.6). Likewise, heifer ADG was highly correlated to G:F. There is a moderately high positive linear relationship between ADG and G: F in cows (Table 4.6), but no significant linear relationship was between DMI and ADG. Therefore, outcomes of predictive models using each of these as dependent variables may lead to similar results in heifers, but not necessarily in cows.

Table 4.6. Spearman correlation coefficients among dependent variables for heifers or cows.[1]

Trait	Age group	ADG	G:F
DMI	Heifer	0.31***	0.17*
	Cow	0.07	-0.19
ADG	Heifer		0.98***
	Cow		0.69***

[1]DMI = Dry matter intake, kg/d; ADG = Average daily gain, kg/d, and G:F = Gain to feed. Level of significance: * is $P < 0.05$, ** is $P < 0.001$, *** is $P < 0.0001$.

4.2.2. Correlation between dependent variables and metabolites in heifers and cows

Correlation coefficients between dependent variables (DMI, ADG and G:F) and metabolites (PUN, GLC and NEFA) in heifers can be found in Table 4.7. Plasma urea-N was positively correlated with DMI across weeks 0 to 8. Also, PUN was positively correlated (r = 0.35 to 0.47) with ADG and G:F throughout weeks 0 to 8. Glucose was negatively correlated with DMI at week 4, but no other linear relationship was detected. Non-esterified fatty acid was negatively correlated with DMI on week 4 and increasingly became more negative from week 4 to week 8. There was a positive relationship between NEFA and G:F at week 0.

Correlation between the dependent variable and metabolites in cows can be found in Table 4.8. Plasma urea-N was positively correlated with DMI at week 0. Non-esterified fatty acids were significant with DMI at a decreasing rate from week 0 to week 2.

Table 4.7. Spearman correlation coefficients between dependent variables and plasma urea-nitrogen (PUN), glucose (GLC), and non-esterified fatty acids (NEFA) in heifers[1]

Traits/week[2]	Metabolites		
DMI; kg/day	PUN; mg/dL	GLC; mg/dL	NEFA; mg/dL
0	0.17*	-0.01	-0.01
2	0.14*	-0.10	-0.12
4	0.15*	-0.15*	-0.24***
6	0.17*	-0.08	-0.39***
8	0.11*	-0.06	-0.67***
ADG; kg/day			
0	0.42***	-0.02	0.24
2	0.35***	-0.01	-0.01
4	0.43***	-0.11	0.15*
6	0.47***	-0.06	-0.06
8	0.46***	-0.05	-0.16*
G:F			
0	0.40***	-0.03	0.25**
2	0.35***	-0.01	0.02
4	0.42***	-0.10	0.21*
6	0.45***	-0.04	-0.01
8	0.44***	-0.03	-0.11

[1]Level of significance: * is $P < 0.05$, ** is $P < 0.001$, *** is $P < 0.0001$.
[2]DMI =Dry matter intake; ADG = Average daily gain and G:F = Gain to feed. The sample population range for PUN was 214 to 331, GLC was 210 to 333, and NEFA was 205 to 333 from week 0 to 8, respectively.

Table 4.8. Spearman correlation coefficients between dependent variables and plasma urea-nitrogen (PUN), glucose (GLC), and Non-esterified fatty acids (NEFA) in mature cows[1].

Traits/week[2]	Metabolites		
DMI; kg/day	PUN; mg/dL	GLC; mg/dL	NEFA; mg/dL
0	0.47**	-0.05	-0.34*
2	0.00	-0.17	-0.59***
ADG; kg/day			
0	-0.09	0.11	0.02
2	-0.01	0.08	0.13
G:F			
0	-0.17	-0.02	0.10
2	-0.04	0.15	0.14

[1]Level of significance: * is $P < 0.05$, ** is $P < 0.001$, *** is $P < 0.0001$.
[2]DMI =Dry matter intake; ADG = Average daily gain and G:F = Gain to feed.60 observations were available for all the metabolites

4.2.3. Correlations within and between Rumen and Cellular metabolism categories

Table 4.9 shows the correlation coefficients within RM category for Spearman Rank and Pearson correlation coefficients in heifers. Outside of TRL, Pearson correlation coefficients ranged from 0.048 to 0.870. On the other hand, Spearman Rank correlation coefficients ranged from0.571 to 0.966 while excluding TRL, indicating normality issues among analyte values that biased Pearson correlation coefficients. Therefore, only Spearman Rank correlation coefficients were used to inform modeling decisions. Trimethylamine had negative correlation coefficients with the majority of other analytes, which ranged from -0.471 to -0.102 in heifers using Spearman Rank method. Given these analytes are not completely correlated (i.e., less than 1.00), all analytes in the RM category were used in subsequent analyses.

Table 4.10 shows the correlation coefficients within CM category. Similar to RM category, Pearson correlation coefficients in heifers ranged from 0.337 to 0.904. On the other hand, Spearman Rank correlation coefficients for those same analytes ranged from 0.706 to 0.963. These

differences indicate again normality issues within analyte data. Spearman Rank correlation coefficients suggests that these analytes depend highly on each other in their operations, but were not perfectly correlated. Therefore, all were used in subsequent analyses.

Correlation coefficient between RM and CM categories in heifers are presented in Table 4.11. Excluding TRL, Spearman Rank correlation coefficients ranged from 0.485 to 0.951 between these two categories. Trimethylamine had only negative correlation coefficients with all other analytes ranging from -0.569 to -0.432.

Table 4.9. Spearman Rank (S, lower diagonal) and Pearson (P, upper diagonal) correlation coefficients of rumen metabolites in heifers[1].

