Evaluation of novel traits influencing the sustainability of the beef industry: greenhouse gas fluxes, feeding and drinking behaviors

by

Elizabeth Anne Dressler

B.S., Kansas State University, 2020

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2022

Approved by:

Major Professor
Dr. Megan Rolf

Copyright

Abstract

Sustainability has recently become an area of increased focus for the beef industry. Feed and water intake behaviors as well as greenhouse gases are novel traits that could impact the sustainability of the beef industry. One of the objectives of this study was to characterize the number of spot samples required to accurately quantify methane, carbon dioxide, and oxygen gas fluxes and metabolic heat production from an individual grazing cow using an open-circuit gas quantification system (OCGQS). One-hundred spot samples from 17 grazing Angus beef cows were used to compute average gas fluxes and metabolic heat production for intervals increasing by 10 visits. The Pearson and Spearman correlations between the full 100 visits and each shortened visit interval were calculated. The recommended number of spot samples needed for the quantification of methane, carbon dioxide, oxygen, and metabolic heat production was 36-38, 40, 38-40, and 36, respectively. Animals in the current study needed 29.5 to 31.8 days to meet the required number of visits for gas fluxes and metabolic heat production. Published literature recommends a similar number of spot samples, however there is a large variation in the average number of visits per day and thus the recommended test duration. For these reasons, protocols for the OCGQS should include the number of spot samples rather than a test duration.

Another objective of this study was to estimate the genetic parameters of feed and water intake behaviors. The feed and water intake behavior phenotypes that were calculated include number of sessions (no/d), intake rate (g/s), session size (kg), time per session (s), and session interval (min) from 830 crossbred steers. Feeding behaviors were heritable and ranged from 0.35 to 0.63; drinking behaviors were also heritable and ranged from 0.54 to 0.88. Phenotypic correlations between traits and genetic correlations with DMI or DWI ranged from low to high. A genome-wide association study was performed for each feeding and drinking behavior.

Candidate genes and previously reported quantitative trait loci related to feed and water intake were identified. Results indicated that feeding and drinking behaviors are controlled by genetic factors and additional research in this area is needed to determine their role in genetic selection for improved feed and water efficiency.

Table of Contents

List of Figures viii
List of Tables ix
Acknowledgements xi
Chapter 1- Literature Review of Methane Production from Beef Cattle 1
Introduction 1
Importance of Methane Production 2
Methane Production 4
Rumen Microbiome and Dietary Factors Effect on Methane Emissions 5
Methods to Quantify Methane Emissions 8
Respiration Calorimetry 8
Sulfur Hexafluoride Tracer Technique 11
Infrared Spectroscopy 12
Prediction Models 13
Open-Circuit Gas Quantification Systems 14
Accuracy of Open-Circuit Gas Quantification systems 17
Diurnal Patterns of Methane Emissions 22
Genetic Parameters 23
Selection Strategies 28
Conclusion 29
Chapter 2 - Characterization of the Number of Spot Samples Required for Calculation of Gas Fluxes and Metabolic Heat Production using an OCGQS (GreenFeed) 31
Abstract 31
Introduction 32
Materials and Methods 34
Study Design 34
Phenotypic Data 36
Results and Discussion 38
Correlations between Intervals for CH_4 emissions 41
Correlations between Intervals for CO_2 emissions 45

Correlations between Intervals for O_2 emissions 48
Correlations between Intervals for Metabolic Heat Production 50
Conclusion 52
Chapter 3 - Genetic Evaluation of Feed and Water Intake Behaviors 64
Abstract 64
Introduction 65
Materials and Methods 66
Study Population and Design 66
Data Filtering 68
Genotyping 69
Breed Composition 69
Carcass Information 70
Behavioral Data 70
Statistical Analysis 72
Phenotypic Model Selection 72
Genetic Model Selection 73
Genetic Parameter Estimation 73
Genome-Wide Association Study 75
SNP Effect Estimation 75
Identification of QTL regions 76
Results and Discussion 76
Season and Bunk Management Phenotypic Model 79
Variance Components and Heritability Estimates 85
Phenotypic Correlations 87
Genetic Correlations 90
Genome-Wide Association Study 92
Feed Traits 92
Water traits 98
Conclusion 101
References 121
Chapter 1 121

Chapter 2 130
Chapter 3 137
Appendix A - Additional Tables 152

List of Figures

Figure 2.1- Panel A- Mean CH_4 emissions (g/d) for all animals throughout the 100 visit test, Panel B- Mean CO_2 emissions (g/d) for all animals throughout the 100 visit test, Panel C- Mean O_2 consumption (g/d) for all animals throughout the 100 visit test, Panel D- Mean metabolic heat production (kcal/d) for all animals throughout the 100 visit test. 60

Figure 2.2- Spearman and Pearson correlations between visit intervals and the full 100 visits. Panel A- CH_4 (g/d), Panel B- CO_2 (g/d), Panel C- O_2 (g/d), Panel D- metabolic heat production (kcal/d). 61

Figure 2.3- Panel A- Mean CH_4 (g/d) for all animals during visits 30 through 40, Panel B- Mean CO_2 (g/d) for all animals during visits 30 through 40, Panel C- Mean O_2 (g/d) for all animals during visits 30 through 40, Panel D- Mean metabolic heat production (kcal/d) for all animals during visits 30 through 40. 62

Figure 2.4- Spearman and Pearson correlations between shortened visit intervals between 30 to 40 visits and the full 100 visits for Panel A- CH_4 (g/d), Panel B- CO_2 (g/d), Panel C- O_2 (g/d), Panel D- metabolic heat production (kcal/d)..... 63

Figure 3.1- Manhattan plot showing the result of univariate genome-wide association mapping with a significance threshold of 4.5 for feed intake traits. A- Number of Sessions (no/d), Panel B- Intake rate (g/s), Panel C- Time per session (s), Panel D- Session Size (kg), Panel E- top 5 SNPs for Session Interval (min), Panel F- Daily feed intake (kg). 119

Figure 3.2- Manhattan plot showing the result of univariate genome-wide association mapping with a significance threshold of 4.5 for water intake traits. Panel A- Number of Sessions (no/d), Panel B- Intake rate (g/s), Panel C- Time per session (s), Panel D- Session Size (kg), Panel E- top 5 SNPs for Session Interval (min), Panel F- Daily water intake (kg). 120

List of Tables

Table 2.1-Summary statistics for gas fluxes and metabolic heat production calculated using the first 100 visits to the open-circuit gas quantification system. 53
Table 2.2- Means (SD) for all animals for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d) and metabolic heat production (kcal/d) across all visit intervals. 54
Table 2.3-Phenotypic variance of the mean forward and reverse values from all animals for each interval for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d) and metabolic heat production (kcal/d). 55
Table 2.4- Spearman and Pearson correlations for each shortened number of visits interval and the full 100 visits for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d), and metabolic heat production (kcal/d). 56
Table 2.5- Means (SD) for each shortened increment between 30 and 40 visits and the full 100 visits for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d), and metabolic heat production (kcal/d). 57
Table 2.6-Phenotypic variance of the mean forward and reverse values from all animals for interval increments between 30 and 40 visits for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d) and metabolic heat production (kcal/d). 58
Table 2.7-Spearman and Pearson correlations for each shortened number of visits interval between 30 and 40 visits and the full 100 visits for methane (CH4), carbon dioxide (CO2), oxygen (O2), and metabolic heat production (kcal/d). 59
Table 3.1-Trial dates, number of animals, season, and bunk management by group. 102
Table 3.2- Covariates that improved model fit determined by forward selection and were included in the model in addition to the base model. Base model for the genetic model included pen nested within group and three breed composition (Bos indicus, continental, and dairy). Base model for the phenotypic model included season and bunk management. 103
Table 3.3- Summary statistics for feed and water intake behavior traits 104
Table 3.4- Variance Components for feed and water intake behavior traits from bivariate analysis. Variance components for daily dry matter intake (DMI) and daily water intake (DWI) calculated as the average from each bivariate. 105

Table 3.5- Phenotypic (below diagonal) and genetic (above diagonal) correlations between feed intake behavior traits. 106
Table 3.6- Phenotypic (below diagonal) and genetic (above diagonal) correlations between water intake behavior traits. 107
Table 3.7- Genomic regions identified by genome-wide association study contributing significantly to feed intake behavior traits and dry matter intake. 108
Table 3.8- Genomic regions identified by genome-wide association study contributing significantly to water intake behavior traits. 109
Table 3.9- Previously reported QTL regions which overlapped QTL in this study identified for feed or water intake attributes. If no QTL were determined to be associated with feed or water intake behaviors, then no QTL were listed in the table. 110
Table 3.10- Significant Single Nucleotide Polymorphism (SNP) associated with feed traits with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. 116
Table 3.11- Significant Single Nucleotide Polymorphism (SNP) associated with water traits with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. 118

Acknowledgements

I would like to thank my committee, Dr. Megan Rolf, Dr. Jennifer Bormann, and Dr. Robert Weaber for all their help and guidance with these projects. Their knowledge and advice were irreplaceable to me during my time working on these projects and writing this thesis.

I'd like to thank all my friends that I have made during my time as a master's student, both in Weber 119 and across other offices and departments. I especially need to thank the students that preceded me, Ashley Hartman, Will Shaffer, Drew Lakamp, and Tamra Kott, for their encouragement, understanding, and knowledge. As well as my peer, Michael Semler for his technical help with the GreenFeed.

Finally, I'd like to thank my family for always pushing me to do my best. Without them, I would not be where I am today. My husband, Chase, is a steadfast support system in all that I do and a voice of reason in trying times.

Chapter 1- Literature Review of Methane Production from Beef Cattle

Introduction

Methane (CH_4) is the second most abundant anthropogenic greenhouse gas (GHG) after carbon dioxide (U.S. EPA, 2021). In 2020, methane accounted for 11% of total U.S. greenhouse gas emissions (U.S. EPA, 2021). In the United States, 27.1% of methane emissions come from enteric fermentation of livestock species (U.S. EPA, 2021). Ruminant livestock species such as cattle, buffalo, sheep, and goats emit methane as part of their normal digestive process. Other sources of methane are landfills, animal manure, coal mining, and natural gas systems.

Greenhouse gases can be compared by their Global Warming Potential (GWP). Global Warming Potential is the amount of energy one ton of emitted gas will absorb over a specified amount of time (normally 100 years) relative to one ton of carbon dioxide (U.S. EPA, 2021). Carbon dioxide has a GWP of one while methane is estimated to have a GWP of 28-36. (U.S. EPA, 2021). This means that one ton of methane warms the atmosphere 28 times more than an equivalent amount of carbon dioxide over 100 years. However, methane lasts in the atmosphere for far less time than carbon dioxide. On average, methane remains in the atmosphere for 12.4 years, while carbon dioxide remains in the atmosphere for thousands of years (Myhre et al., 2013).

Methane is composed of one carbon atom surrounded by 4 hydrogen atoms. The carbon atom within methane plays a role in the natural biogenic carbon cycle. The biogenic carbon cycle is centered around photosynthesis: the plant's ability to absorb and sequester carbon. During photosynthesis, plants convert atmospheric carbon primarily into cellulose. Cattle can consume the human-inedible plant material that contains cellulose and upcycle the carbon for growth,

lactation, and other metabolic processes. As a byproduct of consuming cellulose, that carbon molecule is returned to the atmosphere in the form of CH_4 when cattle eructate. Methane remains in the atmosphere for approximately 12 years before it is converted to CO_2. That carbon atom is now a part of a CO_2 molecule that plants covert to cellulose via photosynthesis. The cycle repeats all over again. Within the biogenic carbon cycle, the carbon atoms within methane are "recycled". Therefore, no "new" carbons are released into the environment as a result of cattle producing methane (CLEAR Center, 2020). Comparatively, when fossil fuels are extracted from the Earth and burned, new carbon is added to the environment and does not return to geological reserves for over 1,000 years (CLEAR Center, 2020).

Importance of Methane Production

Recently, the sustainability of the beef industry has been a popular topic in news and social media. Negative attention has been focused on the environmental impact of beef production. Non-dairy cattle are the largest animal source of enteric CH_4 followed by dairy cattle in the United States (FAOSTAT, 2019). However, there is an opportunity for cattle producers mitigate methane emissions. According to the U.S. EPA (2021), emissions from enteric fermentation has increased by 8.4% (13.9 MMT CO_2 Eq.) from 1990 to 2019. There were periods of time when emissions due to enteric fermentation fluctuated, but these periods usually followed the general trends in cattle population (U.S. EPA, 2021). Capper (2011) assessed the environmental impact of U.S. beef production in terms of resource inputs and waste outputs from 1977 to 2007. Beef production in 2007 only produced 82.3% of the CH_4, a waste output, per billion kg of beef compared to 1977 (Capper, 2011).

Methane production by beef animals impacts all three pillars of sustainability: economic viability, environmental protection, and social equity. As a greenhouse gas, methane has an

obvious impact on the environment. Methane in the atmosphere absorbs and emits radiant energy; this traps heat in the atmosphere and is why methane is considered a greenhouse gas (U.S. EPA, 2021). Social sustainability of the beef industry is entwined with the environmental impacts of methane emissions. Social sustainability includes community and organizational resilience. As a greenhouse gas, CH_4 is related to global warming, which can disrupt the livelihoods of people by making the environment and activities within it less resilient (U.S. EPA, 2021).

Economically, methane production from enteric fermentation in beef cattle represents a decrease in efficiency for cattle production. Ruminants lose 5.5% to 6.5% of gross feed intake to enteric methane production (Johnson & Ward., 1996). Johnson and Johnson (1995) estimate that emissions represent a loss of 2-12% of gross energy intake. McGinn et al. (2004) estimated that 6.5% of gross energy was lost to CH_4 production in cattle fed a barley silage and grain diet. The amount of gross energy lost to CH_4 production depends heavily on the acetic acid to propionic acid ratio in the rumen (Johnson & Johnson, 1995). The acetic acid to propionic acid ratio is altered primarily by feed source; fermentation of cell wall fibers, which is often observed in high forage/roughage diets, leads to a higher acetic acid to propionic acid ratio which causes bigger losses to CH_4 production (Johnson & Johnson, 1995). Diets that use a higher proportion of carbohydrate sources such as feedlot diets, typically lose about half the commonly predicted 6% of energy to CH_4 production (Johnson & Johnson, 1995). Thus, methanogenesis not only creates a greenhouse gas but is an energetically wasteful process. Methane produced by a ruminant does not contribute to any useful metabolic process for that animal. A proportion of the 2% to 12% of gross energy lost could have rather been used by the animal for growth or maintenance. However, methanogenesis is a part of the biological process that allows ruminants to upcycle

forage. Therefore, maintaining animal productivity while mitigating greenhouse gas emissions is crucial. Overall, enteric methane production represents a loss in productivity and efficiency which lessens economic sustainability for producers and the beef industry.

Methane Production

Methane is produced through fermentation by ruminants as a part of their normal digestive processes. Methane is an odorless and colorless gas. Microbes within the rumen work synergistically to convert human indigestible plants into short chain fatty acids and proteins (Janssen, 2010). The main products of fermentation are volatile fatty acids (VFAs) such as acetate, propionate, and butyrate (Janssen, 2010). The short chain fatty acids are primarily absorbed across the rumen wall and provide the animal with energy which allow the animal to maintain homeostasis, reproduce, lactate, and grow. However, there are by-products produced from the fermentation process such as hydrogen, ammonia, and carbon dioxide (Janssen, 2010). Methanogenic archaea in the rumen use by-products from the fermentation process to produce methane (McGovern et al., 2020).

Hydrogen produced from the fermentation process is utilized as an energy source by methanogens to reduce CO_2 to CH_4 (Hunerberg et al. 2015). Methanogens have an important digestive function in the rumen as they are responsible for removal of H_2, which otherwise could accumulate in the rumen and have an inhibitory effect on fermentation rate and microbial function (Van Kessel and Russell, 1996; McAllister and Newbold, 2008) After a feeding event, dissolved hydrogen increases in the rumen fluid. Smolenski and Robinson (1988) found that the normal background hydrogen concentration of forage fed cows was 1.0-1.4 µM but had peaks of 20 µM immediately following feed consumption. This is consistent with the results of Robinson et al. (1981), which reported that a cow fed grain and hay had a hydrogen concentration of 15

μM one hour after feeding, but this value dropped to 1 μM over time. The increase in dissolved hydrogen is followed by a peak in methane production because hydrogen is a substrate for methanogenesis (Hunerberg et al., 2015).

Ruminants produce methane through fermentation in both the rumen and hindgut. According to Murray et al. (1975), 87% of methane is produced in the rumen and 13% is produced in the hindgut. Methane is released from the animal three different ways: 1) methane produced in the rumen and hindgut is absorbed in the blood and released by expiration through the lungs, 2) methane is directly released by eructation, 3) methane is released from the hindgut in flatus (Murray et. al, 1975). Of the methane produced in the hindgut, 89% (11% of the total CH_4 produced) is absorbed into the blood and released through expiration. Only 1-3% of total methane produced is released by flatus (Murray et. al, 1975, Munoz et al. 2012). The methane produced in the rumen is dispersed primarily by eructation and a small amount expiration through the lungs.

Rumen Microbiome and Dietary Factors Effect on Methane Emissions

Cattle produce about 250 L of CH_4 per day (Czerkawski, 1969; McAllister et al., 1996). The exact amount of methane produced by an individual animal is affected by several factors. Rumen pH is one factor that affects methane production. It is well known that the addition of cereal grains/starch to a ruminant's diet causes a decrease in methane production (Van Kessel & Russel, 1996). The addition of starch causes ruminal pH to become more acidic, which likely contributes to lower methane production. A low ruminal pH has an inhibitory effect on methanogenesis (Van Kessel and Russell, 1996). Van Kessel and Russell (1996) found that in vitro CH_4 production from rumen fluid of forage-fed cows stopped when pH was below 6. They hypothesized that the methanogens were not killed at an acidic pH, but entered metabolic stasis

(Van Kessel and Russell, 1996). However, Hunerberg et al. (2015) conducted an in vivo study that measured pH using indwelling pH loggers and found that a pH as low as 5.2 did not inhibit CH_4 production. Daily mean CH_4 emission and ruminal pH were mildly correlated ($R^2 = 0.27$) (Hunerberg et al., 2015). Because of the low R^2, other factors such as propionate formation and passage rate likely contribute to the lower CH_4 emissions in cattle fed high-grain diets compared to high-forage diets (Hunerberg et al., 2015). When cattle are suddenly switched from a forage to grain diet, there is a dramatic drop in ruminal pH, which is called acute acidosis (Owens et al., 1998). During acute acidosis, the fermentation end-products are altered from acetate, propionate, and butyrate to primarily lactate and methanogenesis is paused (Van Kessel and Russel, 1996; Hunerberg et al., 2015). However, intentionally inducing acute acidosis by altering diets to include more grains is not an efficient CH_4 mitigation strategy due to the negative impact on animal health (Hunerberg et al., 2015).