S \ P	3-HDB	3-HDI	3-HDV	ACE	ACT	BUT	D-LAC	FOR	FUM	ISB	MET	PRO	PROG	TRL	URI
3-HDB		0.711	0.742	0.870	0.576	0.761	0.558	0.779	0.673	0.706	0.715	0.810	0.792	-0.328	0.842
3-HDI	0.937		0.562	0.664	0.490	0.614	0.467	0.629	0.605	0.647	0.608	0.661	0.632	-0.252	0.703
3-HDV	0.908	0.920		0.745	0.477	0.717	0.336	0.787	0.610	0.577	0.728	0.746	0.818	-0.138	0.728
ACE	0.966	0.931	0.904		0.601	0.734	0.494	0.843	0.707	0.716	0.75	0.858	0.769	-0.193	0.832
ACT	0.752	0.788	0.770	0.763		0.673	0.623	0.628	0.564	0.744	0.621	0.642	0.633	0.161	0.694
BUT	0.817	0.829	0.795	0.834	0.848		0.655	0.761	0.667	0.804	0.776	0.729	0.85	0.048	0.746
D-LAC	0.595	0.630	0.578	0.599	0.759	0.732		0.484	0.480	0.701	0.530	0.462	0.553	0.141	0.595
FOR	0.883	0.893	0.869	0.878	0.749	0.791	0.620		0.725	0.722	0.811	0.845	0.842	-0.133	0.806
FUM	0.830	0.849	0.817	0.818	0.722	0.762	0.571	0.812		0.691	0.635	0.752	0.697	-0.167	0.768
ISB	0.820	0.862	0.815	0.840	0.794	0.872	0.668	0.799	0.777		0.742	0.725	0.716	-0.070	0.770
MET	0.840	0.847	0.853	0.862	0.787	0.855	0.655	0.837	0.765	0.812		0.760	0.792	-0.105	0.723
PRO	0.906	0.925	0.901	0.903	0.742	0.778	0.578	0.866	0.826	0.826	0.821		0.826	-0.197	0.864
PROG	0.892	0.913	0.915	0.886	0.818	0.833	0.655	0.871	0.852	0.829	0.842	0.910		-0.035	0.797
TRL	-0.471	-0.453	-0.471	-0.456	-0.164	-0.262	-0.102	-0.426	-0.399	-0.394	-0.365	-0.451	-0.367		-0.253
URI	0.905	0.932	0.890	0.904	0.767	0.808	0.627	0.857	0.852	0.830	0.814	0.911	0.907	-0.464	

[1]3-HDB = 3-Hydroxybutyrate; 3-HDI = 3-Hydroxyisobutyrate; 3-HDV = 3-Hydroxyisovalerate; ACE = Acetate; ACT = Acetone; BUT = Butyrate; D-LAC = D-Lactic; FOR = Formate; FUM = Fumarate; ISB = Isobutyrate; MET = Methanol; PRO = Propionate; PROG = Propylene glycol; TRL = Trimethylamine; URI = Uridine. All Spearman and Pearson correlation coefficients were significant at (P < 0.01 to 0.0001).

Table 4.10. Spearman Rank (S, lower diagonal) and Pearson (P, upper diagonal) correlation coefficients of cellular metabolism analytes in heifers[1]

S \ P	CAR	CIT	L-LAC	LAC	MAL	PYR	SUC	sn-GLP
CAR		0.422	0.337	0.504	0.500	0.758	0.480	0.628
CIT	0.776		0.568	0.628	0.814	0.770	0.834	0.853
L-LAC	0.706	0.774		0.627	0.585	0.519	0.537	0.619
LAC	0.779	0.807	0.842		0.512	0.637	0.539	0.669
MAL	0.788	0.934	0.811	0.815		0.797	0.800	0.904
PYR	0.857	0.903	0.788	0.813	0.938		0.802	0.861
SUC	0.830	0.905	0.760	0.775	0.922	0.963		0.837
sn-GLP	0.823	0.922	0.772	0.820	0.934	0.921	0.900	

[1]CAR = Carnitine; CIT = Citrate; L-LAC = L-Lactic acid; LAC = Lactate; MAL = Malonate; PYR = Pyruvate; SUC = Succinate and sn-GLP = sn-Glycero-3-phosphocholine

Table 4.11. Spearman Rank correlation coefficients between rumen and cellular metabolites in heifers[1]

	CAR	CIT	L-LAC	LAC	MAL	PYR	SUC	sn-GLP
3-HDB	0.802	0.934	0.776	0.794	0.936	0.926	0.928	0.915
3-HDI	0.789	0.927	0.806	0.833	0.951	0.922	0.894	0.918
3-HDV	0.786	0.918	0.734	0.778	0.912	0.917	0.893	0.915
ACE	0.809	0.930	0.771	0.795	0.945	0.935	0.939	0.911
ACT	0.591	0.748	0.647	0.607	0.792	0.796	0.788	0.744
BUT	0.701	0.793	0.710	0.647	0.836	0.878	0.873	0.795
D-LAC	0.485	0.587	0.577	0.501	0.647	0.646	0.650	0.580
FOR	0.808	0.880	0.760	0.784	0.893	0.903	0.884	0.873
FUM	0.693	0.832	0.752	0.750	0.842	0.811	0.809	0.807
ISB	0.687	0.822	0.794	0.734	0.858	0.864	0.848	0.825
MET	0.752	0.841	0.694	0.703	0.878	0.891	0.887	0.865
PRO	0.768	0.885	0.792	0.789	0.908	0.880	0.862	0.881
PROG	0.729	0.884	0.755	0.750	0.913	0.900	0.887	0.883
TRL	-0.569	-0.472	-0.487	-0.567	-0.446	-0.463	-0.432	-0.467
URI	0.774	0.890	0.817	0.810	0.931	0.890	0.868	0.885

[1]Column names include cellular metabolites of: CAR = Carnitine; CIT = Citrate; L-LAC = L-Lactic acid; LAC = Lactate; MAL = Malonate; PYR = Pyruvate; SUC = Succinate and sn-GLP = sn-Glycero-3-phosphocholine; Row names include rumen metabolites of: 3=HDB = 3-Hydroxybutyrate; 3-HDI = 3-Hydroxyisobutyrate; 3-HDV = 3-Hydroxyisovalerate; ACE = Acetate; ACT = Acetone; BUT = Butyrate; D-LAC = D-Lactic; FOR = Formate; FUM = Fumarate; ISB = Isobutyrate; MET = Methanol; PRO = Propionate; PROG = Propylene glycol; TRL = Trimethylamine; URI = Uridine

The correlation coefficients within RM category for Spearman Rank and Pearson in cows are reported in Table 4.12. Excluding TRL, Spearman Rank correlation coefficients ranged from 0.687 to 0.914. Trimethylamine had negative correlation coefficients with all analytes and ranged from -0.831 to -0.683). Table 4.13 reports correlation coefficients within CM category, all have strong positive linear correlation coefficients ranging from 0.728 to 0.896. These correlations coefficients suggests that these analytes depend on each other in their operations. Table 4.14 reports the Spearman Rank correlation coefficients between RM and CM categories. Excluding TRL, correlation coefficients ranged from 0.710 to 0.975 between these two categories. Trimethylamine had strong negative correlation coefficients with all the CM analytes (-0.835 to -0.752).

Table 4.12. Spearman Rank (S, lower diagonal) and Pearson (P, upper diagonal) correlation coefficients of rumen metabolites in cows[1].