It is well established that dietary fat causes a decrease in methane production and an increase in propionate formation (Johnson and Johnson, 1995; McGinn et al., 2004). The addition of long chain poly-unsaturated fatty acids to a diet decreases methanogenesis by providing an alternative hydrogen acceptor for the reduction of carbon dioxide (Czerkawski et al., 1966). The addition of supplemental fat such as animal tallow or soybean oil causes decreased CH_4 production (Van der Honing et al., 1981), though the reduction of methane was attributed to decreased fermentable substrate in this study. McGinn et al. (2004) found that when steers fed a forage-based diet were supplemented with sunflower oil, gross energy lost to CH_4 production decreased by 21%. The addition of sunflower oil was also associated with a 20% decrease in total-tract digestibility of neutral detergent fiber (NDF; McGinn et al., 2004). The reduction in fiber digestion is likely what caused the decrease in methane production. In contrast,

Hales and Cole (2017) found that the diet with the highest dietary fat (steam-flaked corn base and 45% wet distillers grains with solubles (WDGS)) in their experiment had the highest hourly and total methane production. The reason for this discrepancy could be the source of fat. The diet Hales and Cole (2017) found to have the highest methane production used WDGS as the primary fat source. The inclusion of WDGS in a diet increases fat concentration but also increases the proportion of protein and NDF, which is likely what caused the increase in hourly CH_4 production for this diet (Hales & Cole, 2017). There are several factors that affect methane production, but source and interactions between these factors are important for an accurate prediction/assumption of CH_4 production.

Feed processing technique is another factor that affects methane production. Blaxter (1989) found that grinding and pelleting a forage source decreased CH_4 production. This is likely because processing the forage increases the passage rate. Processing the feed results in smaller particle sizes so feed exits the rumen more easily and microbial degradation is faster (Janssen, 2010). Hales & Cole (2017) tested hourly CH_4 production rates when feeding diets composed of varying levels of corn processing (dry-rolled, steam-flaked, and WDGS). Starch digestibility increases when corn is more processed. The inclusion of WDGS prolonged peak CH_4 production while cattle fed diets with steam flaked corn had peak CH_4 production sooner (Hales & Cole, 2017).

Another factor that affects CH_4 production is ruminal passage rate or feed disappearance rate. The ruminal passage rate is controlled by feed but also by the individual animal to some extent (Janssen, 2010). In general, concentrate feeds have a higher passage rate than forage feeds, meaning forages move through the rumen and are degraded more slowly. The passage rate is lower for low quality, less readily digestible feeds. When the passage rate is higher, more

emphasis is put on digestion in the abomasum and lower digestive tract. Pinares-Patino et al. (2003) found that in a group of 10 sheep, passage rate and CH_4 production were negatively correlated. Increasing the passage rate through the rumen is associated with lower CH_4 production per unit of feed digested in the rumen (Janssen, 2010). This can somewhat be attributed to undigested feed passing through the rumen at higher passage rates. However, higher passage rates are associated with alternative fermentation pathways that result in more propionate and less hydrogen, and therefore less CH_4.

More propionate is produced as a proportion of total fermentation products when a ruminant is fed a grain diet compared to a forage diet (Beauchemin & McGinn, 2005). Tajima et al. (2000) observed that rumens of cows adjusted to a grain diet contained more bacteria in the *Selenomonas-Succiniclasticum-Megasphaera* group in *Clostridium* cluster IX than cows fed forage diets. This group of bacteria produce more propionate as a major fermentation product (Janssen, 2010). Increases in propionate formation are strongly associated with decreased CH_4 production, because propionate production and methanogenesis are competing pathways (Beauchemin & McGinn, 2005; Janssen, 2010).

Ruminants fed diets with a high proportion of forage or fibrous plant material produce more CH_4 per unit of feed digested (Beauchemin & McGinn, 2005). Johnson and Johnson (1995) determined this difference was due to the lower digestibility of cellulose and hemicellulose in forage.

Methods to Quantify Methane Emissions

Respiration Calorimetry

Respiration calorimetry techniques include whole-animal chambers, head boxes, ventilated hoods, and face masks. Respiration chambers are considered the gold standard for

methane emission measurement, though every system has its strengths and weaknesses. Respiration chambers are a whole-animal open-circuit "room" used to measure respiratory exchange and gas fluxes. Inflowing air is circulated in the chamber and mixed with emitted gases. The amount of gas emitted can be found by comparing the concentration of that gas in the ingoing and outgoing air (Hammond et al., 2016).

The main advantage of whole animal respiration chambers is that it is one of the only techniques that captures both ruminal and hindgut methane emissions. Whole animal respiration chambers capture the estimated 1-3% of emissions that occur in flatus (Murray et al., 1975; Munoz et al., 2012). Respiration hoods and headboxes do not capture hindgut methane emissions. However, respiration chambers are expensive and labor-intensive (Arthur et al., 2017). They are expensive to construct and maintain, and extensive labor is required for animal training and care (Johnson & Johnson, 1995). These factors often limit the number of animals that can be measured. A sufficient sample size is imperative for genetic improvement studies, so these systems pose a major limitation. Studies that use respiration chambers generally have high quality data but require more time and resources to obtain a sufficient sample size compared to other techniques.

Respiration chambers require the animal to be pulled from their normal environment and housed individually. This often causes changes in animal behavior and a lower dry matter intake (DMI). For example, in a study done by McGinn et al. (2004), steers were moved from their normal outside pens to the respiration chambers, resulting in a decrease in DMI of 15% to 19%. Sheep in respiration chambers have 15% to 25% lower feed intake compared to their feed intake the previous week in individual indoor home pens (Bickell et al., 2014). The decrease in DMI associated with respiration chambers is likely due to the stress. Animals using a respiration

chamber can experience stress from relocation and feeding pattern disruption. A lower DMI leads to an underestimation of methane emission, which may be the most severe in the most stressed animals- confounding two different traits. Therefore, the methane production observed in a respiration chamber can be lower than the actual production in the animal's normal environment.

Respiration chambers have two main sources of variation: airflow rate and air mixing in the chamber. Gardiner et al. 2015 found that ducting/airflow and chamber mixing have 15.3% and 3.4% variability between the results of different facilities, respectively. For respiration chambers to be accurate, they must be properly calibrated and have a 100% gas recovery rate (Gardiner et al. 2015). Thus, respiration chambers must be airtight to prevent air loss or entry of outside air. Modern respiration chambers are designed so that the door must be open for up to an hour every day for animal feeding, milking, cleaning, etc. (Hristov et al. 2018). Typically, the one-hour time "gap" is excluded from data analysis. However, this practice could lead to inaccurate methane measurements because methane emission is not constant throughout the day. Methane emissions follow a diurnal pattern dependent upon time of feed intake and have significant hour-to-hour variation. For dairy cows, the peak hourly rate of emission can be three times greater than the minimum hourly rate (Hristov et al. 2018). So, excluding an hour from data analysis near feeding time could lead to error in results.

In addition, the environment in the respiration chamber is artificial and does not represent a production environment. Energy expenditure in respiration chambers is lower than a production setting due to a reduction in space to move and less exposure to variable environmental conditions (Arthur et al., 2018). A reduction in energy expenditure is another possible reason that DMI is reduced for animals in respiration chambers (Llonch et al., 2016).

Respiration chambers, while generally being highly accurate, lack application in a production environment.

Sulfur Hexafluoride Tracer Technique

The Sulfur Hexafluoride (SF_6) tracer technique was one of the first techniques developed to measure gas emissions in an open-air environment without confinement. The SF_6 technique was develop in the early 1990's by Patrick Zimmerman (Zimmerman, 1993). Sulfur hexafluoride is used as a tracer gas to measure CH_4 emissions of ruminants, because it is synthetic and not produced in any biological process. It is also easily measured and traceable at low concentrations, a requirement for a tracer gas technique (Hill et al., 2016). An inert bolus containing liquid SF_6 is placed in the rumen of the animal. The SF_6 is slowly released from the bolus in gaseous form through permeations in the bolus. The animal wears a halter with a capillary tube that is connected to an evacuated sample container on its back or an inflatable neck collar. The vacuum in the sampling container collects the metabolic and tracer gas from the nose and mouth. After the trial, CH_4 and SF_6 concentrations are determined using the known permeation rate of SF_6 from the bolus and the mixing ratio of gases collected in the sampling container (Zimmerman, 1993).

The advantage of the SF_6 technique is that animals are not required to be restrained or enclosed in a chamber (Gunter and Beck, 2018). Therefore, animals are free to move and graze in their normal environment. There are several disadvantages of using the SF_6 technique. One disadvantage is the extensive labor required. The animals must be trained to wear the halter and the sampling container, which is laborious (Gunter and Beck, 2018). In addition, labor is required to insert the bolus into the animal's rumen. Due to the extensive labor, this technique is typically only used in short duration with a small number of animals, which limits possible

applications for genetic improvement. Another disadvantage is that this technique does not account for methane released as rectal flatus (Gunter and Beck, 2018; Murray et. al, 1975). The SF_6 tracer technique often biases the CH_4 measurements due to the diurnal patterns of methane emissions (Gunter and Beck, 2018). The collection canisters are removed and replaced every 24 hours. If the canister is replaced during a distinct bout of feeding and subsequent CH_4 emission, the greatest sampling rate occurs when CH_4 emission is the highest, leading to an overestimation of emissions (Berndt et al., 2014).

The SF_6 tracer technique is dependent upon low background gas concentrations so that differences in CH_4 emissions can be detected (Williams et al., 2011). However, if this technique is used on animals housed indoors, large variability in background gas concentration is often an issue due to poor ventilation (Williams et al., 2011; Dorich et al., 2014). Variability in background gas concentration negatively impacts the precision of the SF_6 tracer technique.

Infrared Spectroscopy

Infrared spectroscopy is a method to measure CH_4 primarily used in dairy cattle. One method, Fourier transform infrared (FTIR) uses infrared transmission spectrum to identify an absorbance spectrum from an air sample (Teye et al., 2009). Then gas densities can be calculated for each sample using the absorbance spectrum. Another infrared spectroscopy method of gas quantification is based on mid-infrared spectra. Infrared spectroscopy methods have the advantage that they are non-invasive, and animals can remain in normal production environments during collection. However, measurements are highly variable and require several hundred measurements during a short period of time to quantify individual animal means (Lassen & Løvendahl, 2015). This is one reason why this method is primarily only used for dairy cows during times of feeding or milking.

Prediction Models

Rather than directly measuring methane emissions using a quantification technique, CH_4 emissions can be predicted using mathematical models. These models can be classified as either empirical/statistical or mechanistic (Kebreab et al., 2016). Empirical models directly relate nutrient intake to CH_4 emissions whereas mechanistic models predict CH_4 emissions by stimulating the underlying process of fermentation (Kebreab et al., 2016).

Measuring CH_4 production is often expensive and requires complex equipment. An advantage to using prediction models is that it does not require any additional equipment to quantify CH_4 (Kebreab et al., 2016). In addition, empirical prediction models are relatively simple and require fewer input variables making empirical models a more practical option compared to mechanistic models (Appuhamy et al., 2016).

A vast number of prediction models exist with a range of different data inputs from DMI to milk production characteristics. For example, Dijkstra et al. (2011) developed methane prediction equations based on milk composition for dairy cattle. Milk fatty acids are suggested to be an indicator of rumen conditions and methane production because certain fatty acids are absorbed into the blood and can be used in the mammary gland for milk fatty acid production (van Engelen et al., 2015). Other prediction models use input variables such as body weight, DMI, or feed characteristics such as total digestible nutrients (Uemoto et al. 2020; Mills et al., 2003; Moe and Tyrrell, 1979).

However, there are several drawbacks to prediction models. The predictive power of the model depends upon the accuracy of the mathematical equation and the data inputs used in that equation (Kebreab et al., 2016). When using mechanistic models, errors in feed intake estimation directly leads to errors in predicted CH_4 (Kebreab et al., 2016). Errors in estimating feed intake,

stoichiometry of volatile fatty acids, and rumen fermentation conditions were identified by Bannink et al (2011) as the most likely sources of uncertainty in mechanistic models. Kebreab et al. (2016) advised that in a pasture system, empirical models are not good predictors of CH_4 because DMI can not be reliably measured in these systems. Empirical models often include a measure of feed intake which is often not available for individual animals in a commercial operation (Hristov et al., 2018). Another disadvantage of prediction models is that the model assumptions may not be met in all situations, especially if trying to apply to a commercial livestock operation (Kebreab et al., 2016). For example, one assumption is that animals are healthy and not effected by environmental conditions. However, this ideal scenario is rarely representative of all animals.

One of the biggest drawbacks to prediction models is that prediction models do not provide individual animal information distinct from differences in feed intake (Lakamp, 2021). A variable that represents feed intake is always included in a prediction model. Therefore, methane production is calculated as a function of feed intake. This is problematic because when feed intake increases, predicted methane will also be higher.

In general, prediction models can be useful especially if all necessary variables are readily available or quantification equipment is not available. However, there are several disadvantages to prediction models and the accuracy of the predicted estimates need to be scrutinized on an individual basis. If possible, a gas quantification technology would be preferred, especially for genetic evaluation.

Open-Circuit Gas Quantification Systems

An open-circuit gas quantification system (OCGQS) is an automated technology that quantifies gas fluxes by exhausting air past the animal's head and into the system (Hristov et al.,

2015). One example of these types of systems is the GreenFeed system (C-Lock, Inc., Rapid City, South Dakota). The GreenFeed measures individual methane, carbon dioxide, hydrogen, and oxygen gas fluxes for a variety of livestock species. The GreenFeed entices animals to visit the unit multiple times a day by releasing a small amount of pelleted feed as bait. Individual animals will insert their head into the hood and the unit will measure these gas fluxes as air is continuously drawn past the head of the animal. Eructated gas concentrations are compared to the background gas concentration. The GreenFeed collects several short-term breath samples throughout the day to calculate gas production rates (Herd et al., 2020). Measurements are an accumulation of spot samples, unlike the continuous sampling of respiration chambers and the SF_6 technique. Data collected by the GreenFeed is stored and uploaded to a remote web interface.

The GreenFeed pasture system is mounted on a trailer, so the system is portable between pastures. A GreenFeed can be moved with a herd during a rotational grazing program. Panels are mounted at the entrance of the GreenFeed so that only one animal can enter at once and the collection is not affected by nearby animals. The GreenFeed is equipped with a wind anemometer and a weather station to adjust to the variables inherent in a changing environment.

One of the main advantages of the GreenFeed is that data can be collected on grazing animals in a pasture setting. It is ideal for methane emissions of grazing animals to be determined at grazing so that data is representative of diet and grazing behavior (Waghorn et. al., 2016). In contrast, cows in respiration chambers fed cut pasture differ in forage and feeding patterns compared to grazing animals (Waghorn et al., 2016) which will affect methanogenesis (Jonker et al., 2014). Another advantage of the GreenFeed is that animals are unencumbered by respiration equipment or respiration chambers and do not require extensive training.

The precision of gas flux data collected by the GreenFeed is influenced by several factors. One factor that can have a major impact on the accuracy and quality of emission estimates is mass airflow rate. The proper air flow rate ensures that the complete breath cloud emitted from the animal is captured by the GreenFeed. Gunter et al. (2017) analyzed emission estimates with a range of airflow rates and found that when the air flow rate was above 26.0 L/s, CO_2 and CH_4 estimates were not affected. However, when air flow rates are below 26.0 L/s, CO2 and CH4 emission estimates decreased as airflow rates decreased ($P < 0.0001$; Gunter et al., 2017). Gunter et al. (2017) speculated that the reason for a decrease in CO_2 and CH_4 emissions during lower air flow rates was due to the animals emitted breath cloud not being completely captured by the GreenFeed. Maintaining clean air filters for proper air flow rates is imperative for accurate estimation of emissions.

Another important aspect of the GreenFeed that needs to be considered is feed bait delivery interval and amount of feed released. Once the animal inserts its head into the intake manifold, feed is released several times as long as the animal maintains adequate head position. The amount of time between feed drops is the bait delivery interval and can be adjusted depending upon the trial. Gunter et al. (2017b) conducted two experiments where alfalfa pellets were fed at eight different timed intervals up to 8 times per visit. Carbon dioxide and oxygen (Exp. 2 only) emission estimates were not affected by timed interval. However, CH_4 estimates and the ratio of CH_4:CO_2 linearly decreased ($P < 0.01$) with an increase time interval in Exp. 1, but were not different in Exp. 2 (Gunter et al., 2017b). Increasing the time interval increased ($P < 0.01$) the total amount of time that animals spent in the intake manifold (Gunter et al., 2017b). Total visit duration can be increased by increasing the time interval between drops. Visits that are longer in duration more accurately capture the CH_4 emissions from eructation events.

The total amount of time that an animal spends with its head in the intake manifold is a crucial aspect of collecting high-quality data. Arthur et al. (2017) compared 2-minute and 3-minute minimum GreenFeed visit durations. Emission records with a minimum visit duration of two minutes were significantly ($P < 0.001$) more heterogeneous than records with a three-minute minimum visit duration (Arthur et al. 2017). The authors concluded that to calculate an animals methane production rate (MPR) and carbon dioxide production rate (CPR), a minimum visit duration of 3 minutes is required. Suggested ways to increase visit duration are: 1) adjusting the interval and amount of feed drops, 2) additional training for cattle using GreenFeed, 3) decrease the number of animals using one unit, 4) switch to a more palatable feed.

The total number of valid collection visits on an individual animal during the trial period needs to be carefully considered. Arthur et al. (2017) evaluated the reduction in variance as more records on an individual are added to a dataset. There was a sharp reduction in variance as more records for each individual animal were added to the dataset (Arthur et al., 2017). The reduction in variance was so rapid that the initial variance at 5 records was reduced by over 50% at 20 records. However, after 30 records (3-min visit duration) were included in the dataset for each animal, there was no substantial reduction in variance. To achieve the same reduction in variance when the visit was only 2-minutes in duration, the minimum number of records needed was 40-45 (Arthur et al., 2017). Arthur et al. (2017) concluded that at least 30 records on an individual animal were needed to reduce variance and increase precision.

Accuracy of Open-Circuit Gas Quantification systems

The accuracy of the GreenFeed has been validated in several studies by comparing the results of the Greenfeed system to respiration chambers and the SF_6 tracer method.

Dorich et al. (2014) compared enteric CH_4 emissions of 16 Holstein cows on a common diet using the GreenFeed system and the SF_6 tracer method. Dorich et al. (2014) found the GreenFeed system to be a reliable method of gas quantification with relatively low coefficients of variation (14.1% to 22.4%) for CH_4 emissions. In comparison, the coefficient of variation for CH_4 emissions using a SF_6 were up to 5-fold greater (16.0% to 111%) than the GreenFeed system (Dorich et al., 2014).