S \ P	3-HDB	3-HDI	3-HDV	ACE	ACT	BUT	D-LAC	FOR	FUM	ISB	MET	PRO	PROG	TRL	URI
3-HDB		0.964	0.937	0.927	0.682	0.919	0.814	0.842	0.842	0.885	0.892	0.886	0.932	-0.917	0.875
3-HDI	0.894		0.955	0.943	0.730	0.930	0.824	0.858	0.878	0.942	0.950	0.927	0.963	-0.940	0.944
3-HDV	0.819	0.778		0.939	0.680	0.872	0.884	0.872	0.942	0.920	0.928	0.932	0.958	-0.911	0.929
ACE	0.894	0.835	0.809		0.703	0.899	0.803	0.843	0.880	0.962	0.950	0.915	0.956	-0.890	0.936
ACT	0.790	0.825	0.742	0.751		0.661	0.514	0.541	0.609	0.712	0.708	0.647	0.748	-0.713	0.669
BUT	0.874	0.868	0.789	0.843	0.807		0.719	0.809	0.792	0.882	0.890	0.855	0.884	-0.901	0.890
D-LAC	0.748	0.807	0.726	0.733	0.757	0.774		0.692	0.915	0.784	0.781	0.849	0.845	-0.770	0.768
FOR	0.716	0.755	0.770	0.737	0.693	0.762	0.687		0.770	0.825	0.835	0.740	0.835	-0.811	0.872
FUM	0.795	0.755	0.796	0.777	0.754	0.792	0.794	0.688		0.897	0.875	0.919	0.916	-0.819	0.880
ISB	0.843	0.901	0.742	0.858	0.789	0.853	0.799	0.721	0.800		0.956	0.931	0.956	-0.868	0.953
MET	0.723	0.739	0.721	0.806	0.721	0.721	0.736	0.715	0.770	0.733		0.907	0.951	-0.890	0.967
PRO	0.893	0.914	0.772	0.855	0.806	0.864	0.808	0.701	0.820	0.909	0.767		0.918	-0.900	0.905
PROG	0.819	0.843	0.753	0.835	0.759	0.772	0.787	0.709	0.738	0.844	0.798	0.803		-0.915	0.944
TRL	-0.831	-0.824	-0.724	-0.776	-0.774	-0.822	-0.719	-0.687	-0.693	-0.798	-0.683	-0.807	-0.776		-0.863
URI	0.763	0.807	0.834	0.845	0.747	0.852	0.786	0.784	0.803	0.861	0.815	0.805	0.817	-0.702	

[1]3-HDB = 3-Hydroxybutyrate; 3-HDI = 3-Hydroxyisobutyrate; 3-HDV = 3-Hydroxyisovalerate; ACE = Acetate; ACT = Acetone; BUT = Butyrate; D-LAC = D-Lactic; FOR = Formate; FUM = Fumarate; ISB = Isobutyrate; MET = Methanol; PRO = Propionate; PROG = Propylene glycol; TRL = Trimethylamine; URI = Uridine

Table 4.13. Spearman Rank (S, lower diagonal) and Pearson (P, upper diagonal) correlation coefficients of cellular metabolism analytes in cows[1].

S / P	CAR	CIT	L-LAC	LAC	MAL	PYR	SUC	sn-GLP
CAR		0.807	0.851	0.769	0.878	0.707	0.827	0.904
CIT	0.833		0.793	0.700	0.920	0.695	0.928	0.891
L-LAC	0.873	0.814		0.878	0.865	0.608	0.763	0.823
LAC	0.832	0.809	0.894		0.731	0.65	0.729	0.709
MAL	0.759	0.811	0.828	0.728		0.751	0.887	0.933
PYR	0.824	0.791	0.884	0.896	0.776		0.739	0.715
SUC	0.811	0.886	0.759	0.769	0.739	0.806		0.899
sn-GLP	0.880	0.851	0.821	0.808	0.728	0.820	0.844	

[1]CAR = Carnitine; CIT = Citrate; L-LAC = L-Lactic acid; LAC = Lactate; MAL = Malonate; PYR = Pyruvate; SUC = Succinate and sn-GLP = sn-Glycero-3-phosphocholine

Table 4.14. Spearman Rank correlation coefficients between Rumen and Cellular metabolites in cows[1].

	CAR	CIT	L-LAC	LAC	MAL	PYR	SUC	sn-GLP
3-HDB	0.805	0.872	0.771	0.752	0.747	0.804	0.975	0.819
3-HDI	0.840	0.898	0.813	0.835	0.762	0.850	0.894	0.826
3-HDV	0.779	0.750	0.724	0.684	0.742	0.732	0.817	0.725
ACE	0.835	0.871	0.755	0.747	0.788	0.776	0.898	0.848
ACT	0.773	0.834	0.778	0.747	0.792	0.765	0.799	0.818
BUT	0.818	0.877	0.809	0.745	0.823	0.837	0.863	0.818
D-LAC	0.822	0.759	0.835	0.879	0.765	0.839	0.770	0.773
FOR	0.825	0.741	0.746	0.745	0.673	0.736	0.751	0.751
FUM	0.799	0.786	0.781	0.741	0.842	0.734	0.805	0.742
ISB	0.854	0.854	0.869	0.819	0.837	0.875	0.831	0.846
MET	0.784	0.810	0.710	0.762	0.802	0.737	0.761	0.813
PRO	0.817	0.855	0.804	0.799	0.804	0.84	0.882	0.823
PROG	0.836	0.832	0.805	0.824	0.752	0.807	0.839	0.841
TRL	-0.788	-0.798	-0.835	-0.763	-0.752	-0.815	-0.815	-0.780
URI	0.853	0.806	0.815	0.772	0.819	0.799	0.761	0.819

[1]Column names include cellular metabolites of: CAR = Carnitine; CIT = Citrate; L-LAC = L-Lactic acid; LAC = Lactate; MAL = Malonate; PYR = Pyruvate; SUC = Succinate and sn-GLP = sn-Glycero-3-phosphocholine; Row names include rumen metabolites of: 3=HDB = 3-Hydroxybutyrate; 3-HDI = 3-Hydroxyisobutyrate; 3-HDV = 3-Hydroxyisovalerate; ACE = Acetate; ACT = Acetone; BUT = Butyrate; D-LAC = D-Lactic; FOR = Formate; FUM = Fumarate; ISB = Isobutyrate; MET = Methanol; PRO = Propionate; PROG = Propylene glycol; TRL = Trimethylamine; URI = Uridine

4.2.4. Correlation between NMR and single metabolites

Spearman Rank correlation coefficients between GLC and PUN at week 8 for heifers and cows, respectively, were calculated with NMR GLC and Urea (i.e., equivalent of PUN) as they were equivalent samples used for analyte data. In heifers, GLC tended to be correlated (r = 0.1065, P = 0.0764) and PUN was moderately correlated (r = 0.2838, P< 0.0001) with their equivalent analyte in NMR. In cows, GLC was not correlated (r = 0.0416, P = 0.7522) and PUN tended to be moderately, but negatively correlated (r = -0.2450, P = 0.0592) with their equivalent analyte in NMR.