Herd et al. (2016) evaluated methane emission traits on animals with a GreenFeed system and in respiration chambers. The GreenFeed was set up in feedlot conditions where animals had access to an ad-libitum grain-based diet whereas within the respiration chambers the animals had two trials: a restricted grain-based diet and a restricted roughage diet. Methane yield (MY) and residual methane production (RMP) had moderate positive phenotypic correlations (0.54 - 0.58) between the GreenFeed feedlot test and the roughage respiration chamber test (Herd et al., 2016). In this study, the GreenFeed feedlot test was performed 73 d after the chamber roughage test suggesting strong repeatability.

Hammond et al. (2013) compared CH_4 production in growing dairy heifers using a GreenFeed to values found using a respiration chamber or SF_6 technique and reported that they had similar ($P > 0.10$) CH_4 emission (g/d) estimates. However, Hammond et al. (2013) did find that the SF_6 technique estimated higher ($P < 0.001$) methane emission values than the GreenFeed. The authors speculated that the difference could have been due to different housing conditions (grazing vs. indoor) or the accuracy of the SF_6 technique data. The patterns of methane emissions were comparable for the GreenFeed and the respiration chamber (Hammond et al., 2013). Overall, Hammond et al. (2013) concluded that the GreenFeed system can accurately estimate CH_4 emissions from livestock.

In 2015, Hammond et al. found differing results to their previous study mentioned above published in 2013. Hammond et al. (2015) aimed to compare measurements from a GreenFeed to a respiration chamber and SF_6 technique. There were three experiments, all with different diets. Two experiments used four animals and the third experiment used 12 animals total. The average methane emissions (g/d) measured using the GreenFeed were numerically similar to the averages from the respiration chamber, however they were not statistically significant (203 g/d vs. 213.5 g/d). However, the Lin's concordance correlation coefficient, which determines if the difference between the respiration chamber and GreenFeed for each experiment was different from zero, was poor (0.1043) (Hammond et al., 2015). The GreenFeed did not identify the significant treatment effects on methane emission that the respiration chamber and SF6 technique did (Hammond et al., 2015). The authors discussed that the reason the GreenFeed did not detect the treatment effects was due to a limited number of animals and the timing of measurements taken with the GreenFeed.

Validating GreenFeed as a gas quantification system has been done by other groups of researchers by comparing the GreenFeed to respiration chambers and the SF_6 technique. Jonker et al. (2016) compared CH_4 and CO_2 measurements from a respiration chamber, a GreenFeed, and the SF_6 technique with a small sample size of eight beef heifers. The CH_4 yield estimates from the GreenFeed did not differ from the estimates from the respiration chamber (Jonker et al., 2016). However, the CH_4 yield estimates from the GreenFeed were greater ($P < 0.02$) than the estimates from the SF_6 technique (Jonker et al., 2016). Measurement method did not affect CO_2 values expect for in one period out of five periods total (Period 2; $P < 0.001$), in which the respiration chambers had higher values than the GreenFeed and SF_6 technique (Jonker et al., 2016). Jonker et al. (2016) concluded that the GreenFeed system provided CH_4 yields that were

not different from the respiration chamber, evidence that the GreenFeed is a comparable technique to measure gas fluxes.

Waghorn et al. (2013) measured CH_4 emissions of grazing dairy cows using a GreenFeed system. Methane emissions were calculated on individual animals from predictive equations based on milk production and body weight change. Measurements from the GreenFeed and calculated CH_4 emissions were positively correlated ($R^2 = 0.72$; $P = 0.004$).

Nitrate has been identified as an alternative non-protein nitrogen source to urea and has evidence for reducing enteric methane emissions (Nolan et al., 2010). Velazco et al. (2013) supplemented feedlot steers with either urea or nitrate and measured gas fluxes with the GreenFeed system. Dietary nitrate supplementation significantly ($P < 0.05$) reduced methane yield (g CH_4/ kg DMI) (Velazco et al., 2013). This is evidence that the GreenFeed system can detect differences in emissions from mitigation strategies.

Alemu et al. (2017) grouped animals into a high-residual feed intake (RFI) or low-RFI group based on data from the GrowSafe system (Calgary Alberta, Canada). Animals had gas flux measurements taken using both the GreenFeed system and a respiration chamber. The respiration chamber had greater variation (CV = 19.9%) in CH_4 estimates among animals compared to the GreenFeed (CV = 14.3%). Estimates for CH_4 production (g/d) were greater ($P < 0.001$) for the GreenFeed than the respiration chamber for both groups and CH_4 yield (g/kg DMI) differed ($P = 0.01$) for the high-RFI group (Alemu et al., 2017). This means that the GreenFeed and respiration chambers had similar estimates for CH_4 yield but differed on daily CH_4 emissions (Alemu et al., 2017). The authors hypothesized that the reason the GreenFeed estimated greater CH_4 emissions than the respiration chamber was because animals in the respiration chambers had 19% to 20% lower DMI than the animals housed in group pens (Alemu et al., 2017). The differences in CH4

emissions between the respiration chamber and GreenFeed were likely due to the conditional differences between the systems, proving the difficulty in directly comparing the two systems.

McGinn et al. (2021) used a novel method to compare the GreenFeed system to a respiration chamber. Instead of using animals, McGinn et al. (2021) used a mass flow controller to release known concentrations of CH_4. The mass flow controller released CH_4 into the GreenFeed in an open environment meant to represent a pasture setting with low background concentrations. McGinn et al. (2021) also directly compared the respiration chamber to the GreenFeed by placing the GreenFeed inside of the respiration chamber. The mass flow controller released a known amount of CH_4 inside the respiration chamber and collected CH_4 emission rates (g/d) from the GreenFeed and the respiration chamber. This was designed to represent a barn with the potential to have higher background concentrations. There was a significant difference between the CO_2 emission rates between the GreenFeed and the mass flow controller (outside of the respiration chamber; $P = 0.013$) and between the GreenFeed and the respiration chamber ($P = 0.007$; McGinn et al., 2021). When CH_4 emission rates were compared, there was not a significant difference ($P = 0.726$) between the GreenFeed and the mass flow controller. There was a small, but significant difference in CH_4 emission rates between the GreenFeed and the respiration chamber (328 vs. 323 g/d; $P = 0.019$). McGinn et al. (2021) later found that the difference in CO_2 emission rates between the GreenFeed and the mass flow controller was due to a systematic error. The authors concluded that the GreenFeed system has the potential to accurately measure emission rates in both an open environment and a barn (McGinn et al., 2021).

A meta-analysis done by Huhtanen et al. (2019) aimed to compare CH_4 production measured from the GreenFeed to CH_4 production prediction equations. Some datasets included respiration chamber values which allowed for a direct comparison to the GreenFeed. Eighteen

different empirical equations based on intake and nutrient composition were selected from literature to represent different datasets. Equations to predict CH_4 production were selected based on the variables available in the dataset: feed intake only, intake and nutrient composition, and CH_4 yield and intake (Huhtanen et al., 2019). The meta-analysis included 83 treatment means from both dairy and growing beef animals with a wide range of diet, nutrient composition, housing type, and environmental conditions. Huhtanen et al. (2019) found that methane production measured by the GreenFeed and predicted by the equations were closely related (R^2 values in most cases > 0.90). In direct comparisons (n = 20), methane production measured by the GreenFeed and respiration chamber were closely associated with a high R^2 ($R^2 = 0.92$) and a high concordance correlation coefficient (CCC = 0.95; Huhtanen et al., 2019). These results indicate that the GreenFeed measured CH_4 emissions that agreed well with emissions calculated from empirical models from respiration chamber data. This indirectly suggests that the GreenFeed can accurately measure CH_4 emissions (Huhtanen et al., 2019).

Diurnal Patterns of Methane Emissions

One concern with the GreenFeed is related to the variation in methane emissions due to circadian rhythms. Methane is emitted in pulses that vary in concentration and volume, unlike carbon dioxide which is emitted more constantly (Gunter and Beck. 2018). Methane release rates for grazing ruminants follow a "diurnal biphasic pattern" with peaks in the mid-morning and late afternoon (Hegarty 2013). The peaks in emissions likely correspond with periods of more intensive grazing (Champion et al., 1994).

The rate and variation of methane production throughout the day is highly influenced by feed intake. Typically, cattle that are "meal-fed" (feedlots) have CH_4 production that is different than grazing cattle because cattle that eat intermittently while grazing throughout the day have

smaller changes in CH_4 production over the day compared to "meal-fed" cattle. Baxter and Clapperton (1965) found that sheep with constant feeding had small day-to-day variation in CH_4 production. Cattle that have access to ad libitum feed have more consistent CH_4 production therefore, the GreenFeed should be able to accurately measure CH_4 emissions using "spot samples" (Gunter and Beck, 2018).

Genetic Parameters

Heritability and genetic parameter estimates for methane production in literature are fairly limited for beef cattle. Some research has been done in dairy and concentrate-fed confined beef cattle; however, research is limited for grazing beef cattle. Methane emissions vary widely depending upon feed intake, body weight, feed type, etc., which are all factors that are different between beef and dairy cattle. However, heritability estimates from dairy cattle can give insight into beef cattle as they are both the same species.

Three CH_4 phenotypes including CH_4:$C0_2$ ratio, CH_4 production (g/d) measured over a week, and CH_4 intensity (g CH_4/L milk produced), were measured for 3,121 Holstein dairy cows using an automatic milking system and FTIF detection (Lassen & Løvendahl, 2015). The FTIF method uses an infrared transmission spectrum to find what wavelength an air sample absorbs and then is calibrated to provide the gas density of that air sample (Lassen & Løvendahl, 2015). Both CH_4 production and CH_4 intensity had heritabilities of 0.21 ± 0.06 and CH_4:$C0_2$ ratio had a heritability of 0.16 ± 0.04 (Lassen & Løvendahl, 2015). Interestingly, CH_4 production and CH_4:$C0_2$ ratio had strong genetic correlations to fat- and protein-corrected milk yeild, (0.43 ± 0.10 and 0.37 ± 0.07 respectively; Lassen & Løvendahl, 2015). This suggests that CH_4 production in dairy cattle is a heritable trait and that a strong genetic potential for milk production could mean a strong genetic potential for CH_4 emission.

Methane production can be predicted using milk composition in dairy cows. van Engelen et al. (2015) used milk fatty acid profile in three different methane yield prediction equations to estimate CH_4 yield (g/kg DMI) of 1,905 Holstein-Friesian cows. The heritability estimates from the three different equations for CH_4 yield were 0.12 ± 0.06, 0.20 ± 0.07, and 0.44 ± 0.10 (van Engelen et al., 2015). Methane yield based on milk fat composition is heritable.

Pickering et al. (2014) used feed intake, milk yield, live weight, and condition scores to predict methane emissions of 1,726 dairy cows. Laser methane detector data was used to find repeated measurements from 57 cows. Predicted methane emissions (PME) was calculated daily from morning and evening milkings then averaged for each week of lactation. The laser methane detector was found to not be suitable for genetic prediction due to the small number of observations available in this study (Pickering et al., 2014). PME (g/d) had a mean heritability of 0.13 ± 0.04 across 44 weeks of lactation (Pickering et al., 2014). Interestingly, the heritability of PME stayed relatively stable across the 44 weeks of lactation measured.

Methane emissions were predicted from feed intake, milk and body weight data on 548 Holstein-Friesian heifers (de Haas et al., 2011). Predicted CH_4 emissions gradually increased throughout lactation until it reached a plateau around 400 g/d in mid-lactation until the end of lactation (de Haas et al., 2011). de Haas et al. (2011) estimated that predicted CH_4 emissions had a heritability of 0.35 ± 0.12 for week 0 through week 42 of lactation. Heritabilities estimates varied between weeks of lactation from 0.29 to 0.42 with standard errors ranging from 0.10 to 0.12 (de Haas et al., 2011). Feed intake data collected from an automated feeders was used to calculate residual feed intake (RFI) and dry matter intake (DMI). Predicted CH_4 emissions had a strong positive phenotypic correlation with RFI, indicating that animals with lower RFI also would have lower predicted CH_4 emissions (de Haas et al., 2011).

Kandel et al. (2017) studied two milk mid-infrared based CH_4 proxies, PME and log-transformed CH_4 intensity (LMI). The fatty acid profile was predicted using mid-infrared spectrometry and then an equation developed by Vanlierde et al. (2015) was used to find PME given the mid-infrared milk information (Kandel et al., 2017). Log-transformed CH_4 intensity was found by log-transforming the ratio of PME over daily methane yield. Kandel et al. (2017) studied both first (n = 56,957) and second (n = 34,992) parity cows. The heritability of PME was moderate and slightly decreased from first to second lactation, 0.25 ± 0.01 and 0.22 ± 0.01 (Kandel et al., 2017). The heritability of LMI was 0.18 ± 0.01 for first lactation and 0.17 ±0.02 for second lactation (Kandel et al., 2017). Between first and second lactation, PME increased (433 g/d vs. 453g/d) while LMI decreased (2.93 vs. 2.86; Kandel et al., 2017). The authors suggested that the rankings of animals were similar between the two lactations based on the high Spearman correlation values for PME and LMI, 0.92 and 0.95 respectively (Kandel et al., 2017). Kandel et al. (2017) explained that the differences in values observed between first and second lactation were due to changes in feed intake, feed efficiency, energy partitioning, and milk production.

Although a different species, sheep are a grazing ruminant animal that also produce methane. Sheep are typically less expensive to manage and are easier to handle offering a potential proxy to cattle for CH_4 emissions research. Pinares-Patiño et al. (2013) measured methane production (g CH_4/d) and methane yield (g CH_4/DMI) from 1225 sheep in respiration chambers. The heritability of CH_4 production and CH_4 yield was 0.29 ± 0.05 and 0.13 ± 0.03, respectively (Pinares-Patiño et al., 2013). Measurements in respiration chambers were repeated 14 days later to assess repeatability. Methane production and CH_4 yield had repeatabilities of

0.55 ± 0.02 and 0.26 ± 0.02, respectively (Pinares-Patiño et al., 2013). The results of this study indicate that CH_4 emission traits are heritable and repeatable for sheep.

The cow-calf sector of the beef industry includes the largest number of animals compared to other sectors. The cow-calf sector is estimated to contribute 68% to 80% of the total greenhouse gas emissions from the beef life cycle (Stackhouse-Lawson et al., 2012; Beauchemin et al., 2010). Therefore, it is imperative that methane production heritability estimates are representative the sector of the beef industry that is producing the majority of CH_4 emissions.

Hayes et al. (2016) derived genomic estimated breeding values (GEBV) for methane traits on 747 Angus cattle with a validation set of 273 Angus cattle. All animals in this study were born and raised on pasture, expect for the period of methane measurement where they were fed a roughage diet consisting of alfalfa and oaten hay chaff. Methane production rate (MPR; g/d), methane yield (MY; g/kg), and four residual methane traits were measured in respiration chambers. The estimated heritability derived from genomic information for MPR was 0.28 ± 0.06 and 0.20 ± 0.05 for MY (Hayes et al., 2016). Heritabilities were also derived from pedigree information for MPR (0.27 ± 0.06) and MY (0.22 ± 0.06). The accuracies of GEBV calculated from genomic BLUP for all traits included in the analysis ranged from 0.26 to 0.38 (Hayes et al., 2016).

Manzanilla-Pech et al. (2016) estimated heritabilities for a variety of methane traits on 1,020 Angus beef cattle collected with respiration chambers and in two validation populations of Holstein dairy cows collected with the SF_6 tracer technique. The CH_4 traits evaluated for the Angus population were methane production (MeP; g/d), methane yield (MeY; g/ kg DMI), methane intensity (MeI; g/ kg product), residual phenotypic methane (RPM), and residual genetic methane (RGM). The estimated heritabilities for MeP, MeY, MeI, RPM, and RGM in the

Angus population were 0.30 ± 0.06, 0.20 ± 0.05, 0.25 ± 0.06, 0.19 ± 0.05, and 0.15 ± 0.05, respectively (Manzanilla-Pech et al., 2016). Heritabilities for the Holstein population were only evaluated for 3 methane traits and different values were found. The estimated heritability for MeP, MeY, and MeI were 0.23, 0.30, and 0.42, respectively (Manzanilla-Pech et al., 2016). It is unknown whether the difference in heritability estimates was due to genetics or the smaller population size and higher associated standard errors (approximately 0.23). The authors concluded that CH_4 is a moderately heritable trait, and several factors need to be evaluated to determine which trait is the "best" measure of CH_4 emissions.

Donoghue et al. (2016) found genetic and phenotypic variances and covariances estimates for methane emission traits. Using largely the same animals as Manzanilla-Pech et al. (2016) and Hayes et al. (2016), this study included data on Angus 1,046 animals that were born and raised on pasture. Methane emissions were measured in a respiration chamber for two days while animals ate a roughage-based diet. The traits evaluated were MPR, MY and 4 residual methane production traits as well as production traits such as birth weight (BW), weaning weight (WW), yearling weight (YW), final weight (FW). Carcass traits such as ultrasound measures of eye muscle area (EMA), rump fat depth, rib fat depth, and intramuscular fat were also included. One objective of this study was to estimate phenotypic and genetic correlations between the methane and production traits (Donoghue et al., 2016). Donoghue et al. (2016) estimated the heritability of MPR and MY to be 0.27 ± 0.07 and 0.22 ± 0.06, respectively. All four forms of residual methane had an estimated heritability of 0.19. Methane production rate and MY had a phenotypic correlation of 0.68 ± 0.02; this indicates that animals with high MPR also have high MY. Donoghue et al. (2016) hypothesized that reducing MY will not impact DMI because the two traits are not genetically correlated (-0.04 ± 0.18), however reducing MY will have a

correlated effect on MPR because the two traits have a strong genetic correlation (0.50 ± 0.14). Interestingly, Donoghue et al. (2016) found that MPR had a weaker phenotypic correlation with BW (0.26 ± 0.04) than later in life growth trait such as WW (0.53 ± 0.03), YW (0.61 ± 0.03), and FW (0.56 ± 0.03). Genetic correlations between MPR and production traits were moderate to strong: BW (0.36 ± 0.18), EMA (0.40 ± 0.16), WW (0.84 ± 0.09), YW (0.86 ± 0.06), and FW (0.79 ± 0.08; Donoghue et al., 2016). Donoghue et al. (2016) speculated that the strong genetic correlations between MPR and animal weight traits is likely due to the strong association between MPR and DMI. This means that reducing MPR will have a correlated reduction in animal weight for the progeny. Instead, the authors proposed the mitigation strategy of selecting for reduced MY or residual methane because it should reduce methane production without a negative effect on DMI (Donoghue et al., 2016).

Due to the sparse literature on CH_4 emissions from grazing beef cattle, genetic parameters from sheep should be considered. Robinson et al. (2010) evaluated 708 grazing ewes for 1-hour methane emissions using a sealed polycarbonate booth. Heritability of 1-hour methane production (dL/hour) after adjustments for live weight was 0.13 with a repeatability of 0.32 (Robinson et al., 2010).

Selection Strategies

The objective is to reduce CH_4 emissions from beef cattle to maximize productivity, profitability, and sustainability. However, CH_4 production is a natural digestive process of ruminants that allows cattle to digest and ferment human non-edible plant material. Therefore, it is vital that the optimum balance between CH_4 production and animal productivity is reached.

High feed intake is associated with high methane production rate (MPR) in ruminants (Blaxter & Clapperton, 1965). Production traits such as growth are highly correlated with feed

intake (Arthur et al., 2001). Therefore, reducing MPR could have an unfavorable impact on animal productivity due to the correlation with feed intake. Herd et al. (2014) evaluated several ways to measure methane independent of feed intake for their phenotypic relationships with production traits. The methane traits evaluated were MPR (L/d), methane yield (MY; MPR/DMI), and four forms of residual MPR (RMP). MPR was positively correlated with DMI, MY, RMP, growth traits, and body composition traits (0.65 ± 0.02; 0.72 ± 0.02; 0.65 to 0.79; 0.19 to 0.57; 0.13 to 0.29). However, MY was not correlated with DMI, growth traits, or body composition traits (-0.02 ± 0.04; -.03 to 0.11; 0.01 to 0.06). These results suggest that reducing MPR as a mitigation strategy would have a negative impact on growth and body composition traits. However, MY was not correlated with DMI, but was positively correlated with MPR. This indicates that reducing MY would have no effect on DMI or animal productivity but have a correlated reducing effect on MPR.

Development of a selection index for methane production would be the most advantageous mitigation strategy. A well-constructed index with properly weighted traits would allow for optimum selection to reduce methane production without compromising important production traits. Further research is required in this area to define economic values for methane production and evaluate its place in a selection index.

Conclusion

Methane is a potent greenhouse gas with adverse effects on the environment due to warming potential of the atmosphere. Enteric fermentation from ruminant animals is a source of methane production and represents an energetic loss for that animal. Several methods to quantify methane emissions from cattle exist including respiration calorimetry, the sulfur-hexafluoride tracer technique, prediction models, and OCGQS. The OCGQS has been proven to accurately

quantify methane gas flux and has the advantage that animals are unencumbered by equipment and can remain in their normal production environment. This allows for the quantification of methane emissions from grazing cattle. Although, methane production represents an energetic loss to animals, it is also a component of fermentation- an important digestive process. Therefore, adverse effects on production should be considered when selecting to reduce methane production. Methane emissions is a heritable trait that allows for genetic progress to made with selection which would best be done using a well-constructed selection index.

Chapter 2 - Characterization of the Number of Spot Samples Required for Calculation of Gas Fluxes and Metabolic Heat Production using an OCGQS (GreenFeed)

Abstract

Enteric fermentation from cattle results in greenhouse gas production that not only is an environmental concern but also an energetic loss. Several methods exist to quantify gas fluxes, however an OCGQS allows for unencumbered quantification of methane, carbon dioxide, and oxygen from grazing cattle. While previous literature has proven the accuracy of an OCGQS, little work has been done to establish the minimum number of spot samples required to best evaluate an individual grazing animal's gas fluxes and metabolic heat production. Seventeen grazing animals with 100 spot samples each were collected using a GreenFeed system (C-Lock, Inc). The mean gas fluxes and metabolic heat production were computed starting from the first 10 visits (forward) and increasing by increments of 10 visits. Mean gas fluxes and metabolic heat production were also computed starting from visit 100 (reverse) using the same approach. Pearson and Spearman correlations were computed between the full 100 visits and each shortened visit interval. A large increase in correlations were seen between the 30 and 40 visit intervals. Mean forward and reverse gas fluxes and metabolic heat production were also computed starting at 30 visits and increasing by 2 visits until 40 visits. The minimum number of spot samples was determined when correlations with the full 100 visits was greater than 0.95. The results indicated that the minimum number of spot samples needed for accurate quantification of methane, carbon dioxide, and oxygen gas fluxes are 36-38, 40, and 38-40, respectively. Metabolic heat production can be calculated with gas fluxes collected with the

GreenFeed with 36 spot samples. Published literature recommends a similar number of total spot samples. However, large variation exists around the average number of spot samples for an animal per day, therefore a wide range of test durations are published to meet the same number of spot samples. For this reason, protocols for the OCGQS should be based on the total number of spot samples, rather than a test duration.

Introduction

Methane (CH_4) is the second most abundant greenhouse gas emitted annually following carbon dioxide (CO_2). Methane accounted for 11% of U.S. greenhouse gas emissions in 2020 (U.S. EPA, 2021). The atmospheric lifetime of CH_4 is approximately 12 years, which is much shorter than CO_2 (which can last in the atmosphere for thousands of years). However, CH_4 has a Global Warming Potential (GWP) of 27-30, meaning that 1 ton of CH_4 will absorb 27-30 times more energy than 1 ton of CO_2 over 100 years (U.S. EPA, 2021). While GWP is a measure of the potency of gases, another important consideration is that CH_4 is a flow gas, meaning atmospheric concentration stays stagnant in part due to its role in the biogenic carbon cycle (Allen et al. 2018; Mitloehner, 2021).

When categorized by emission source, 27% of U.S. CH_4 emissions were produced from enteric fermentation of livestock species, making it the second largest source of CH_4 in the United States behind natural gas and petroleum systems (U.S. EPA, 2021). Enteric fermentation is a part of the natural digestive process for animals, particularly ruminant species such as cattle, buffalo, sheep, goats, and camels. Microbial fermentation that occurs in the rumen allows ruminants to utilize forages and plant material to create high-quality protein sources from materials not digestible by monogastric animals, including humans. Methane is produced as a

by-product of this fermentation process and expelled from the animal by flatus (1-3%) and primarily eructation (Murray et. al, 1975, Muñoz et al. 2012).

In addition to environmental concerns, there is also an economic loss associated with CH_4 production. Johnson & Johnson (1995) estimate that CH_4 emissions represent a loss of 2-12% of gross energy intake. A portion of this loss could have potentially been used towards muscle or milk production, thus saving producers feed and increasing profitability. However, enteric fermentation is a very useful process that allows ruminants to upcycle low quality forages into a high-quality protein product. Therefore, it is important that in the process of reducing CH_4 production, animal productivity is not sacrificed, or that sacrifices in performance are utilized to achieve an optimum balance between methane emissions and productivity that maximizes profitability and environmental sustainability.

There are a few different ways to measure CH_4 emissions from cattle such as the sulfur-hexafluoride tracer technique and respiration calorimetry techniques like whole-animal chambers and headboxes, as well as prediction models to estimate CH_4. However, another technique to measure CH_4 emissions is available: an open-circuit gas quantification system (OCGQS). These systems measure individual animal gas fluxes (methane, carbon dioxide, oxygen, hydrogen) from livestock species. There are several factors that affect the accuracy of the gas emission data that is measured. One of the most important factors is the collection protocol. There are several protocol parameters that need to be established prior to use of the OCGQS. One important parameter is the total number of emissions records for an animal from the trial period. Most of the protocols that exist in literature are from animals in confinement (feedlot and dairy). Substantial research has been done to validate and assess the accuracy of emission spot-measurements from the OCGQS; however, little work has been done to evaluate the total number

of emission records necessary to best evaluate an individual grazing animal's gas emissions and metabolic heat production for the purpose of genetic evaluation.

The objective was to determine the minimum number of gas flux records required to accurately estimate CH_4, CO_2, and oxygen (O_2) gas fluxes and metabolic heat production from an individual grazing beef cow.

Materials and Methods

Study Design

Methane, CO_2 and O_2 fluxes were collected using a OCGQS (GreenFeed, C-Lock, Inc., Rapid City, South Dakota). Collection of daily CH_4, CO_2, was performed on grazing mature Angus beef cows (n = 23) from the Kansas State Purebred Unit (PBU) near Manhattan, KS from May 23, 2021 to September 9, 2021. Three animals refused to use the system, giving a refusal rate of 13%. Seventeen of the twenty animals actively using the system achieved at least 100 visits during the trial and were used in this analysis. All animal procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University (protocol 4463) in accordance with Federation of Animal Science Societies (FASS, 2010) guidelines.

The OCGQS system used was equipped with two units mounted side-by-side on a bumper-pull trailer. The system has two alleyways, one leading to the feed dish of each unit to ensure that only one animal has access to a unit at a time. Wind barriers were mounted on either side of the feed dish as well as high-density polyethylene boards fastened to both sides of the alleyways to minimize wind. Ten feet by five feet cattle panels surrounded the trailer to limit animal access to the technical equipment on the trailer. The batteries that power the units were charged by a generator.

Before using the OCGQS, each animal received a half-duplex radio frequency electronic ID (Allflex USA Inc.) and a body condition score was taken. Prior to gas flux collection, animals were exposed to the OCGQS during an acclimation period of approximately two weeks. During the acclimation period, alleyways were stowed upright so animals had easier access to the feed bins. The acclimation period was considered complete when approximately 75% of the cows used the OCGQS frequently. After the acclimation period was complete, alleyways were lowered to ensure only one animal had access to a unit at a time.

During collection, cows freely grazed the pasture and were provided a mineral supplement. Animals were enticed to visit the OCGQS using alfalfa pellets approximately 7 mm in diameter. When an animal inserted its head into the feed dish, the system read the RFID tag, identified the animal, and released feed. The feed provided an incentive for animals to keep their head in the system so that the gas fluxes could be collected for each individual animal. Other studies have dropped 50g to 55g of feed at 45 second intervals up to 5 times in one feeding (Dorich et al., 2015; Rischewski et al., 2017; Hammond et al., 2015). However, in an effort to extend the total visit duration to better capture an eructation event, the protocol can be altered by decreasing the amount of feed dropped, shortening the drop interval, and increasing the number of feed drops. The system was programmed to drop approximately 25 grams of feed every 30 seconds up to eight times during one visit. In this study, animals were allowed to visit the system up to 5 times per day with a minimum of two hours between visits, which encourages animals to visit during different times of the day to better capture the diurnal pattern of CH_4 emissions (Gregorini, 2012).

Raw collection data was checked and validated by C-Lock, Inc. The visit validation process includes checking that head proximity is higher than the low head proximity cutoff, the

CO_2 response must be higher than the low CO_2 response cutoff and the visit must be at least two minutes in duration. This process formed the preliminary dataset. During the final review process, there are several checks on standard gas calibrations, CO2 recovery tests, airflow correction, and wind correction.

Phenotypic Data

The OCGQS calculates the emission rates of gases (Q_c) in order to calculate the gas flux (Q_m;g/d). The Q_c were calculated using the following equation (Huhtanen et al., 2015):

$$Q_c = \left[C_p * (Conc - BConc) * Q_{air}\right]/10^6$$

where C_p is the fractional capture rate of air, Conc is the concentration of captured gas measured by the OCGQS gas sensor, BConc is the background concentration of gas measured by the OCGQS gas sensor, and Q_{air} is the volumetic airflow measured by the air velocity transmitter. Once Q_c is known, the Q_m, is determined as follows (Huhtanen et al. 2015; McLean and Tobin, 1987):

$$Q_m = Q_c * 273.15/(273.15 + T_{air}) * p_c$$

where T_{air} is the air temperature and p_c is the density of gas at 1 atm and 273.15 K.

Only the first 100 visits were used in this analysis, therefore visits that exceeded 100 for an animal were truncated. Using the daily gas fluxes calculated as described above, average CH_4, CO_2, and O_2 for each animal were computed for increasing visit intervals in 10 visit increments starting with the first 10 visits and increasing until the full 100 visit data set (forward) was utilized (F10, F20, F30, F40, F50, F60, F70, F80, F90, F100). Average CH_4, CO_2, and O_2 were also calculated starting from visit number 100 (reverse) using the same approach (R10, R20, R30, R40, R50, R60, R70, R80, R90, R100) to determine if there were substantial differences due to collection time.

Metabolic heat production was calculated for each animal for each interval using the following equation (Brouwer, 1965):

$$HP(Mcal/day) = 3.866 * O_2(L) + 1.2 * CO_2(L) - 0.518 * CH_4(L) - 1.431 * N$$

where O_2, CO_2, and CH_4 were the average values for each animal by interval. In the current study, information on N (nitrogen) was unavailable and was omitted from the calculation. Metabolic heat production was calculated for each animal and each interval, both forward and reverse.

Means and standard deviations for CH_4, CO_2, O_2, and metabolic heat production were calculated for each shortened visit interval within animal using mean and standard deviation functions in R Studio (R Core Team, 2022). Pairwise comparisons between forward and reverse mean gas flux and metabolic heat production values for each interval were made using the PROC GLM procedure in SAS 9.4 with the LSMEANS statement. Phenotypic (Pearson and Spearman) correlations were also calculated for each visit interval compared to the full 100 visits with the correlation function in R Studio (R Core Team, 2022). In this study, the minimum recommended number of visits for CH_4, CO_2, O_2, and metabolic heat production was determined when the Pearson and Spearman correlation was greater than 0.95, in accordance with the level used for the Beef Improvement Federation guidelines for feed intake and weight gain (BIF, 2021). Spearman correlations were utilized to determine the extent of reranking of animals between visit intervals and the full 100 visits. The phenotypic variance of CH_4, CO_2, O_2 and metabolic heat production for the group of animals was calculated for all intervals.

After the initial correlation analysis between each interval and the full 100 visits, the 0.95 Pearson correlation was between 30 and 40 visits for each gas and metabolic heat production, but the increase in correlation between 30 and 40 visits tended to be fairly large. Thus, the 30 to 40

visit interval was split further into increments of two visits for both forward (F32, F34, F36, F38) and reverse (R32, R34, R36, R38) in a second correlation analysis for CH_4, CO_2, O_2 and metabolic heat production. Means and standard deviations for CH_4, CO_2, O_2 and metabolic heat production were calculated using the mean and standard deviation functions of R Studio (R Core Team, 2022) for each increment. Pairwise comparisons between forward and reverse mean gas flux and metabolic heat production values for each increment between 30 and 40 visits were made using the PROC GLM procedure in SAS 9.4 with the LSMEANS statement. Pearson and Spearman phenotypic correlations between the full 100 visits and the increments between 30 and 40 were calculated using the correlation function in R Studio (R Core Team, 2022). The phenotypic variance of CH_4, CO_2, O_2 and metabolic heat production for the group of animals was calculated for interval increments between 30 and 40 visits.

Results and Discussion

Summary statistics for gas fluxes (CH_4, CO_2, and O_2) and metabolic heat production are presented in Table 2.1. The average CH_4 emissions was 353.8 g/d with a range from 106.0 g/d to 599 g/d. In a maize silage maturity study with 60 continental crossbred steers, McGeough et al. (2010) reported a range of CH_4 emissions (g/d) from 228 to 304, depending upon diet treatment. The low end of the range of CH_4 emissions (g/d) from McGeough et al. (2010) was from a group of steers with ad libitum access to concentrate. This average is lower than the current study; however, that could be due to the forage-based diet eaten by cows in this study, which is known to be associated with higher CH_4 emissions (Beauchemin and McGinn, 2005). Huhtanen et al. (2015) reported an average CH_4 emissions of 453 g/d from Swedish Red dairy cows using a GreenFeed. This average is higher than the current study, which is reasonable because lactating dairy cows typically produce more CH_4 than beef cows (Broucek 2014) due to higher nutritional

requirements (National Research Council, 2001). However, lactating Holstein-Friesian cows grazing paddocks in Waghorn et al. (2016) had methane emissions (g/d) ranging from 298 to 334 depending upon stocking rate, which is similar to the average in the current study. Published meta-analyses from Jonker et al. (2020) and Huhtanen et al. (2019) reported average methane emissions from dairy cows of 293 g/d and 378 g/d, respectively. The average CH_4 in the current study falls within that reported range.

Literature including CO_2 and O_2 emissions is more limited compared to CH_4. The average CO_2 emissions was 10428.1 g/d with a range from 5585 g/d to 14996 g/d. Grazing Holstein-Friesian cows had average CO_2 emissions ranging from 9360 g/d to 11500 g/d depending upon stocking rate (Waghorn et al. 2016). The average CO_2 emissions from the current study falls within that reported range, though the minimums and maximums are more extreme. Huhtanen et al. (2015) reported an average CO_2 emission of 11619 g/d for lactating dairy cows using a GreenFeed which is slightly higher than the current study, which is reasonable as lactating dairy cows have higher nutritional requirements compared to the current study's population (National Research Council, 2001). Manafiazar et al. (2016) reported a range from 6422 g/d to 6532 g/d for beef heifers using a GreenFeed which is lower than the current study. Arthur et al. (2018) reported average CO_2 emissions from heifers to be 5760 g/d and steers to be 8939 g/d. The CO_2 emissions reported from Manafiazer et al. (2016) and Arthur et al. (2018) are lower than the average CO_2 emissions from the current study. However, this is expected as the study populations were housed in a dry lot setting whereas the current study used grazing cows which are expected to have higher CO_2 production due to additional energy expenditure from walking and grazing (Agnew and Yan, 2000; Brosh et al. 2010). In addition, differences between body weight and DMI for cows compared to heifers could be another reason

for different CO_2 emissions. Using the sulfur-hexafluoride tracer technique, Pinares-Patino et al. (2007) reported average CO_2 emissions of grazing dairy cows to be 9363 or 10496 depending on stocking rate. The larger average CO_2 emissions reported by Pinares-Patino et al. (2007) for high stocking rate is very similar to the average CO_2 emissions in the current study.

Unlike CH_4 and CO_2 emissions that are eructated and expired from the animal, O_2 emissions are a measure of O_2 consumption during respiration. In the current study, the average O2 flux was 7713.2 g/d with a range from 3913 g/d to 11629 g/d. The average O_2 consumption of eight young Charolais bulls in Guarnido-Lopez et al. (2022) ranged from 4173 L/d to 4509 L/d which equates to 5963.2 g/d to 6443.3 g/d. The average O2 consumption reported by Guardnido-Lopez et al. (2022) could be lower because the population studied was young Charolais bulls with an average BW of 382 kg while the current study used mature cows. A meta-analysis of respiration chamber gas flux data from dairy cows found an average O_2 consumption of 5544 L/d (7922.38 g/d), which is very similar to the average O_2 consumption in the current study (Aubry and Yan, 2015).