4.2.5. Correlation between DMI and Rumen and Cellular metabolism categories

Instead of considering analytes individually, two NMR categories (RM and CM) were summarized into group scores. After assigning quartile number (1 = minimum to 25th percentile; 4 = 75th percentile to maximum) to each analyte in a given category, the average score for that NMR category was calculated and used. The average quartile score (standard deviation) for heifers and cows, respectively, for RM was 2.497 (0.889) and 2.529 (0.806), and for CM was 2.497 (1.013) and 2.506 (1.004).

Table 4.15reports the correlation coefficients between DMI, ADG, and G:F and with RM and CM category quartile scores in heifers and cows. Correlation coefficients between RM and DMI are similar in heifers and cows. On the other hand, CM with DMI, ADG and G:F differs between heifers and cows. Heifers' correlation coefficients were all low, negative and non-significant. Cows had higher and positive associations with ADG and G:F, with RM score significantly associated.

Table 4.15. Correlation between dry matter intake (DMI), average daily gain (ADG), and gain to feed (G:F) ratio with Rumen and Cellular metabolism categories quartile score at week 8[1].

	Trait	Metabolism category	
		Rumen	**Cellular**
Heifers	DMI; kg/day	-0.058	-0.093
	ADG; kg/day	-0.061	-0.048
	G:F	-0.027	-0.009
Cows	DMI; kg/day	-0.086	0.009
	ADG; kg/day	0.259*	0.237
	G:F	0.288*	0.236

[1]Level of significance: * is $P < 0.05$, ** is $P < 0.001$, *** is $P < 0.0001$.

4.3. Statistical modelling of DMI with different metabolic predictors

4.3.1. Modelling of DMI with PUN, GLC and NEFA in heifers and cows

When modeling DMI using the GLM procedure of SAS, fixed effects of frame size category, ancestral breed group, and year born for heifers resulted in an adjusted R^2 of 52.87 with 335 data records. Similar modeling in cows with 60 records resulted in an adjusted R^2 of 56.45. Inclusion of metabolite predictors per week changed the adjusted R^2 in both heifers and cows as reported in Table 4.16. Sample sizes of heifers decreased based on inclusion of the specific metabolite and week as reported in Tables 4.3 and 4.4.

The metabolite analysis utilized 335 and 60 data records for heifers and cows, respectively. The NMR analysis utilized 280 and 60 data records for heifers and cows respectively. Weeks 4, 6 and 8 had a close adjusted R^2 values across all metabolites in heifers, as did weeks 0 and 2 for PUN and GLC in cows (Table 4.16). However, the inclusion of PUN for heifers did not account

for any increase in the base model except for week 6, which accounted for 0.13% increase in the

base model.

Table 4.16. Predictions for DMI with Adjusted R^2 among weeks in metabolites and NMR[1] category in heifer and cow.

Traits/week	Metabolites			Metabolism	
DMI; kg/day	PUN; mg/dL	GLC; mg/dL	NEFA; mg/dL	Rumen	Cellular
Heifers[2]					
0	42.8	42.9	43.4		
2	43.2	43.8	44.5		
4	52.5	53.4*	57.0*		
6	53.0*	52.5	58.1*		
8	52.8	52.5	54.4*	51.9	52.0
Cows[3]					
0	59.5*	57.0*	64.4*		
2	57.5*	57.2*	70.6*	55.6	55.8

[1]Values with a superscript (*) shows increase over the base model.
[2]Base model (fixed effects of frame size category, ancestral breed group, and year born) under metabolites for heifers had an adjusted R^2 of 52.87 with a total of 335 read in data and an adjusted R^2 of 56.45 with a total of 60 read in data in cows
[3]Base model (fixed effects of frame size category, ancestral breed group, and year born) under metabolism for heifers had an Adjusted R^2 of 51.88 with a total of 280 read in data and an Adjusted R^2 of 56.45 with a total of 60 read in data in cows.
NOTE: Weeks 0 and 2 in cows is equivalent to weeks 6 and 8 in heifers given the data were taken at the same time of the year (between the 3rd week in July and the 1st week in August).

A similar observation was seen for GLC, except for week 4 (0.53% increase) in heifers. On the other hand, the inclusion of NEFA in weeks 4, 6, and 8 increased the variation explained by the model more, ranging from 1.53% to 5.23% increase in adjusted R^2 in heifers.

The PUN and GLC models show an increase of 3.05% and 0.75%, respectively, in the adjusted R^2 value for cows. In predicting DMI, the NEFA model accounted for the most variation, accounting for 7.95% to 14.15% increase in the adjusted R^2 value over the base model, which was much higher compared to PUN and GLC increases. The NEFA models in heifers and cows accounted for the most variation and explain DMI better. Fit plots were employed to make further decisions on which specific week explains DMI better; showing the association between raw DMI

values and predicted DMI values. Given the adjusted R^2 outcomes, fit plots for week 4 to 8 for heifers and both weeks of cows were generated. Figure 4.1illustrates the fit between DMI and NEFA for weeks 4, 6, and 8, respectively, in heifers, whereas Figure 4.2 illustrates the fit between DMI and NEFA for weeks 0 and 2, respectively, in cows.
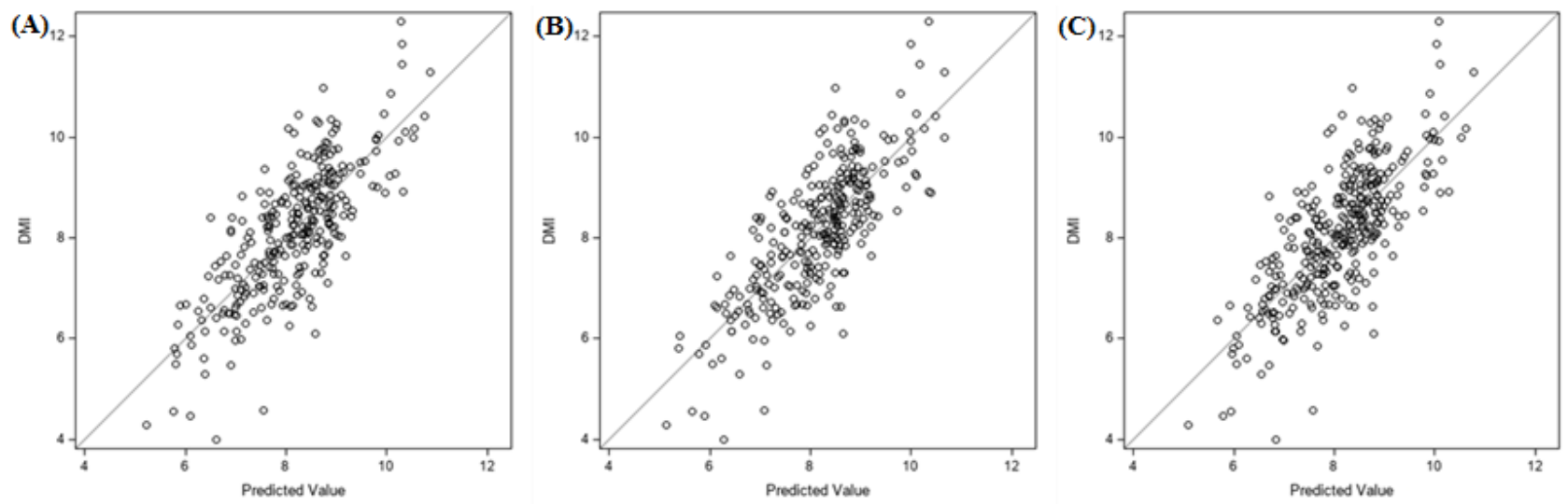
Figure 4.1. Fit plot for dry matter intake (DMI) and predicted values of non-esterified fatty acids (NEFA) model on weeks 4 (A), 6 (B) and 8 (C) in heifers.
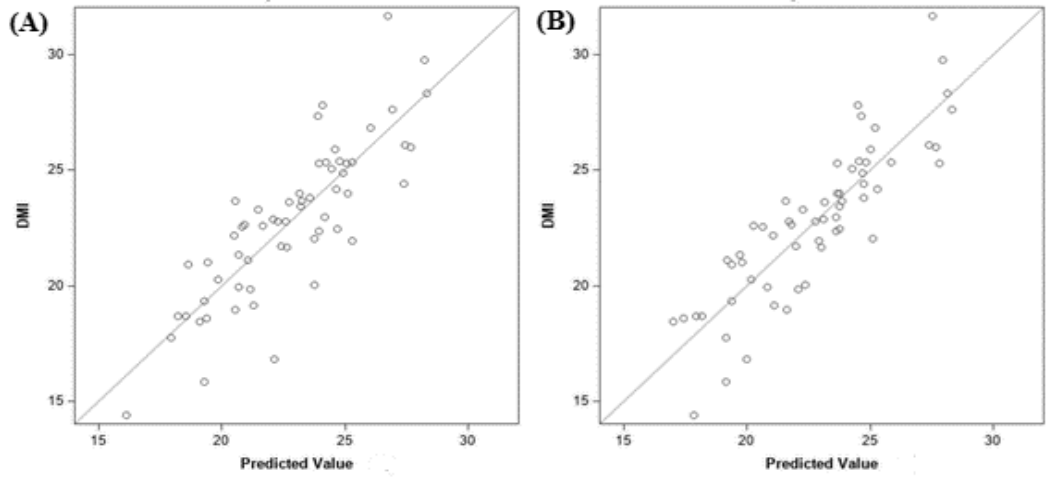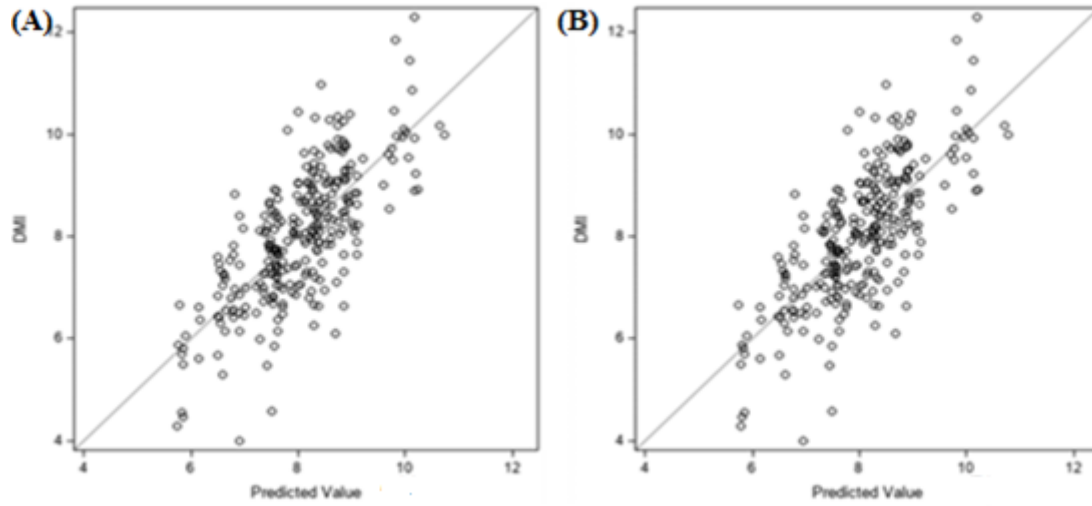

Figure 4.2. Fit plot for dry matter intake (DMI) and predicted values of non-esterified fatty acids (NEFA) model on weeks 0 (A)and 2 (B) in cows.

4.3.2. Modelling of DMI with NMR quartiles averages for Rumen and Cellular metabolism categories quartile score in heifers and cows

The adjusted R^2 from each model in heifers and cows in RM or CM category are presented in Table 4.16. For RM in heifers, the adjusted R^2increased slightly by 0.02%, whereas the CM adjusted R^2 increased by 0.12% from the base model. For RM and CM in cows, the adjusted R^2 reduced by 0.80%and 0.65%.m the base model, respectively. Cellular metabolism models in heifers had only a little increase in the value of R^2. Fit plots (Figures 4.3 and 4.4) were further explored to see how the raw DMI values align linearly with their predicted values.

Results from Spearman correlation coefficients between nuclear magnetic resonance (glucose [NMRglc] and urea [NMRurea]) and metabolites analyzed individually (PUN and GLC) in heifers and cows. There was a positive relationship (r = 0.284, P<.0001) between NMRurea and PUN in heifers. Glucose and NMRglc were not correlated (r = -0.011, P = 0.8530). Glucose and NMRglc had a mean and SD of (3.967 ± 0.510) and (12.216 ± 10.496) respectively in heifers. Plasma urea-N and NMRurea had a mean and SD of (9.839 ± 4.950) and (26.383 ± 22.096) respectively in heifers. There was positive a negative relationship (r = -0.245, P = 0.0592) between NMRurea and PUN in cows. Glucose and NMRglc were correlated (r = 0.042, P = 0.7522). Glucose and NMRglc had a mean and SD of (71.062 ± 7.769) and (10.148 ± 10.975) respectively in cows. Plasma urea-N and NMRurea had a mean and SD of (13.785 ± 2.310) and (31.666 ± 33.583) respectively in cows.