The average metabolic heat production in the current study is 27,278.2 kcal/d with a range from 22,068.22 to 32,391.34 kcal/d. Kumar et al. (2016) reported a range of heat production per metabolic body weight of Sahiwal and Karan Fries heifers from 118.20 kcal/$kg^{0.75}$ to 134.83 kcal/$kg^{0.75}$ depending upon feeding regime. Heat production ranged from 5,858.01 to 8,634.67 kcal/d, which is much lower than the average in the current study. However, this is expected as heifers generate far less metabolic heat than cows (West, 2003). In addition, Kumar et al. (2016) performed their study in India- a different country with different management, feeding practices, and breeds, all factors that could affect metabolic heat production. In addition, Kumar et al. (2016) used gas fluxes collected with a three-way valve and face mask to calculate

metabolic rate whereas the current study used an OCGQS which could be another reason estimates were different. Nkrumah et al. (2006) reported heat production per metabolic body weight for feedlot steers ranging from 129.32 kcal/$kg^{0.75}$ to 163.97 kcal/$kg^{0.75}$. Steers from Nkrumah et al. (2006) had metabolic BW ranging from 105.01 $kg^{0.75}$ to 110.22 $kg^{0.75}$. Therefore, the top of the range from Nkrumah et al. (2006) is 18,072.77 kcal, which is lower than the current study's average. However, Nkrumah et al. (2006) studied feedlot steers fed a concentrate diet in a confinement setting, whereas the current study used mature cows grazing forages on pasture. Reynolds et al. (1991) reported that heifers consuming a concentrate diet generated less heat energy than heifers consuming a forage diet which could be the reason for different metabolic heat production between the current study and Nkrumah et al. (2006). Herd et al. (2020) reported metabolic heat production for steers (104 MJ/d or 24,856.59 kcal/d) and heifers (67 MJ/d or 16,013.38 kcal/d). Steers from Herd et al. (2020) had similar metabolic heat production to the cows in the current study.

Correlations between Intervals for CH_4 emissions

Means and the corresponding standard deviations for CH_4 emissions (g/d) for each interval are shown in Table 2.2 and an illustration of the means are shown in Figure 2.1 Panel A. The phenotypic variance of the mean forward and reverse CH_4 values for all intervals are shown in Table 2.3. The CH_4 (g/d) interval means calculated from the beginning of the study were numerically greater than the CH_4 (g/d) interval means calculated from the end and became closer in value as the number of visits increased towards 100, although not significantly different. The numeric difference in mean values from forward and reverse approaches could have been due to a difference in collection time. This study took place from May to September, which was likely accompanied by decreasing forage quality and the maturation of warm-season grasses (George et

al. 2001). Mature forages have a reduced soluble carbohydrate content and more lignified plant cell walls which promotes acetate production in the rumen and increases CH_4 production per unit of forage digested (Beauchemin et al. 2009; Pinares-Patino et al. 2003; Pinares-Patino et al. 2007; Jonker et al. 2016). However, reduced forage quality is normally associated with reduced intake, therefore it may be that reduced forage quality does not increase the amount of CH_4 produced as a percentage of gross energy intake (Pinares-Patino et al. 2003). Therefore, the cows could have had lower CH_4 emissions towards the end of the trial due to reduced intake stemming from reduced forage quality making the mean CH_4 emissions from the reverse approach lower than the forward approach.

The correlation between each shortened visit interval and the full 100 visits are shown in Table 2.4 and an illustration of correlations is shown in Figure 2.2 Panel A. The Spearman and Pearson correlations between the first 10 visits and 100 visits was 0.69 and 0.68, respectively for the forward approach. The correlation with 100 visits is still reasonably high even with the small number of spot samples. This result agrees with the results from Arthur et al. (2017) which found that with only 20 visits the variance of CH_4 was reduced by 54% compared to the variance at 5 visits. A 0.95 correlation with the full 100 visits was attained in the interval of 20 visits for reverse Pearson. All correlations reached 0.95 in the interval of 30 to 40 visits. Interestingly, between the 30 and 40 visit interval there was a large increase in correlation. For the forward approach, the Spearman correlation increased from 0.87 to 0.98, while the Pearson correlation increased from 0.93 to 0.97. To investigate the large increase in correlation between the 30 and 40 visits intervals and extract a more precise recommendation for number of visits, the 30 to 40 visit interval was split into smaller increments.

The means and standard deviations for the 30 to 40 visit increments are shown in Table 2.5 and an illustration of the means is shown in Figure 2.3 Panel A. The phenotypic variance of the mean forward and reverse CH_4 values for the 30 to 40 visit increments are shown in Table 2.6. Forward means were numerically greater than the reverse means between 30 and 40 visits, which follows the same trend with all intervals. The correlation between the 30 to 40 visits increments and the full 100 visits are shown in Table 2.7 and an illustration of correlations is shown in Figure 2.4 Panel A. The correlation with the full 100 visits first reached 0.95 at 34 visits for the Pearson correlation with a forward approach. At 36 visits all other combinations for direction of approach and type of correlation (Pearson or Spearman) had reached 0.95 except the Spearman correlation with a forward approach. All correlations were above 0.95 with 38 visits. In a grazing cattle trial, it is difficult to control the exact number of visits from each animal or stop the trial at an exact number of visits, although it is important to establish a minimum number of visits that must be completed before the trial ends. The recommended minimum number of visits for calculation of CH_4 emissions is 36-38. Additional records beyond 38 visits may not be providing much additional information or needed for an accurate estimate of average daily CH_4 emissions. Renand and Maupetit (2015) suggested that approximately 50 spot-measures would be sufficient for calculation of CH_4 emissions. The recommendation of 36-38 visits is very similar to the recommendation from Arthur et al. (2017). Arthur et al. (2017) found a 70% reduction in variance after 30 records relative to the initial variance of 5 records. There was no substantial reduction in variance after 30 records (Arthur et al. 2017). Arthur et al. (2017) completed this study using both steers and heifers in a confined lot setting, which contrasts the current study which included cows in a grazing setting. These differences could be why the

recommendation in the current study is slightly greater than the recommendation from Arthur et al. (2017).

In the current study, cows utilized the OCGQS for an average of 29.5 ± 8.7 days to reach the recommended 36-38 visits for calculation of CH_4 emissions. Gunter and Bradford (2017) reported using a power analysis that for grazing heifers 4.8 to 6.3 days are required to accurately calculate CH_4 flux. This recommendation is much lower than the recommendation from the current study and other published literature. Gunter and Bradford (2017) made their recommendation as animals were visiting the OCQGS 2.4 times a day whereas animals in the current study visited less frequently (1.2 visits/d). One reason Gunter and Bradford (2017) had a higher number of visits per day may be because animals were first penned with the OCQGS for one week during acclimation and then released to freely graze the pasture. This could have acclimated the animals to use the OCQGS quicker and more frequently, therefore less days were needed to quantify CH_4 emissions. Renand and Maupetit (2016) reported that CH_4 emissions calculated from 2 weeks of testing had a 0.69 correlation with emissions calculated from 8 weeks of testing and recommended a 2 week test duration with at least 50 spot samples for calculation of CH_4 emissions. Renand and Maupetit (2016) conducted their study in confinement whereas the current study was conducted in a pasture which could be why the recommended test duration is different. A study in France (Arbre et al. 2016) reported that 17 days were required to achieve a repeatability of 0.70 for CH_4 emissions. Gunter and Beck (2018) reported that quality CH_4 emissions could be calculated during a 14 day period when grazing animals visit the OCGQS 2.5 times per day. Animals that visit 2.5 times per day for 14 days would have a total of 35 visits, which is very close to the recommendation from the current study. Although, animals in the current study took a greater number of days to reach the recommendation than animals from

Gunter and Beck (2018). The discrepancy in the number of visits an animal makes per day is one reason that spot sample recommendations should be a total number of visits instead of a test duration.

Correlations between Intervals for CO_2 emissions

Means and the corresponding standard deviations of all animal's CO_2 emissions (g/d) for each interval are shown in Table 2.2 and an illustration of the means is shown in Figure 2.1 Panel B. The phenotypic variance of the mean forward and reverse CO_2 values for all intervals are shown in Table 2.3. Mean CO_2 emissions calculated with the forward approach were numerically greater than mean CO_2 emissions calculated with the reverse approach, although not significantly different. There was a large numeric increase in mean CO_2 emissions from 10 visits to 20 visits for both forward and reverse approaches. Then as the number of visits increased, the mean CO_2 from forward and reverse approaches gradually became closer numerically.

Carbon dioxide is produced by mammals as a part of cellular respiration. Vital nutrients are converted to ATP in the presence of O_2 and CO_2 is a byproduct of the reaction. Therefore, CO_2 production is influenced by feeding level and nutrient composition of the diet (Brouwer, 1965, Aguerre et al. 2011). Manipulating CO_2 emissions using various feed supplements has been attempted such as ensiled crimped grape marc (Caetano et al. 2019) and protein (Shreck et al. 2021), among others. Pickett et al. (2020) reported that supplementation with grain and ionophores did not affect CH_4 emissions however, supplementation did increase CO_2 emissions. Arthur et al. (2018) found that carbon dioxide production is strongly correlated with body weight and dry matter intake for steers (0.87 and 0.83, respectively) and heifers (0.84 and 0.84, respectively) in a confined lot setting. The reason CO_2 emissions were higher when calculated

with the forward approach could also be attributed to the same reasons CH_4 was higher with the forward approach.

The correlation between each shortened visit interval and the full 100 visits are shown in Table 2.4 and an illustration of the correlations is shown in Figure 2.2 Panel B. The correlation between the first 10 visits and the full 100 visits ranged from 0.67 to 0.92 depending upon direction of analysis and type of correlation with reverse approach correlations being higher than forward approach correlations, then eventually leveling out to approximately the same around 30 visits. A 0.95 correlation with 100 visits was first achieved between 30 and 40 visits with all correlations except for reverse Spearman. The 30 to 40 visit interval was split into smaller 2 visit increments.

The means and standard deviations for the 30 to 40 visit increments are shown in Table 2.5 and an illustration of the means is shown in Figure 2.3 Panel B. The phenotypic variance of the mean forward and reverse CO_2 values for the 30 to 40 visit increments are shown in Table 2.6. Forward approach means were numerically greater than the reverse approach means between 30 and 40 visits, which follows the same trend as the analysis with all intervals and CH_4 emissions trends. The correlation between the 30 to 40 visit increments and the full 100 visits are shown in Table 2.7 and an illustration of correlations is shown in Figure 2.4 Panel B. A 0.95 correlation with the full 100 visits was first accomplished at 34 visits with the forward Spearman correlation and remained the only correlation greater than 0.95 for 36 visits as well. At 38 visits, both forward correlations were above 0.95, while both reverse correlations still had not met the 0.95 threshold. It is important to mention that forward correlations may be more important for determining the minimum number of visits needed because all trials proceed forward. All

correlations were above 0.95 at 40 visits except for the reverse Spearman correlation. The recommended number of visits for calculation of CO_2 emissions is 40 visits.

The recommendation of 40 visits for CO_2 is similar to the recommendation of 36-38 visits for CH_4 in the current study. Carbon dioxide and CH_4 were found to have a strong linear relationship in dairy cattle (Aubry and Yan, 2015) and a correlation of 0.48 (Liu et al. 2012). Thus, it is reasonable that similar number of visits would be necessary for CH_4 and CO_2. However, the residual variability is typically larger for CH_4 emissions compared to CO_2 emissions because of the circadian patterns of CH_4 emission (Renand and Maupetit, 2016), whereas CO_2 is emitted in more constant manner (Gunter and Beck, 2018). It is interesting that the required number of visits for CO_2 is larger than the number of visits for CH_4. Arthur et al. (2017) made a similar recommendation of a minimum of 30 records for calculation of CO_2 emissions. Once again, the study population and environment were different for the current study compared to Arthur et al. (2017) which could be why there is a slight difference in the number of visit recommendations.

In the current study, an average of 31.8 ± 9.2 days were needed for animals to meet the recommended 40 visits to the OCGQS for quantification of CO_2 gas flux. Using a power analysis, Gunter and Bradford (2017) reported that 3.4 to 3.8 days were required to quantify CO_2 emissions when animals visited the OCGQS 2.4 times per day. The recommendation from Gunter and Bradford (2017) is much lower than the recommendation from the current study. Once again, Gunter and Bradford (2017) utilized a unique acclimation procedure which could be why animals visited the OCGQS more frequently and perhaps why less visits were needed. Gunter and Beck (2018) reported that CO_2 emissions could be accurately calculated during a 14 day period when grazing animals visited the OCGQS 2.5 times per day. If an animal visits the

OCGQS 2.5 times per day for 14 days, the total number of visits is 35, which is very close to the recommendation from the current study.

Correlations between Intervals for O_2 emissions

Means and the corresponding standard deviations for O_2 consumption (g/d) for each interval are shown in Table 2.2 and an illustration of the means is shown in Figure 2.1 Panel C. The phenotypic variance of the mean forward and reverse O_2 values for all intervals are shown in Table 2.3. Means calculated with a forward approach are numerically greater than means calculated with a reverse approach, although not significantly different. Means gradually become closer as number of visits increased towards 100. Unlike CH_4 and CO_2 which are waste by-products of biological processes exhaled from the body, O_2 is inhaled as an essential reactant for the production of ATP. However, similar to the trends for CH_4 and CO_2, animals in the current study consumed less O_2 towards the end of the trial making means from the reverse approach numerically smaller. Lower O_2 consumption is expected as feed intake decreases (Blaxter, 1962). As previously discussed, cows could have had lower intake at the end of the trial due to a decrease in forage quality, resulting in lower CH_4, CO_2, and O_2 gas fluxes lower.

The correlation between each shortened visit interval and the full 100 visits are shown in Table 2.4 and an illustration of the correlations is shown in Figure 2.2 Panel C. A 0.95 correlation with the full 100 visits was first achieved at 20 visits for the reverse Pearson correlation, while others still ranged from 0.81 to 0.91 at 20 visits. The reverse Pearson correlation dropped to 0.94 at 30 visits and then returned to 0.95 at 40 visits. Between 30 and 40 visits, all correlations with the full 100 visits were above 0.95. Both forward and reverse Spearman correlations experienced a large increase in correlation between 30 and 40 visits which is why the 30 to 40 visit interval was split into 2 visit increments and examined more precisely.

The means and standard deviations for the 30 to 40 visit increments are shown in Table 2.5 and an illustration of the means is shown in Figure 2.3 Panel C. The phenotypic variance of the mean forward and reverse O_2 values for the 30 to 40 visit increments are shown in Table 2.6. Following the same trend as other gases, the means calculated with the reverse approach were numerically smaller than the means from the forward approach. The correlation between the 30 to 40 visit increments and the full 100 visits are shown in Table 2.7 and an illustration of correlations is shown in Figure 2.4 Panel C. The reverse Pearson correlation was the first correlation to reach 0.95 at 32 visits. At 34 and 36 visits, 2 correlations had reached 0.95. All correlations were above 0.95 at 38 visits except for reverse Spearman. At 40 visits, all correlations with the full 100 visits had reached 0.95. The minimum recommended number of visits for calculation of O_2 consumption is 38-40 visits.

The recommendation of 38-40 visits for the calculation of O_2 consumption is similar to the current study's recommendation for CH_4 and CO_2. Aubry and Yan (2015) found that CO_2 and O_2 had a strong positive linear relationship ($R^2 = 0.92$) as did CH_4 and O_2 ($R^2 = 0.86$). Thus, it is logical that number of visit recommendations would be similar among gases. One of the only other studies that evaluated the optimal number of visits for gas flux data collection from an OCGQS (Arthur et al. 2017) only examined CH_4 and CO_2, not O_2.

Animals in the current study needed an average of 30.52 ± 9.1 days to achieve the recommended 38-40 spot samples for the quantification of O_2 gas flux. Gunter and Bradford (2017) reported that 3.7 to 4.1 days were required to calculate O_2 consumption when animals visited the OCGQS 2.4 times per day based on a power analysis. This is a much lower recommendation than the current study and other published literature. Gunter and Beck (2018) reported that O_2 consumption can be calculated in 14 days when grazing animals visited the

OCGQS 2.5 times per day for a total of 35 visits, which is similar to the recommendation in the current study.

Correlations between Intervals for Metabolic Heat Production

Means and the corresponding standard deviations for metabolic heat production (kcal) for each interval are shown in Table 2.2 and an illustration of the means is shown in Figure 2.1 Panel D. The phenotypic variance of the mean forward and reverse metabolic heat production values for all intervals are shown in Table 2.3. The mean metabolic heat production calculated from the forward approach were numerically greater than the means from the reverse approach, although not significantly different. This follows the same trend as CH_4, CO_2, and O_2 which is reasonable and would be expected as these gasses were used in the calculation of metabolic heat production. Feed intake and muscular activity are two factors that influence metabolic heat production in domestic animals (Blaxter, 1989). If animals had lower intake during the end of the trial, gas fluxes would be lower as well as metabolic heat production.

The correlation between each shortened visit interval and the full 100 visits are shown in Table 2.4 and an illustration of the correlations is shown in Figure 2.2 Panel D. A 0.95 correlation with the full 100 visits was first found at 20 visits for the reveres Pearson correlation but then dropped below 0.95 for 30 visits. The same pattern was seen in the O_2 consumption correlations, which makes sense as oxygen is highly influential in the calculation of metabolic heat production (Brouwer et al. 1965). Three correlations were above 0.95 at 40 visits and all correlations were above 0.95 at 50 visits. A large increase in correlation with the full 100 visits was found between 30 and 40 visits therefore the 30 visit interval was again split into 2 visit increments for analysis.

The means and standard deviations for the 30 to 40 visit increments are shown in Table 2.5 and an illustration of the means is shown in Figure 2.3 Panel D. The phenotypic variance of the mean forward and reverse metabolic heat production values for the 30 to 40 visit increments are shown in Table 2.6. Following the same trend as the gas fluxes, the forward approach means for metabolic heat production were numerically higher than the reverse approach means. The correlation between the 30 to 40 visit increments and the full 100 visits are shown in Table 2.7 and an illustration of correlations is shown in Figure 2.4 Panel D. A 0.95 correlation with the full 100 visits first was reached at 36 visits with all correlations except reverse Spearman. Thus, the minimum recommended number of visits for the calculation of metabolic heat production using gas fluxes is 36 visits. The recommendation of 36 visits for the calculation of metabolic heat production is similar to the number of visit recommendations for other gases in the current study. This is expected as the CH_4, CO_2, and O_2 gas fluxes were used for the calculation of metabolic heat production.

In the current study, animals needed 29.5 ± 8.7 days to reach the recommended 36 spot samples for the calculation of metabolic heat production. Herd et al. (2020) used an OCGQS to collect gas fluxes for calculation of metabolic heat production in steers and heifers. Herd et al. (2020) collected gas fluxes on steers for 10 weeks as a part of a larger feeding test and on heifers for 15 days following acclimation. However, Herd et al. (2020) did not evaluate the accuracy of metabolic heat production calculated from these test durations. Currently, no other published literature is available on the recommended number of visits to an OCQGS required to calculate metabolic heat production for comparison.

Conclusion

The results from the current study suggest that the number of spot samples required for accurate calculation of CH_4, CO_2, and O_2 gas fluxes with an OCGQS are 36-38, 40, and 38-40 spot samples, respectively. This recommendation is similar to previously published recommendations. This study also suggests that metabolic heat production can be calculated from gas fluxes collected with an OCGQS with 36 spot samples. If only collecting CH_4 gas flux, there is an opportunity to only collect 36 spot samples. However, if collecting all gases simultaneously, 40 spot samples are needed to meet the recommendation for CO_2 and O_2.

Animals met the required number of visits for quantification of CH_4 emissions and metabolic heat production in 29.5 ± 8.7 days. It took animals 30.5 ± 9.1 days and 31.8 ± 9.2 days to meet the required number of visits for calculation of O_2 and CO_2, respectively. A large range of test durations exist in the literature and there is variation in the average number of visits per day which is why protocols for the OCGQS should include the number of spot samples rather than a test duration (d).

Table 2.1-Summary statistics for gas fluxes and metabolic heat production calculated using the first 100 visits to the open-circuit gas quantification system.

	n	Mean	Minimum	Maximum	Standard Deviation
CH_4, g/d	17	353.8	106.0	599	83.7
CO_2, g/d	17	10,428.1	5,585	14,996	1,754.7
O_2, g/d	17	7,713.2	3,913	11,629	1,325.1
Metabolic Heat Production, kcal/d	17	27,278.2	22,068.2	32,391.3	3,089.2

Table 2.2- Means (SD) for all animals for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d) and metabolic heat production (kcal/d) across all visit intervals.

Gas	Direction	10 visits	20 visits	30 visits	40 visits	50 visits	60 visits	70 visits	80 visits	90 visits	100 visits
CH_4, g/d	Forward	358.2[a] (41.9)	362.3[a] (46.0)	358.0[a] (48.6)	358.3[a] (49.4)	357.7[a] (50.8)	358.1[a] (51.0)	355.9[a] (51.0)	354.3[a] (50.0)	353.7[a] (49.9)	352.9[a] (50.0)
	Reverse	346.0[a] (59.8)	347.0[a] (52.0)	345.0[a] (48.7)	345.3[a] (49.3)	348.0[a] (50.6)	349.6[a] (52.1)	351.3[a] (52.4)	350.9[a] (52.7)	352.4[a] (52.5)	352.9[a] (50.0)
CO_2, g/d	Forward	10,493.4[a] (1,339)	10,618.2[a] (1,164)	10,634.1[a] (1,221)	10,623.6[a] (1,256)	10,622.4[a] (1,242)	10,580.9[a] (1,227)	10,510.9[a] (1,193)	10,456.9[a] (1,164)	10,432.6[a] (1,132)	10,399.6[a] (1,134)
	Reverse	10,083.8[a] (1,286)	10,163.2[a] (1,061)	10,126.6[a] (1,076)	10,130[a] (1,080)	10,180.5[a] (1,093)	10,257.5[a] (1,116)	10,305.5[a] (1,144)	10,352.3[a] (1,181)	10,390.6[a] (1,162)	10,399.6[a] (1,134)
O_2, g/d	Forward	7,815.1[a] (946)	7,855.0[a] (893)	7,845.9[a] (951)	7,858.2[a] (996)	7,863.9[a] (976)	7,836.4[a] (957)	7,779.5[a] (927)	7,737.2[a] (908)	7,715.2[a] (889)	7,693.6[a] (887)
	Reverse	7482.4[a] (969)	7,511.8[a] (855)	7,481.1[a] (854)	7,484.8[a] (843)	7,530.0[a] (844)	7,590.2[a] (862)	7,630.1[a] (896)	7,656.4[a] (919)	7,680.0[a] (911)	7,693.6[a] (887)
Metabolic Heat Production, kcal	Forward	27,663.1[a]	27,848.8[a]	27,838.6[a]	27,864.9[a]	27,880.1[a]	27,778.1[a]	27,580.5[a]	27,432.2[a]	27,357.5[a]	27,278.2[a]
	Reverse	26,507.1[a]	26,637.2[a]	26,532.3[a]	26,544.2[a]	26,696.9[a]	26,908.8[a]	27,046.3[a]	27,148.3[a]	27,236.0[a]	27,278.2[a]

[a]Indicate no significant differences between forward or reverse interval means ($P < 0.05$).

Table 2.3-Phenotypic variance of the mean forward and reverse values from all animals for each interval for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d) and metabolic heat production (kcal/d).

Gas	Direction	10 visits	20 visits	30 visits	40 visits	50 visits	60 visits	70 visits	80 visits	90 visits	100 visits
CH_4, g/d	Forward	1,658.9	1,995.0	2,232.0	2,304.9	2,430.0	2,454.3	2,456.6	2,355.7	2,345.8	2,361.4
	Reverse	3,375.4	2,551.7	2,237.1	2,288.5	2,414.8	2,560.9	2,591.2	2,615.6	2,595.1	2,361.4
CO_2, g/d	Forward	1,688,418	1,276,015	1,403,901	1,485,665	1,452,722	1,418,761	1,341,212	1,276,968	1,208,146	12,10876
	Reverse	1,558,351	1,059,527	1,090,063	1,099,193	1,126,003	1,173,556	1,232,387	1,314,619	1,272,180	12,10876
O_2, g/d	Forward	843,395.3	751,093.2	852,799.6	934,983	898,316.1	862,381.6	809,918.1	777,401.1	744,727.6	742,131.2
	Reverse	885,489.3	689,431	686,723.5	670,339.2	671,134.9	700,410.4	755,765.7	795,191.3	782,122.4	742,131.2
Metabolic Heat Production, kcal	Forward	10,727,114	9,172,749	10,382,150	11,299,402	10,893,190	10,480,824	9,848,375	9,429,231	9,000,816	8,982,075
	Reverse	10,914,998	8,216,359	8,254,679	8,107,512	8,142,437	8,504,256	9,132,183	9,647,215	9,454,059	8,982,075

Table 2.4- Spearman and Pearson correlations for each shortened number of visits interval and the full 100 visits for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d), and metabolic heat production (kcal/d).

Gas	Direction	Analysis	10 visits	20 visits	30 visits	40 visits	50 visits	60 visits	70 visits	80 visits	90 visits	100 visits
CH_4, g/d	Forward	Spearman	0.69	0.82	0.87	0.98	0.99	0.99	0.99	0.98	0.99	1
		Pearson	0.68	0.86	0.93	0.97	0.98	0.99	0.99	0.99	0.99	1
	Reverse	Spearman	0.83	0.90	0.89	0.96	0.96	0.96	0.96	0.97	0.98	1
		Pearson	0.89	0.95	0.96	0.98	0.98	0.98	0.99	0.99	0.99	1
CO_2, g/d	Forward	Spearman	0.70	0.86	0.93	0.95	0.95	0.98	0.98	0.99	0.99	1
		Pearson	0.67	0.82	0.93	0.96	0.97	0.98	0.99	0.99	0.99	1
	Reverse	Spearman	0.89	0.97	0.88	0.93	0.92	0.95	0.95	0.97	0.98	1
		Pearson	0.92	0.95	0.94	0.95	0.96	0.97	0.98	0.99	0.99	1
O_2, g/d	Forward	Spearman	0.65	0.81	0.94	0.97	0.98	0.98	0.99	0.99	0.99	1
		Pearson	0.72	0.86	0.94	0.96	0.97	0.98	0.99	0.99	0.99	1
	Reverse	Spearman	0.88	0.91	0.90	0.95	0.96	0.98	0.98	0.99	1	1
		Pearson	0.93	0.95	0.94	0.95	0.97	0.98	0.98	0.99	0.99	1
Metabolic Heat Production, kcal/d	Forward	Spearman	0.67	0.81	0.90	0.95	0.97	0.99	0.99	0.99	1	1
		Pearson	0.71	0.86	0.94	0.96	0.97	0.98	0.99	0.99	0.99	1
	Reverse	Spearman	0.85	0.91	0.91	0.94	0.96	0.98	0.99	0.99	0.99	1
		Pearson	0.93	0.95	0.94	0.95	0.97	0.97	0.98	0.99	0.99	1

Table 2.5- Means (SD) for each shortened increment between 30 and 40 visits and the full 100 visits for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d), and metabolic heat production (kcal/d).

Gas	Direction	30 visits	32 visits	34 visits	36 visits	38 visits	40 visits
CH_4, g/d	Forward	358.07[a] (48.69)	359.05[a] (49.48)	358.98[a] (50.06)	358.49[a] (49.94)	357.91[a] (48.95)	358.33[a] (49.48)
	Reverse	345.07[a] (48.75)	343.93[a] (48.89)	344.62[a] (49.14)	344.83[a] (48.84)	344.79[a] (49.13)	345.35[a] (49.31)
CO_2, g/d	Forward	10,634.1[a] (1,221.3)	10,653.5[a] (1,225.3)	10,640.0[a] (1,225.7)	10,629.1[a] (1,234.1)	10,619.1[a] (1,242.6)	10,623.6[a] (1,256.3)
	Reverse	10,126.6[a] (1,076.1)	10,134.4[a] (1,077.7)	10,113.9[a] (1,068.6)	10,114.9[a] (1,069.9)	10,122.4[a] (1,079.5)	10,130[a] (1,080.6)
O_2, g/d	Forward	7,845.9[a] (951.8)	7,861.8[a] (952.8)	7,852.3[a] (962.7)	7,854.9[a] (971.3)	7,852.3[a] (984.5)	7858.2[a] (996.7)
	Reverse	7,481.1[a] (854.1)	7,490.4[a] (846.3)	7,470.8[a] (835.7)	7,471.6[a] (835.6)	7,477.4[a] (840.4)	7484.8[a] (843.9)
Metabolic Heat Production, kcal/d	Forward	27,838.6[a]	27,893.4[a]	27,858.9[a]	27,859.3[a]	27,846.4[a]	27,864.9[a]
	Reverse	26,532.3[a]	26,563.5[a]	26,496.7[a]	26,499.2[a]	26,526.7[a]	26,544.2[a]

[a]Indicate no significant differences between forward or reverse interval means ($P < 0.05$).

Table 2.6-Phenotypic variance of the mean forward and reverse values from all animals for interval increments between 30 and 40 visits for methane (CH_4 g/d), carbon dioxide (CO_2 g/d), oxygen (O_2 g/d) and metabolic heat production (kcal/d).

Gas	Direction	30 visits	32 visits	34 visits	36 visits	38 visits	40 visits
CH_4, g/d	Forward	2,232.0	2,304.4	2,359.0	2,347.2	2,255.8	2,304.9
	Reverse	2,237.1	2,249.6	2,273.0	2,245.0	2,272.0	2,288.5
CO_2, g/d	Forward	1,403,901	1,413,144	1,414,053	1,433,540	1,453,437	1,485,665
	Reverse	1,090,063	1,093,258	1,074,826	1,077,369	1,096,802	1,099,193
O_2, g/d	Forward	852,799.6	854,560.2	872,383.2	888,021.7	912,264.8	934,983
	Reverse	686,723.5	674,207.9	657,453.1	657,262.5	664,828.6	670,339.2
Metabolic Heat Production, kcal/d	Forward	10,382,150	10,411,648	10,577,131	10,763,610	11,033,407	11,299,402
	Reverse	8,254,679	8,141,997	7,952,487	7,953,584	8,057,674	8,107,512

Table 2.7-Spearman and Pearson correlations for each shortened number of visits interval between 30 and 40 visits and the full 100 visits for methane (CH4), carbon dioxide (CO2), oxygen (O2), and metabolic heat production (kcal/d).

Gas	Direction	Analysis	30 visits	32 visits	34 visits	36 visits	38 visits	40 visits	100 visits
CH_4, g/d	Forward	Spearman	0.87	0.90	0.89	0.92	0.95	0.98	1
		Pearson	0.93	0.94	0.95	0.96	0.96	0.97	1
	Reverse	Spearman	0.89	0.90	0.94	0.97	0.96	0.96	1
		Pearson	0.96	0.97	0.97	0.98	0.98	0.98	1
CO_2, g/d	Forward	Spearman	0.93	0.93	0.95	0.95	0.95	0.95	1
		Pearson	0.93	0.94	0.94	0.94	0.95	0.96	1
	Reverse	Spearman	0.88	0.89	0.89	0.90	0.91	0.93	1
		Pearson	0.94	0.94	0.94	0.94	0.94	0.95	1
O_2, g/d	Forward	Spearman	0.94	0.93	0.95	0.94	0.97	0.97	1
		Pearson	0.94	0.94	0.94	0.95	0.96	0.96	1
	Reverse	Spearman	0.90	0.92	0.92	0.94	0.94	0.95	1
		Pearson	0.94	0.95	0.95	0.95	0.95	0.95	1
Metabolic Heat Production, kcal/d	Forward	Spearman	0.90	0.93	0.94	0.95	0.96	0.96	1
		Pearson	0.94	0.94	0.94	0.95	0.96	0.96	1
	Reverse	Spearman	0.91	0.93	0.93	0.94	0.94	0.94	1
		Pearson	0.94	0.94	0.94	0.95	0.95	0.95	1

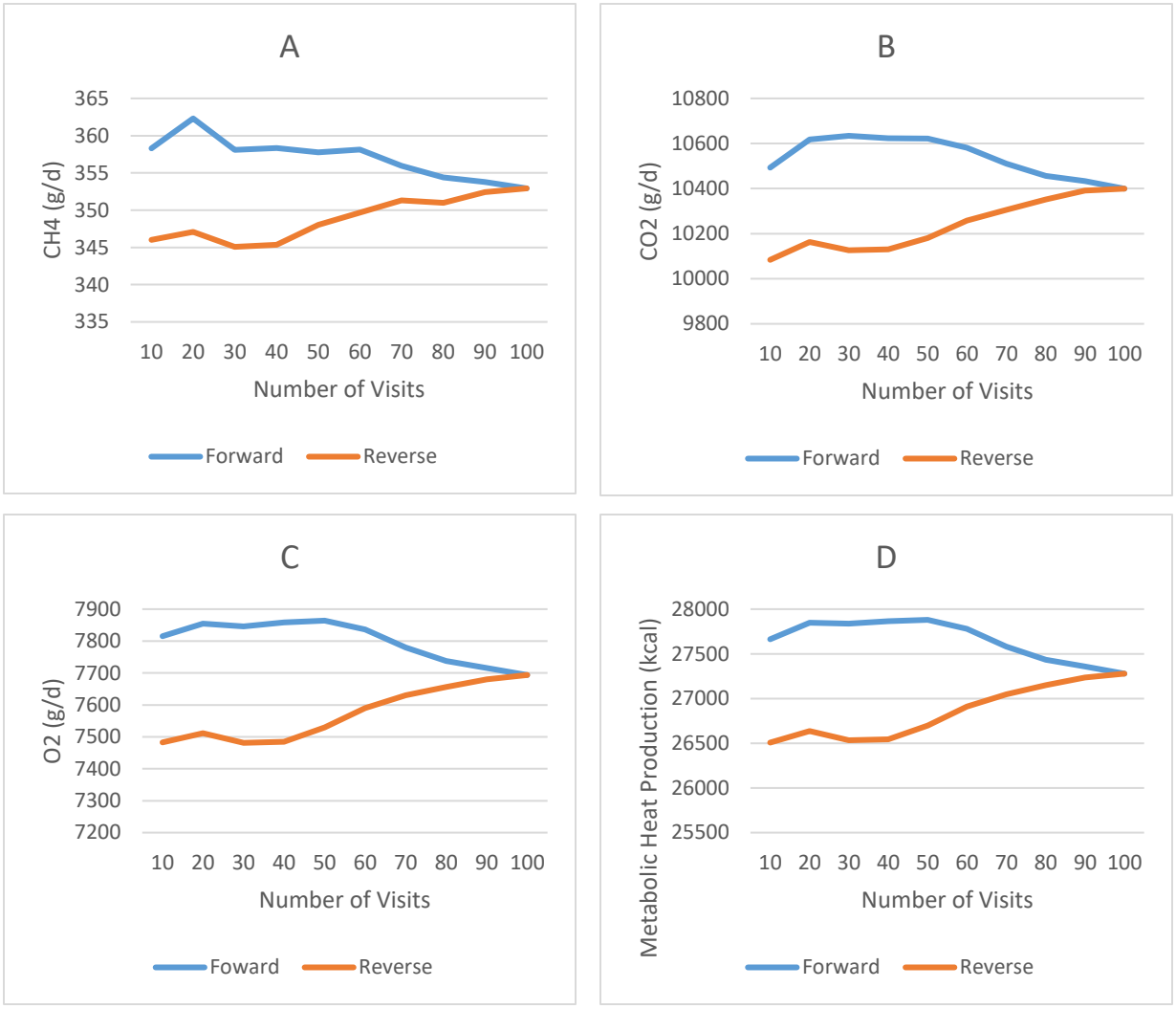

Figure 2.1- Panel A- Mean CH_4 emissions (g/d) for all animals throughout the 100 visit test, Panel B- Mean CO_2 emissions (g/d) for all animals throughout the 100 visit test, Panel C- Mean O_2 consumption (g/d) for all animals throughout the 100 visit test, Panel D- Mean metabolic heat production (kcal/d) for all animals throughout the 100 visit test.