Figure 4.3. Fit plot for dry matter intake (DMI) and predicted values of (A) Rumen and (B) Cellular metabolism model on weeks 8 in heifers.
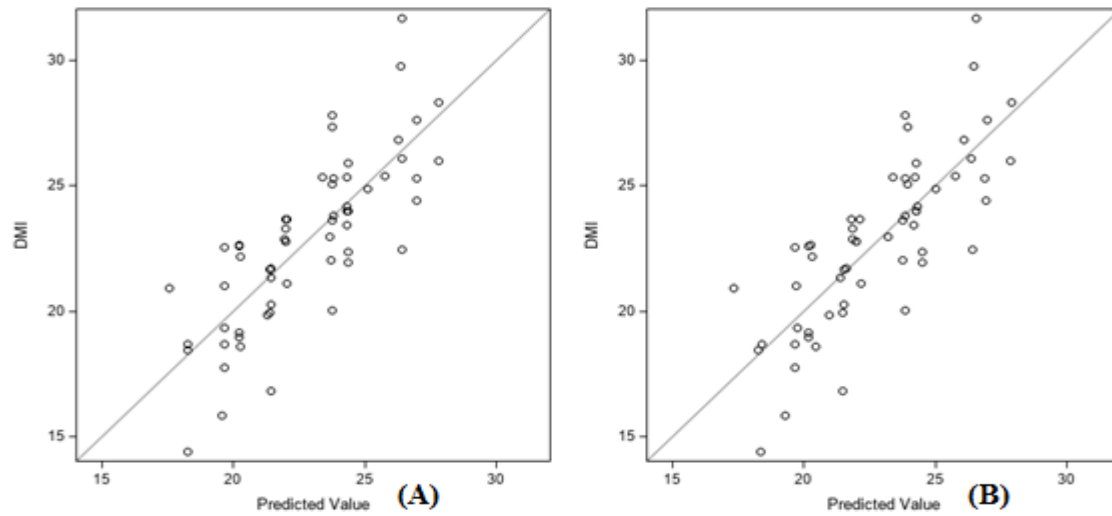
Figure 4.4. Fit plot for dry matter intake (DMI) and predicted values of (A) Rumen and (B) Cellular metabolism model on weeks 8 in cows.

5. DISCUSSION

Feeding beef cattle is a producer's greatest cost, representing the major part of the total costs in beef cattle production systems (Miller et al., 2001). Also, feed efficiency has moderate to high heritability (Schenkel et al., 2004), suggesting genetic improvement is a possibility. However, the intensive labor and rising cost associated with the assessment of feed efficiency in beef cattle are limiting factors towards effectively selecting feed efficient cattle (Herd and Arthur, 2009), suggesting the need of alternatives for assessing feed efficiency.

5.1. Basic relationships between dependent and independent variables in heifers and cows

Before the arrival of heifers and cows to the BCRC, heifers were housed in a pen area and fed hay containing 12% CP with salt and mineral whereas cows were on calving pastures feeding on grass with a salt and mineral supplement. Up on arrival to the BCRC, diet was formulated based on growth or maintenance goals. In heifers, this meant gaining weight, whereas cows maintained it. The formulated diet for the feeding trial contained grass hay, corn silage, dry rolled corn, fine ground corn, dried distiller's grain with solubles, salt and supplements. Summary statistics of PUN, GLC and NEFA presented in Table 4.3 shows that both heifers and cows had an increase in PUN across weeks. The more CP an animal takes in, the more it passes in urine. Bohnert et al. (2011) suggested that the increased forage intake of animals is in response to protein supplementation of low-quality forages depends on the forage type.

Glucose and NEFA levels generally decreased across weeks for heifers and cows although time points for these animals were not the same. These changes can be as a result of the changes in feed or environment since animals were mostly on pastures at DREC. Some studies show that mid-lactating cows that exhibit lower feed intake and stress have no increase in plasma NEFA (Shwartz et al. 2009; Calamari et al. 2013). This might be a reason for the decline in NEFA for

cows. Glucose, as one of the important biochemical components of the energetic metabolism also decline. Heifers might not be having enough feed thereby reducing their intake since the method of feeding at the BCRC with the Insentec is new to them. Temporary or prolonged energy deficit can reduce glucose production, which can lead to hypoglycemia (O'Neill et al., 2018). This is a possibility in the animals used in this study as glucose levels dropped after their arrival at the BCRC. Also, cows could allocate energy to other physiological process like milk production, thereby causing a reduction in glucose levels.

Linear correlation coefficient in statistics is a tool used to assess what relationship exists between two variables. It measures the direction and strength of the relationship and this "trend" is represented by a correlation coefficient. Due to distribution concerns with variables, Spearman's rank correlation method was run to see the variables considered are linearly related asit is a distribution free test of independence. The moderate correlation coefficients between DMI and ADG or G:F, respectively, indicates that DMI has a linear relationship with gain on forage-based diets in young beef heifers during the growing phase. Cassady et al. (2016) reported a simple phenotypic correlation coefficient of 0.58 between forage DMI and grain DMI, which suggests that DMI is repeatable across stages in cattle development and a measure that can be recorded in both heifers and cows. Beef cattle consume an average of 3% of their body weight to gain 1.5 to 3 pounds on a daily basis (Putnam et al., 1964). Retallick et al. (2017) reported similar findings to this study between heifers and cows (0.98 over 0.69) that heifers had greater correlations between feed intake and gain than steers in their study. This is similar because one-year old heifers are in their growing stage and constantly convert energy gotten from feed into growth. We recorded a significant correlation (0.31) between DMI and ADG in heifer. However, in cows, we observed weak, non-significant correlation coefficients between DMI and ADG or G:F. The negative

correlation with G:F might be associated with mature animal size, varying breeds and year. Although a useful management tool, the use of G:F as a measure of feed efficiency in programs has been questioned because of its strong correlation with BW and, consequently, its adverse effect on mature animal size and feed intake (Cantalapiedra-Hijar et al., 2018).There has been many studies comparing heifers and steer or feedlot cattle but there are very few comparing heifers and mature cows. Freetly et al. (2020) compared the estimate of feed intake in beef cattle; heifers and cows. The results show that heifers had higher ADG estimate of 0.53 ± 0.12 than cow's 0.34 ± 0.11, which supports what was observed in our study. Gain to feed has been one of the most traditional measures of feed efficiency in growing beef cattle with its inverse; feed to gain, commonly used as a selection tool for growing animals due to its moderate heritability (Schenkel et al., 2004). Other measures of feed efficiency that could be explored in the cow-calf production system with animals fed forage is the pounds of calves weaned per acre. Heitschmidt et al. (1990) conducted a 6year-long study on cow-calf production using varying stocking rates on pasture across years. The summary of the research pointed that stocking rate was responsible for differences in production output and could affect economic returns, therefore conception rate, weaning calf average, weaning weights and pounds per acre decreases as stocking rate increases.