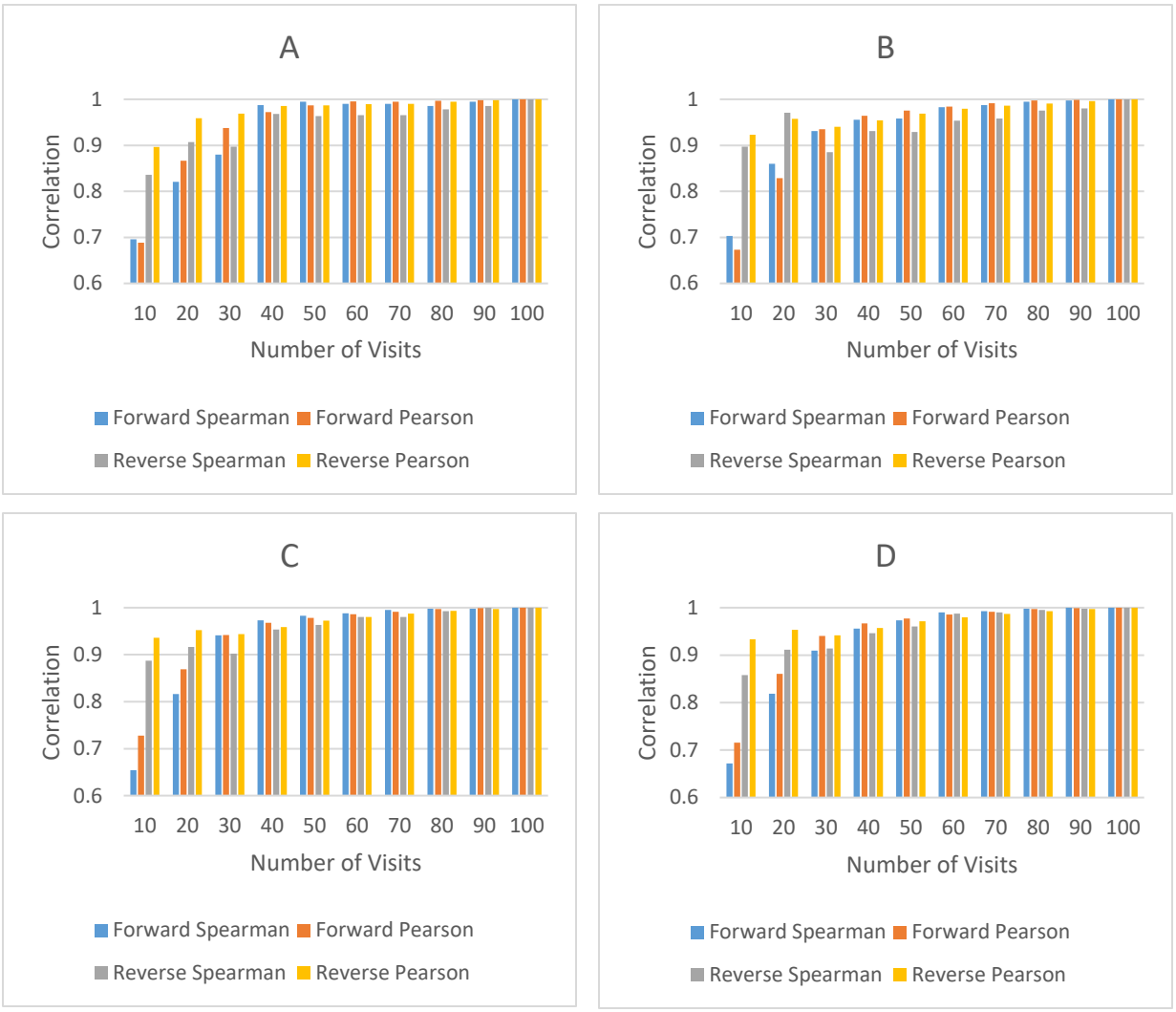

Figure 2.2- Spearman and Pearson correlations between visit intervals and the full 100 visits. Panel A- CH_4 (g/d), Panel B- CO_2 (g/d), Panel C- O_2 (g/d), Panel D- metabolic heat production (kcal/d).

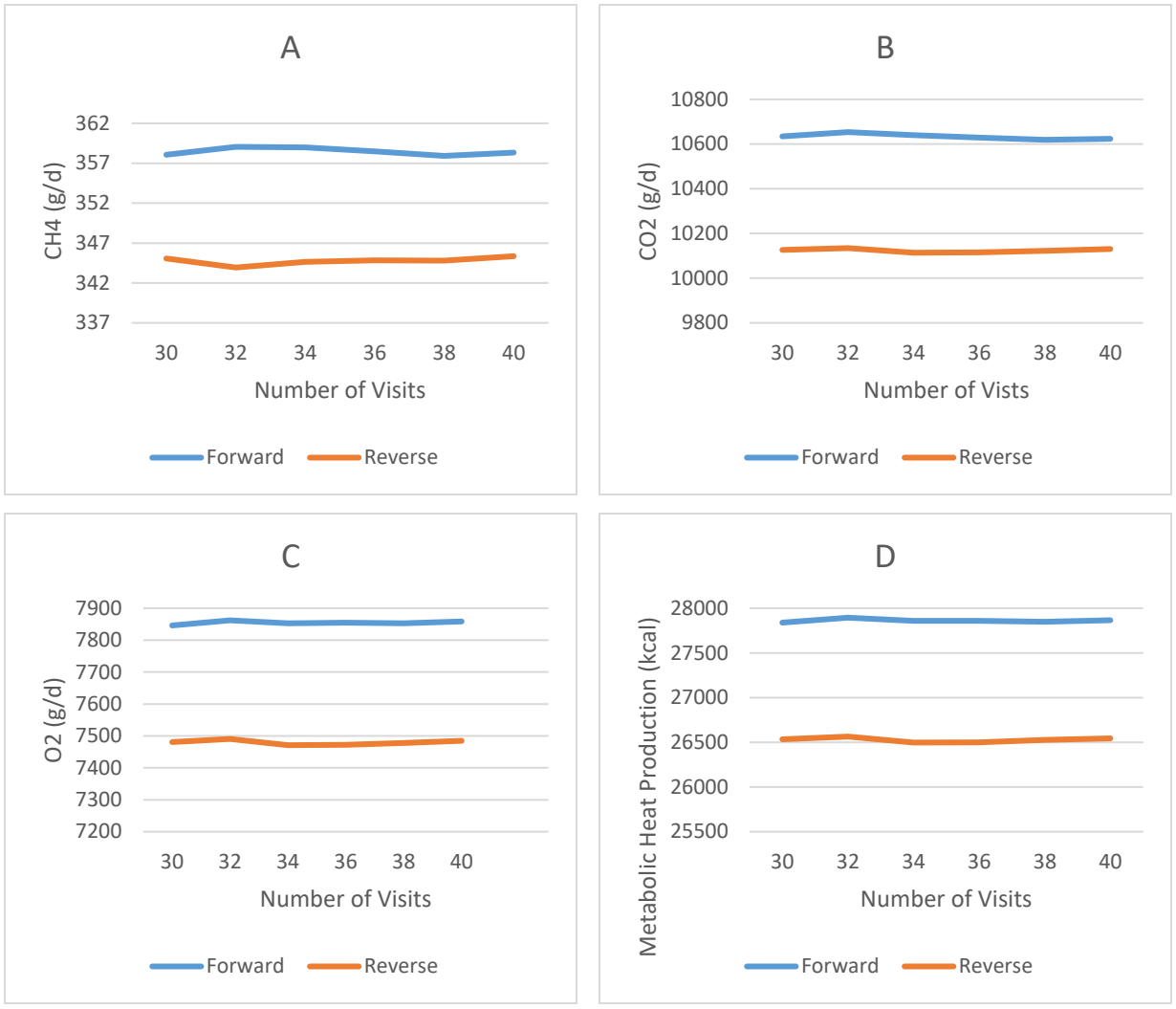

Figure 2.3- Panel A- Mean CH_4 (g/d) for all animals during visits 30 through 40, Panel B- Mean CO_2 (g/d) for all animals during visits 30 through 40, Panel C- Mean O_2 (g/d) for all animals during visits 30 through 40, Panel D- Mean metabolic heat production (kcal/d) for all animals during visits 30 through 40.

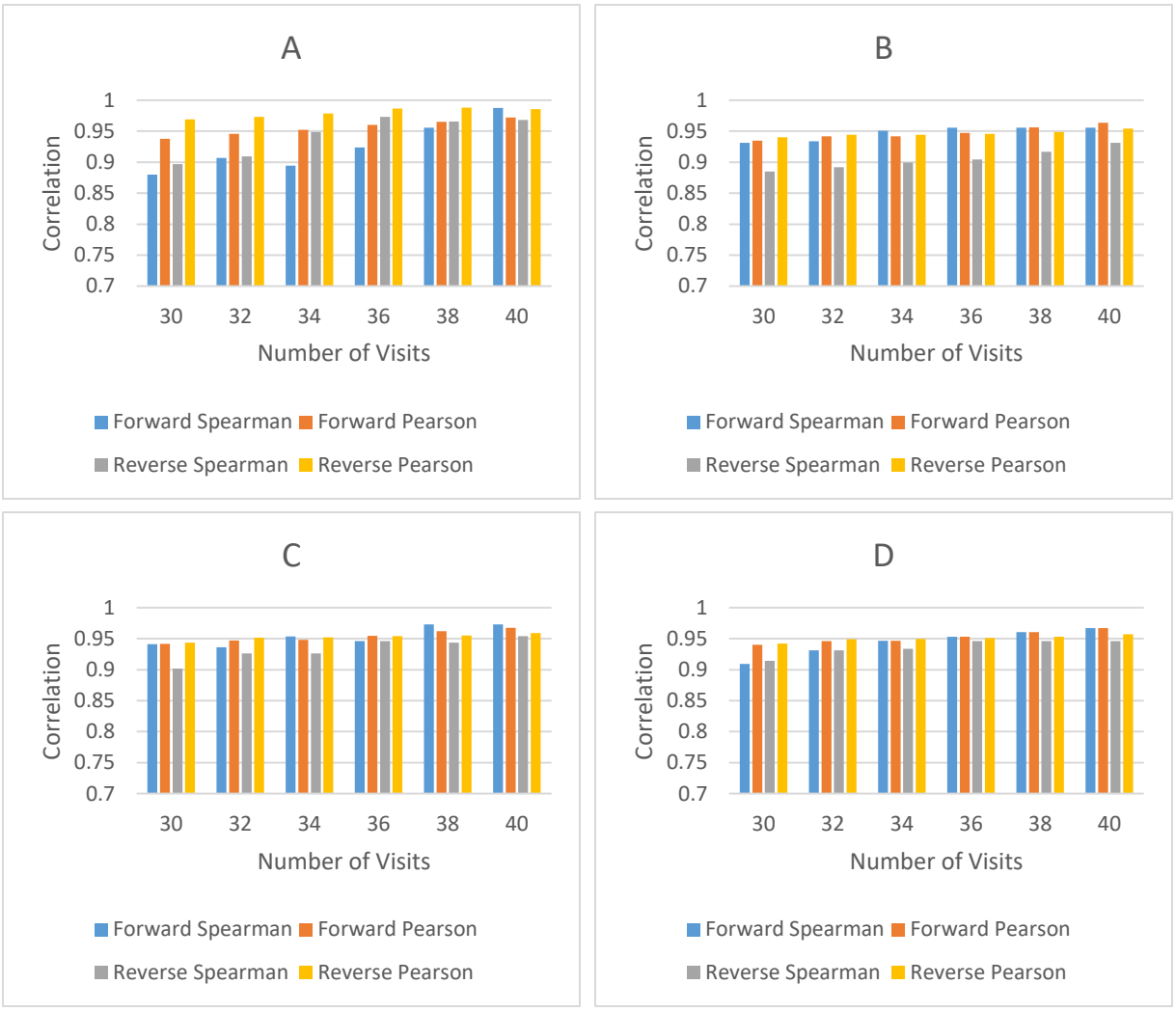

Figure 2.4- Spearman and Pearson correlations between shortened visit intervals between 30 to 40 visits and the full 100 visits for Panel A- CH_4 (g/d), Panel B- CO_2 (g/d), Panel C- O_2 (g/d), Panel D- metabolic heat production (kcal/d).

Chapter 3 - Genetic Evaluation of Feed and Water Intake Behaviors

Abstract

Feed and water intake are two important aspects of cattle production that greatly impact the profitability, efficiency, and sustainability of producers. Feed and water intake have been studied previously, however there is little research on their associated animal behaviors and there is a lack of standardized phenotypes for these behaviors. Feed and water intakes obtained with an Insentec system (Hokofarm Group, The Netherlands) from 830 crossbred steers were used to compute five intake behaviors for both feed and water: number of sessions (NS), intake rate (IR), session size (SS), time per session (TS), and session interval (SI). Variance components and heritabilities were estimated for each trait. Heritabilities for feed intake behaviors were 0.50 ± 0.12, 0.63 ± 0.12, 0.40 ± 0.13, 0.35 ± 0.12, and 0.60 ± 0.12 for NS, IR, SS, TS, and SI, respectively. Heritabilities for water intake behaviors were 0.56 ±0.11, 0.88 ± 0.07, 0.70 ± 0.11, 0.54 ± 0.12, and 0.80 ± 0.10 for NS, IR, SS, TS, and SI, respectively. Dry matter intake (DMI) and daily water intake (DWI) had heritabilities of 0.57 ± 0.11-0.12 and 0.44 ± 0.11. Phenotypic correlations varied between pairs of traits. Genetic correlations between DMI and feed intake behaviors were moderate to high, while genetic correlations between DWI and water intake behaviors were low to moderate. Several significant SNPs were identified for the feed and water intake behaviors. Genes and previously reported quantitative trait loci near significant SNPs were evaluated. The results indicated that feed and water intake behaviors are influenced by genetic factors and are heritable therefore, improvement could be made through genetic selection.

Introduction

Several factors affect the sustainability of the beef industry, including genetic selection for economically relevant, heritable traits. Genetic improvement of feed and water efficiency are two traits that could have a large impact on the sustainability of the beef industry. For example, feed is the largest single cost item of a livestock operation (USDA NASS, 2019). One way to reduce feed costs is to improve feed efficiency. In addition, water is often viewed as a cheap and unlimited resource in a beef operation and is therefore ignored. However, access to clean drinking water is becoming more critical as drought and other environmental events lead to variable water availability and quality (Nardone, 2010). Improving the efficiency of feed and water use in the beef industry could help sustain individual farm and ranch operations.

One aspect of feed and water intake is the associated animal behavior. There is limited research on feed and especially water intake behavior. Standard feed intake behavior phenotypes have not been established in the industry; however, number of meals (per d), time at feeder (min/d), time per meal (min), meal size (g DM), eating rate (g DM/min), and visits to the feeder (per d) are feed behavior characteristics previously defined in literature (Montanholi et al. 2010; Robinson and Oddy, 2004, Nkrumah et al., 2007). Early work in feeding behavior required animals to be penned and fed individually with a form of bunk attendance monitoring. Schwartzkopf-Genswein et al. (2002) used a GrowSafe radio frequency antenna system to monitor bunk attendance frequency and duration and found cattle that spent more time at the bunk ate more and gained weight faster. Nkrumah et al. (2007) used the GrowSafe automated feed intake system to measure feed intake and feeding behaviors, which indicated that feeding behavior could be a source of variation in growth efficiency

The development of systems that track individual feed and water intake has been rapidly advancing research in this area. This equipment can provide insight into feeding behaviors of individual animals and variation in performance not available from pen data. This equipment also allows animals to remain in groups within a pen so that group dynamics and social behavior remain intact.

Literature available on water intake behavior is even more scarce than on feed intake behavior. Ahlberg et al. (2019) reported that water intake is moderately heritable (0.39). Water intake behavior is affected by animal health (Sowell et al. 1999) and environmental factors such as temperature humidity index (Arias and Mader, 2011; Ahlberg et al. 2018), but the relationship between behavior and water intake has not yet been explored.

The objectives of this study were to identify feed and water intake behavior traits, estimate heritability and variance components for these traits, examine their relationship to feed and water intake and perform a genome-wide association study to identify regions of the genome associated with feed and water intake behaviors.

Materials and Methods

Study Population and Design

Water and feed intakes were collected at the Willard Sparks Beef Research Center at Oklahoma State University in Stillwater, OK from 830 steers using an Insentec system (Hokofarm Group, The Netherlands) over seven feeding periods from May 2014 to May 2018 (Table 3.1). Steers within each group were placed into four pens that were equipped with one water bunk and six feed bunks per pen. Pens were 11.27 m by 31.85 m (358.95 m^2) with 103.0 m^2 of shaded space. Bunks were in the shaded area of the pen.

Steers were sourced from sale barns in the south and great plains as well as Oklahoma State University herds. Steers were visually evaluated and those with *Bos indicus* attributes (such as excessively loose skin and/or large elongated ears) were removed from the trial because *Bos indicus* cattle are known to consume less water (Winchester and Morris, 1956; Brew et al., 2011). Upon arrival, a plastic identification tag and a passive half-duplex radio frequency electronic identification (eID; Allflex USA Inc., Dallas-Fort Worth, TX) were placed in the left ear of all steers. During processing, steers were weighed (iBW) and implanted with Compudose (Elanco Animal Health, Greenfield, IN), containing estradiol 17ß (E2 ß). Following processing, animals were grouped by iBW into high iBW and low iBW groups and randomly assigned one of four pens, with approximately 30 steers per pen. Animals were blocked by iBW as a proxy for size so that bunk gates could be set to the appropriate height; if gates were set too low to accommodate the smaller animals, larger animals could access the feed bunks without the system reading their tag. Animals acclimated to the pens and the Insentec system for 21 d prior to the start of each feeding period, and those that failed to acclimate were removed from the trial. The acclimation period was followed by a 70 d feed and water intake testing period in accordance with the Beef Improvement Federation standard test guidelines for feed intake and body weight (BW) gain (BIF, 2021). Individual BW was measured at the end of the acclimation period, every 14 d during the trial, and at the end of the trial period.

Steers were fed a growing ration consisting of 15% cracked corn, 51.36% wet corn gluten feed, 28.44% prairie hay, and 5.20% supplement for the duration of the trial. Ration samples were taken weekly, composited at the end of the trial and analyzed by Dairy One, Inc. (Ithaca, New York) to determine dry matter and gross energy estimates. Gross energy values varied from 18.26 to 19.91 MJ/kg of dry matter. The average dry matter for groups ranged from 70.04% to

74.02%, which was used to calculate dry matter intake (DMI) from feed intake. Bunk management for groups 1-3 followed a slick bunk protocol and groups 4-7 had ad libitum access to feed. Animals had ad libitum access to water, regardless of feed bunk management. Groups 1, 3, 4, and 6 were on trial during the summer season and groups 2, 5, and 7 were on trial during the winter season. Environmental variable averages for each group are described in Ahlberg et al. (2018). All animal procedures were approved by the Institutional Animal Care and Use Committee at Oklahoma State University (protocol AG13-18) in accordance with Federation of Animal Science Societies (FASS, 2010) guidelines.

Data Filtering

The Insentec bunk system recorded individual feed intake and water intake events (kg) on a per animal basis using eID tags and the Roughage Intake Control software system. The Roughage Intake Control software calculates an intake event record by subtracting the ending bunk weights from the starting weights and also records the start and end times for the visit. During the trial, animals were allowed an unlimited number of visits to any bunk. Feed intake and water intake events were filtered to ensure data quality as described in Ahlberg et al. (2018) and Allwardt et al. (2017). Briefly, records that had a greater end weight than start weight were removed. Records with a length of time shorter than 5 seconds or longer than 3600 seconds were removed, and intake records with values substantially larger than the bunk capacity were also edited out of the dataset. If an animal was ill and required treatment, the records on the day before, day of, and day after treatment were removed. All records from days animals were weighed or when equipment malfunctioned were removed. Feed intake and water intake event records were summed by animal for each day to obtain daily feed intake and daily water intake

(DWI). Daily feed intakes were converted to daily dry matter intake (DMI) using the average feed dry matter percentage for each group.