The major concern for this study was to find the week within 4-weeks to the breeding season that accounted for the most variation in DMI. Several metabolites have been associated with feed efficiency phenotypes in heifers (Gonano et al., 2014; Montanholi et al., 2007) and in cows (Wood et al., 2014). However, the association between DMI and PUN found in the present study was low (r=0.15, 0.17 and 0.11) for weeks 4, 6 and 8 in heifers, while cows had a moderate correlation coefficient (r = 0.47) for week 6. Kelly et al. (2010) found a positive correlation between PUN and DMI in Limousin $\times$ Holstein-Friesian heifers and also had a moderate

correlation coefficient (r = 0.38) between PUN and RFI. Since the major physiological states of concern in cattle are lactation, growth, gestation and maintenance, the measurement of urea-N concentration is mostly applied to lactating, growing and finishing beef cattle (Hammond, 1983).Nitrogen content of diet, energy content of diet, N degradability of diet and the level of feeding can contribute to the changes in urea-N concentration in beef cattle (Cross et al., 1974; Richardson and Kegel., 1980) as seen between heifers and cows in relations to dry matter intake.

Only week 4 showed (r = -0.15; P = 0.013) a significant correlation coefficient between DMI and GLC in heifers while there was no significance in cows. Kelly et al. (2010) reported relationship between glucose and other measures of feed efficiency measures but the correlation with DMI was not observed in heifers. Wood et al. (2014) considered the evaluation of residual feed intake measured in mid- to late-gestation mature beef cows and found no relationship between DMI and glucose concentration in mature beef cows. This aligns with our study given there was no significant relationship between DMI and GLC in cows.

There was a negative correlation between DMI and NEFA (r = -0.24, -0.39 and -0.27) for weeks 4, 6 and 8 in heifers and (r = 0.34 and -0.59) for weeks 6 and 8 in cows, respectively. This supports the negative correlation found in growing beef heifers (Kelly et al., 2010) and mature cows (Wood et al., 2014) between NEFA concentrations and RFI while investigating the relationship between feed efficiency and blood metabolic variables. When feed is offered for ad libitum intake, ruminants tend to store excess energy as body reserves. This energy can be later used by animal or used towards their offspring later (Ingvartsen et al., 2000; Owens et al., 2014; Ginane et al., 2015). There has been research done on evaluating feed efficiency measures and metabolites in heifers (Kelly et al., 2010), bulls (Kelly et al., 2011), and cows (Wood et al., 2014). There has not been any study out there comparing heifers and cows.

5.2. Prediction models using PUN, GLC and NEFA in heifers and cows

The base model for our metabolites was DMI = $\beta 0$ + $\beta 1$(SIZE) + $\beta 1$(BREED) + $\beta 3$(YEAR)after considering all the class effects in heifers. The 15th week in the heifer and cow feeding trial was removed because heifers were already in the breeding season at the time. Week 0 and 2 recorded lower values compared to weeks 4, 6 and 8. These were the weeks animals were conditioned to their newly formulated feed for the trial and new environment. Upon arrival to the BCRC from DREC, animals were stressed from hauling. This can affect results negatively. Donley et al. (2009) recorded reduced ADG and elevated PUN in feedlot heifers which was as a result of stress after being shipped to the Kansas State University. Schwartzkopf-Genswein et al. (2012) reported that loading density, animal age, trailer micro climate, transportation duration and loading density can cause stressful conditions that can reduce the ability of an animal to be reproductive and impede production performance standards. Week 8 data was observed and compared against the other weeks before breeding heifers and cows. The base model had an adjusted $R^2$of 52.87%. However, the PUN model for week 6 in heifers and cows explained DMI with the cow model explaining more variation than heifers. There was a similar trend in heifers and cows given their response with the GLC model. Week 4 in heifers and weeks 6 and 8 in cows, which were the weeks leading up to breeding, had only a little increase in adjusted $R^2$of 0.53% to 0.75%, explaining variation in DMI. Although, the GLC model accounted for some level of variation but this is small. There were negative correlations with DMI and these might be because ruminant animals do not take up high amount of glucose from their feed and only use glucose as respiratory fuel (Cerrilla et al., 2003). In ruminants, carbohydrates gotten from diets are fermented into short chain volatile fatty acids in the rumen. Less than 10% of the glucose required for the animal is absorbed from the digestive tract (Nafikov and Beitz., 2007; Luthfi et al., 2014). Since the GLC model shows

some differences between week 4 and the remaining weeks leading to breeding, which is classified within the category of 4 weeks to breeding, this might be an indicator that GLC metabolism could be of use in explaining DMI in growing beef heifers, especially 4 weeks into breeding. With the NEFA prediction model, there was a distinctive increment from the base model (57.0%, 58.1% and 54.4%) at week 4, 6 and 8 for heifers and at weeks 6 and 8 for cows (64.4% and 70.6%). These values are within the time frame of 4 weeks before heifers go into breeding. This is important because heifers and cows are given pre-breeding vaccinations approximately 4 weeks before breeding to avoid early-term abortion, fetal malformation, and the development of persistently infected calves. Figures 4.1 shows the fit plot for DMI and predicted values for NEFA model on weeks 4, 6 and 8, respectively, in heifers. The plots display a strong linear fit between DMI and its predicted values. According to Fontoura et al. (2017), NEFA seemed highly dependent on DMI. Since weeks 4 to 8 predict similar values in heifers and cows and their fit plots are linearly the same, they could all be used for explaining DMI since they all appear before breeding. Farmers can use this information in that if there is a time frame just before breeding when vaccinations can be given to animals and blood draws can be done simultaneously to predict DMI, this information can be used for better management of animals. This reduces multiple entry into the chute, stress on the animals and labor for farm workers. Although NEFA did not account for a 100% variation, the prediction plot clearly shows an agreement between the predicted and raw values of DMI. This information can be used to aid in allotment of animals to pasture in addition to other considerations such as animal size and stocking rate. Elliot (2021) reported that in managing pasture, producers need to destock; this does not means selling animals, but the animals are either moved to a new pasture, purchased or rented, so that all animals can have a chance at food. Fox et al. (1991) suggested that measuring plasma NEFA may be a useful indirect indicator of feed intake in

ruminants. In this study, cows accounted for more variation (14.15%) in DMI over heifer (5.23%) even though they were both subjected to a forage diet. Schöbitz et al., (2013) reported that NEFA concentration increased due to pasture grazing, which further decreased feed intake because of the low-mass pasture.

5.3. Basic linear relationships between Rumen and Cellular metabolism in heifers and cows

Under the NMR summary statistics, our study reported ACT, FUM and TRL in heifers and 3-HDV, ACT, BUT, D-lactic acid (D-LAC), FUM and isobutyrate (ISB) in cow's had concentrations less than 1. Winterbach et al. (1993) considered the cyclic fluctuation in acetone concentrations in blood and milk. The ratio of acetone concentration in milk compared to that in blood had a minimum of 0.114 and a maximum of 1.656 and also found out that blood-milk acetone concentration correlation varied from 0.721 to 0.992 from 6 different cows. Our minimum and maximum was 0.000 to 0.051, and 0.001 to 0.009 in heifers and cows, respectively. This was lower than reports by Winterbach et al. (1993), and there has not been much reports about acetone levels in beef cattle. Both heifers and cows had fumarate maximum values lower than 0.05. Fumarate is a major precursor for propionate formation and it plays a vital role in rumen hydrogen metabolism. Although fumarate can be in low concentration, López et al. (1999), while studying the influence of fumarate on rumen fermentation reported that fumarate increases the dry matter digestibility of basal diet and can stimulate the multiplication of cellulolytic bacteria and digestion of fibre. There has been limited reports on the level of fumarate in beef cattle. Trimethylamine has low concentrations is heifers and cows. The formation of trimethylamine N-oxide is caused by the gastrointestinal degradation of choline, betaine, and carnitine results in humans and dairy cows (McFadden and Myers., 2020). Benedet et al. (2019) explained that most studies applied the threshold of 1.2 mmol/L of hydroxybutyrate concentration in blood. Several authors have

considered hydroxybutyrate concentrations between 1.2 and 2.9 mmol/L (Benedet et al., 2019) with the maximum of 3-HDV in cows at 2.4 in our study. This 3-HDV fall in the same category as many results from researchers. There has been sparse publication on the concentration levels in cows.

Correlations within and between RM and CM categories were done to see the level of relationship between them. Rumen metabolites were highly correlated with each other however, not one analyte was dropped off since none of this relationship was a perfect one (1), there was more to be accounted for. Trimethylamine had a negative correlation with all other analytes ranging from -0.471 to -0.102. Correlations within the CM category also highly correlated to each other. Spearman's correlation was done between the RM and CM category and there was high correlations between analytes. It was not understood why trimethylamine was negatively correlated with all other analytes. There has not been reports on the relationship between trimethylamine and Rumen or Cellular analytes. Kulshreshtha et al. (2017) reported that trimethylamine is widely used as indicators of fish and meat spoilage. Trimethylamine can be primarily gotten from dietary choline and L-carnitine by bacteria in the intestine and absorbed into the hepatic portal circulating blood (Zeisel and Warrier, 2017). Trimethylamine can be further oxidized by enzymes flavin monooxygenases in the liver and then produces trimethylamine N-oxide, which is the end product and cannot be metabolized further (Bennett et al., 2013). The majority of trimethylamine N-oxide can be excreted unchanged in the urine by the kidney within twenty-four hours. There are no concluded reasons to why trimethylamine could be negatively correlated to other analytes.

Correlations between dependent variables and NMR categories were explored. There was no significant relationship found in heifers. However, there was a relationship between ADG

(0.259), G:F (0.288) and RM. The rumen is responsible for fermentation, mixing, digesting and conversion of feed through methane and CHO to produce VFAs for the animal. Du et al. (2019) reported a significant increase in ADG levels and a positive correlation (0.012) between ADG and rumen fluid in sheep. Our study reports a larger correlation with RM. This might be due to the differing size of the animals also, digestibility of feed might be related to ADG and G:F.

Spearman's correlation between NMR (glucose and urea) and single metabolites (PUN and GLC) was explored. There was a positive relationship (r = 0.2838, $P<.0001$) between NMRurea and PUN in heifers. Other relationships were not significant both in heifers and in cows. it is not understood why NMRurea and PUN had such low correlation. This is a path to explore whether higher correlations between single metabolites and NMR analytes sustain the ability to increase the adjusted R^2 over the base model thereby accounting for more variation in our dependent variables.

5.4. Prediction models using NMR categories in heifers and cows

There was a small increase in the amount of variation captured in DMI in the base model from 51.88% to 51.93% in heifers and a decrease from 56.45% to 55.0% in cows in the RM category. This suggests more variation that could not be explained by the inclusion of the RM quartile average. There was a little increase from the CM average quartile base model from 51.88% to 52.03% in heifers and a decrease from 56.45% to 55.83% in cows in the CM quartile average. However, blood metabolites have become growing areas of interest especially in their use in diagnosing human diseases and some metabolites of higher concentration can show changes in different organs in the body. For example, glucose associated with glycosynthesis in the metabolic process in muscle tissue (Zhao et al., 1996). There was a high concentration of acetate in both heifer and cow's NMR profiles compared to other metabolites in the Rumen metabolism section.

Tasha and Joslyn (2019) reported that cattle fed a forage-based diets produce a higher concentration of acetate than propionate. These metabolites are volatile fatty acids (VFAs) produced in the rumen and used by cattle for energy. The rumen wall absorbs the VFAs which provides a large proportion of the cow's energy requirements (Tasha and Joslyn., 2019). There have also been some studies that have shown that high concentration of metabolites in ruminal fluid are VFAs such as acetate, propionate, and butyrate, which are associated with microbial fermentation (Mao et al., 2012). Given the poor results we saw using the rumen metabolism approach in this study for NMR, using blood analytes might be a better and more practical approach because when the body breaks down food, it travels through the blood. Kim et al. (2021) observed metabolite profile in 5 biofluids. There was high concentration in acetate, propionate and butyrate in ruminal fluids and high concentration in lactate and glucose, and a low concentration in acetate in serum fluid. However, blood plasma and serum contain vast ranges of macromolecules that can match with peaks from small molecule metabolites in NMR under some physiological states (Beckwith-Hall et al., 2003). In the case of metabolic analysis, the use of blood plasma is preferred because it is more sensitive, useful for protein estimation, reproducibility measure and shows effective results compared to rumen fluids in biological studies.

6. CONCLUSION

This study has shown that NEFA improves prediction of DMI in both growing heifers and mature cows, which may be a tool in long-term selection of heifers or management of cows given their DMI levels. On the other hand, PUN, GLC, and NMR categories of RM and CM were not as effective in predicting DMI. Based on fit plots, the prediction of DMI using NEFA accounted for a strong prediction opportunity at any time (week 4 to 8) prior to breeding. This information can help with management categories of heifers and/or cows using NEFA for producers. Additional research is needed to explore how effective these management categories are and validate them for use in the production system. Furthermore, appropriate use of NMR data for prediction of DMI should be explored further given drawbacks of the current study.

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