Genotyping

On a day when steers were weighed, two blood samples were taken from the jugular vein to obtain DNA. Blood samples were collected in a 10 mL BD vacutainer tube containing 1.5 mL of the anticoagulant acid citrate dextrose. Whole blood samples were centrifuged to obtain white blood cells from which DNA was extracted using a phenol:choloroform:isoamyl alcohol extraction and ethanol precipitation. Five hundred nanograms of DNA was sent to GeneSeek (Lincoln, NE) for genotyping on the GeneSeek Genomic Profiler Bovine High Density genotyping array (GGP HD150K). Quality control filtering of genotypes included removing markers with a minor allele frequency less than 0.05 and markers with a single nucleotide polymorphism (SNP) call rate less than 0.90, leaving 123,912 SNPs for analysis.

Breed Composition

Full details on breed composition calculations are described in Ahlberg et al. (2019). Briefly, true breed composition of most animals was unknown, so breed composition was estimated for all animals using individual genotypes within a regression framework developed by Chiang et al. (2010). The following model was used to predict breed composition:

$$y = Xb + e$$

where y is a vector containing the scaled number of copies of allele B for an animal, X is a 36,403 by 16 matrix of frequencies for allele B (36,403 allele frequencies for 16 breeds) and b is a vector of regression coefficients that represents the percentage of each breed for each individual animal in y, and e is a vector of random residuals. The 16 breeds were as follows: Angus, Red Angus, Brahman, Braunvieh, Chiangus, Charolais, Gelbvieh, Hereford, Limousin,

Maine Anjou, Salers, Shorthorn, Simmental, Holstein, Jersey, and Brown Swiss. Percentage estimates were grouped into four different biological types (British, Continental, *Bos. indicus*, and dairy) because most breeds had relatively low levels of inclusion, which limits estimation of breed-specific effects.

Carcass Information

Following the initial 70 d intake trial used in this analysis, animals also went through a 70 d period of incremental water restriction (Shaffer, 2022). After the completion of the trial, steers were removed from the Insentec facility, placed in finishing lots to be managed as a group, and transitioned to a finishing ration. The finishing diet consisted of 57.5% cracked corn, 30% wet corn sweet bran, 7.0% prairie hay and 5.5% supplement. Final body weights were taken within 24 hours of steers being sent to a processing plant. Carcasses were chilled at the plant for at least 24 hours post slaughter before they were evaluated for marbling (MARB), rib eye area (REA), and back fat thickness measured at the 12^{th} rib (BFAT). Marbling scores, REA, and BFAT were collected using a camera system at the harvest plant or by trained personnel. Marbling scores that were not recorded as a numeric value were converted as reported by Nephawe (2004) so that all MARB scores were on the same scale.

Behavioral Data

Five behavior traits were analyzed for both feed and water intake: number of sessions (NS), session size (SS), time per session (TS), session interval (SI), and intake rate (IR). Session is defined in this study as one visit to the Insentec bunk (feed or water) that passed data filtering (longer than 5 seconds and shorter than 3600 seconds).

Number of sessions describes the total number of visits to the Insentec bunk in a day. The number of sessions to the feed bunk (NS_f) and the number of sessions to the water bunk (NS_w)

were summed per day for each individual. Then the daily NSf and NSw were averaged from the entire 70 d trial to obtain a period average for each individual.

Session size describes the amount eaten (kg DMI) or drank (kg) in one session. The session size for feed intake (SS_f) and the session size for water intake (SS_w) was averaged for all sessions recorded for each day. Then the mean of the daily averages from the 70 d feeding period were utilized as the variable for analysis.

Time per session describes the amount of time (s) an animal spends at the bunk during one session. Time per session at the feed bunk (TS_f) and time per session at the water bunk (TS_w) were averaged for each individual each day. Then the daily TS_f and TS_w were averaged for the entire 70 d feeding period to obtain the period average for each animal used in analysis.

Session interval describes the amount of time (minutes) between two sessions at the bunk. The session interval was calculated for the sessions in one 24 hour time period, therefore the interval between the last visit of the previous day and the first visit of the following day was not included. The session interval between feed intake sessions (SI_f) and the session interval between water intake sessions (SI_w) were averaged daily for each individual. The daily SI_f and SI_w for each individual were averaged for the 70 d feeding period to obtain a period average that was used for analysis.

Intake Rate describes the amount eaten (g DMI) or drank (g) as a function of the amount of time (s) an animal spent in the bunk for that specific visit. In other words, intake rate is session size divided by time per session. The feed Intake Rate (IR_f) and water Intake Rate (IR_w) for each session in a day was averaged. The mean of IRf and IRw daily averages over the 70 d trial was calculated to obtain the value used in the analysis for each animal.

Statistical Analysis

Phenotypic Model Selection

Many factors affect feed and water intake and their associated behaviors such as environmental factors (Arias and Mader, 2011; Koknaroglu et al., 2008), group dynamics (Grant and Albright, 2001, Proudfoot et al., 2009), diet (DelCurto-Wyffels et al., 2020), and bunk management (Pritchard and Bruns, 2003; Schwartzkopf-Genswein et al., 2002), among others. All available factors describing these attributes were extracted from the data and used in model selection for each behavior trait. Model selection was performed using forward selection stepwise regression. Forward selection starts with a base model and each step adds the variable that improves the model the most, until no more terms can be added that substantially increase model fit. The final model was considered complete when the addition of another variable did not reduce the AIC by 3 or more.

Environmental factors such as season (Arias and Mader, 2011; Koknaroglu et al., 2008; Ahlberg et al. 2018) and bunk management (Pritchard and Bruns, 2003; Schwartzkopf-Genswein et al., 2002) are two factors known to impact feed and water intake. To determine if these factors also have an influence on feeding and drinking behaviors, a simple linear model including only phenotypic data was fit for each behavior trait. This was performed using the SAS 9.4 GLM (general linear model) procedure with a LSMEANS statement. Ten models were used (one for each behavior trait) with a base model as follows:

$$y = b_0 + b_1 Season + b_2 Bunk\ Management$$

where y is the trait being evaluated, b_0 was the intercept, *Season* was either winter or summer, and *Bunk Management* was either slick or ad libitum. The following covariates were tested and added to the base model if found to improve model fit: average daily gain (ADG), BFAT, REA,

MARB and initial body weight. The covariates determined to improve model fit through forward selection (Table 3.2) were added to the phenotypic base model for each behavior trait.

Genetic Model Selection

A separate base model was utilized for genetic evaluation. Bunk management and season were not included in the genetic model and were only used in the phenotypic model because of confounding with group. The genetic base model for each trait included pen nested within group as a fixed effect, and breed composition covariates (*Bos indicus*, continental, and dairy) as follows:

$$y_{ijk} = S_i + group_j(pen_k) + Bos\ indicus_i + Continental_i + Dairy_i + e_{ijk}$$

where y_i is the phenotype being evaluated for the i^{th} animal in the j^{th} group and k^{th} pen, S_i is the random animal effect, group(pen) is the k^{th} pen nested within j^{th} group, Bos indicus is the percent of British breed composition for the i_{th} individual, Continental is the percent of continental breed composition for the i_{th} individual, Dairy is the percent of dairy breed composition for the i^{th} individual and *e* is the random residual. The same covariates that improved model fit for feed and water intake behavior traits in the phenotypic model were added to the genetic base model for each trait outlined in Table 3.2.

Genetic Parameter Estimation

First, univariate animal models were generated based on the base model and the covariates selected from forward selection for each trait to determine starting values for subsequent bivariate analysis. Variance components were estimated using average information restricted maximum likelihood (Misztal et al. 2014). The genomic relationship matrix, G was constructed in the BLUPF90 suite (Misztal et al. 2014) as described by VanRaden (2008). The G matrix is constructed as follows:

$$G = \frac{ZZ'}{2\Sigma p_i(1-p_i)}$$

where Z is a matrix of alleles centered around the mean allele effect, and p_i is the frequency of the second allele at SNP i. In this analysis, pedigree relationships between animals were unknown, so the analysis was strictly a genomic best linear unbiased prediction (GBLUP) where the identity matrices replaced the pedigree numerator relationship matrices in the H^{-1}matrix as follows:

$$H^{-1} = I + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - I_{22} \end{bmatrix}$$

where G^{-1} is as previously described, I is an identity matrix with row and column dimensions equal to the number of genotyped animals, and I_{22} is an identity matrix with row and columns equal to the number of genotyped animals and the dimensions of G. Following the univariate analyses, a bivariate animal model was fit with the BLUPF90 software package (Mizatal et al. 2014) for all pairwise combinations of feed intake behavior traits and DMI and also for all pairwise combinations of water intake behavior traits and DWI. For the bivariate analyses, the base model was used and all covariates from the individual traits used for the univariate analyses were also included (Table 3.2).

For the estimation of variance components, heritabilities, and genetic correlations, the following bivariate animal model was utilized:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 b_1 \\ X_2 b_2 \end{bmatrix} + \begin{bmatrix} Z_1 u_1 \\ Z_2 u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where y_i is a vector of observations for each trait, X_i is an incidence matrix relating observations to fixed effects, b_i is a vector of fixed effects outlined in Table 3.2, Z_i is an incidence matrix relating observations to additive direct genetic effects, u_i is a vector of additive direct genetic effects, and e_i is a vector of random residuals.

Genome-Wide Association Study

SNP Effect Estimation

Animal effects for the single-step Genome-Wide Association Study (GWAS), as described by Wang et al. (2012), were predicted as follows:

$$a = Zu$$

where a is a vector of breeding values for genotyped animals, Z is a matrix relating individuals to phenotypes and u is a vector of SNP marker effects. The variances of a genotyped animal can then be written as follows:

$$var(a) = var(Zu) = G\sigma_a^2 = ZIZ'\lambda$$

where G is the genomic relationship matrix, I is an identity matrix, λ is the ratio of the SNP marker effect variance and the breeding value variance (Masuda, 2019). The SNP effects were predicted as follows:

$$\hat{u} = IZ'(ZIZ')^{-1}\hat{a}$$

where u is a vector of SNP marker effects, I is an identity matrix, Z is a matrix relating individuals to phenotypes, and a is a vector of breeding values for genotyped individuals (Masuda, 2019).

The BLUPF90 family program suite was used to conduct the GWAS (Misztal et al. 2014). Specifically, the postGSf90 program was used to estimate single nucleotide polymorphism (SNP) effects, prediction error variance, and p-values. SNP effects were obtained from each univariate analysis for all 10 feed and water intake behavior traits. The significant SNP threshold was set at $-\log_{10} > 4.5$. In addition, the $-\log_{10}$ p-values were converted to p-values so that the False Discovery Rate (FDR) could be calculated. There were no significant SNPs at an FDR of less than 0.05. When possible, SNPs were identified by their corresponding rs

number. Using estimated SNP effects, Manhattan plots were generated in R using the qqman package (Turner, 2018).

Identification of QTL regions

QTL regions were formed ± 250 kb around the genomic positions of significant SNP to account for linkage disequilibrium (Bovine HapMap Consortium, 2009). Gene candidates were identified within each QTL region using the GALLO package in R (R Core Team, 2013) and the National Center for Biotechnology Information (NCBI) bovine GFF annotation file for the USDA ARS-UCD 1.2 genome assembly (Rosen et al., 2020; accession GCF_002263795.1). All gene functions were identified based on the molecular and biological functions outlined by The UniProt Consortium (2019). Genes were compared with previously reported trait associations within the Cattle QTLdb (Hu et al., 2019).

Results and Discussion

Summary statistics for feed and water intake behavior traits, DMI, and DWI are reported in Table 3.3. The mean NS_f is 39.21 ± 12.11 sessions/d. Means reported in the literature vary widely. Robinson and Oddy (2004) reported mean number of sessions (no/d) from 7.9 ± 2.6 to 18.8 ± 7.9 depending on breed. Schwartzkopf-Genswein et al. (2002) reported that cattle made 16.8 ± 0.1 visits to the feed bunk per day on average. Mantanholi et al. (2010) reported a much higher number of sessions at the feed bunk at an average of 53.0 ± 13.55. Another mean from Schwartzkopf-Genswein et al. (1999) of 29 ± 11.8 visits per day to the feed bunk is closest to the mean for NS_f reported in the current study. Although the mean NS_f is high, it does not necessarily indicate that an animal ate a substantial amount of feed during every visit. In the current study, on average 12% of an animal's visits resulted in an intake of 0 kg, which were filtered out of the dataset. However, any visit resulting in an intake amount greater than 0 kg was

included but may not have been a full meal for that animal. Schwartzkopf-Genswein (1999) observed that only 55.8% of visits to the feed bunk were associated with a feeding activity and the remaining visits involved other behavior such as scratching, licking, and rubbing.

The mean NS_w is 6.39 ± 2.06 sessions/d which is much lower than the mean NS_f. The difference in number of visits between water and feed could have been influenced by the number of bunks available; There were six feed bunks per pen, but only one water bunk. The means available in literature for number of visits to the water bunk are not necessarily from water intake behavior studies but rather studies of water quality or water bunk design. For example, Lardner et al. (2013) examined different water types offered to cattle and reported a range of 2.92 to 10.92 visits per day depending on water type. Coimbra et al. (2009) examined different water tank designs and reported a means from 0.57 to 5.10 depending on design and trial. In dairy cows, the number of drinking bouts per day ranged from 6.8 ± 2.7 to 7.9 ± 2.8 depending on the stocking density of the pen (Cardot et al. 2008). Although there has been a range of means published influenced by a variety of factors, the mean in the current study fits reasonably within the range of previously published studies.

The mean IR_f is 1.67 ± 1.99 g/s. Both Robinson and Oddy (2004) and Schwartzkopf-Genswein (2002) reported eating rate (g/min) whereas the current study examined IR_f (g/s). The eating rate (g/min) ranged from 126 ± 32 to 158 ± 37 depending upon breed and sex (Robinson and Oddy, 2004). Schwartzkopf-Genswein et al. (2002) reported faster intake rate, with means ranging from 203 ± 20.9 to 242.4 ± 18.4 for different feeding regimens and sexes. The lowest end of the range published in literature is a mean eating rate of 97.10 ± 18.24 reported by Lancaster et al. (2009). If our IR_f data was converted to grams per minute to be on the same scale as previous literature, the mean IR_f is 100.79 ± 26.97 which is very similar to the mean published

by Lancaster et al. (2009). The mean IR_f in the current study could be lower due to a high NS_f where the intake is low, making the mean IR_f lower. The mean intake rate is much higher for water (78.00 ± 34.26 g/s) compared to feed which is likely due to a difference in the weight of the material. Unfortunately, there are no IR_w averages for feedlot steers available in published literature for comparison.

The mean TS_f is 203.07 ± 79.36 s. Oliveira et al. (2018) reported an average visit duration (s/visit) of 157.0 ± 2.6 which is shorter than the current study. Oliveira et al. (2018) studied dairy heifers, whereas the current study used feedlot steers which could be why the mean TS_f are different. Time per session in the current study was calculated as seconds per session whereas others have published a similar trait, meal duration, as the total minutes at the feed bunk in one day. Means published in literature for meal duration (min/d) range from 77 ± 19 to 112.1 ± 1.6 (Robinson and Oddy, 2004; Schwartzkopf-Genswein et al. 2002; Lancaster et al., 2009). If the average TS_f in this study is converted from seconds to minutes and multiplied by the average NS_f, animals spent on average 130 minutes at the feed bunk per day which is similar to the highest value in the literature range. The mean TS_w is 101.18 ± 37.41 s. Coimbra et al. (2009) reported that animals in small pasture paddocks only drank for 115.23 to 167.23 seconds per day. Lardner et al. (2013) reported a wide range of drinking duration (s/d) from 108.7 to 627.9 when comparing different water types. The mean for TS_w in the current study was 100.57 seconds per visit and the mean NS_w was 6.34 visits per day hence, on average animals spent an average of 637.61 seconds at the water bunk per day which is very similar to the top of the range reported by Lardner et al. (2013).

For SS_f, which is the amount of dry matter eaten per session, the mean is 0.3127 ± 0.105 kg. Montanholi et al. (2010) reported a mean meal size (g DM) of 1,000 or 0.1 kg which is lower

than the mean in the current study. The mean session size is slightly larger for water (6.50 ± 1.85) compared to feed which is also likely a function of the weight of the material. In two studies with dairy cattle by Cardot et al. (2008) and Jago et al. (2005) reported larger average water intake per drinking bout than the current study (12.9 L and 14.9 L, respectively). For session interval (min), animals spent a much shorter amount of time between visits to the feed bunk (21.5 ± 8.14) compared to visits to the water bunk (155.22 ± 39.77). Published literature that reports average SIw for feedlot steers in sparse, making comparisons difficult.

Steers in the study had an average daily DMI of 10.73 ± 1.47 kg. This is close to the averages previously published in other feeding behavior studies such as Schwartzkopf-Genswein et al. (2002) and Lancaster et al. (2009), which were 9.7 ± 0.1 and 9.46 ± 1.31, respectively. Steers drank an average of 37.50 ± 10.5 kg of water per day, which is equivalent to 37.5 ± 10.5 L. This mean is very close to the mean water intake of purebred male Senepol cattle (37.11 ± 4.75 L/d) reported by Pereira et al. (2021). Arias and Mader (2011) reported an average DWI in the summer (32.4 ± 0.1 L/d) and in the winter (17.3 ± 0.1 L/d). In a two-year study, Parker et al. (2000) found the average water usage to be 40.9 L/d. Lardner et al. (2013) reported a wide range for DWI from 13.9 L/d to 58.1 L/d. The mean water intake in the current study falls within the range of means previously published in literature.

Season and Bunk Management Phenotypic Model

The mean NS_f was significantly different between seasons ($P = 0.0036$). In the current study, animals visited the feed bunk 2.90 more times per day in the winter compared to the summer. During winter conditions, maintenance energy requirements increase due to exposure to cold, wet, or windy environments (National Research Council, 2016). Perhaps animals visited the feed bunk more times per day in the winter as a part of thermoregulation This contrasts with

the effect season had on NS_w. The mean NS_w was significantly different between seasons ($P < 0.0001$), however, animals visited the water bunk 2.0 more times in the summer compared to the winter. It is logical that perhaps due to the higher temperatures in summer months (Ahlberg et al. 2018) animals opted to visit the water bunk instead of the feed bunk. Arias and Mader (2011) found that maximum and minimum ambient temperatures as well as the temperature-humidity index are primary environmental factors influencing DWI in finishing cattle and that DWI increases during the summer. The primary reason DWI increases in the summer is attributed to animals attempting to reduce the thermal load (Beede and Collier, 1986). These results align with the current study and help explain why the NS_w are greater in the summer compared to the winter.

The mean NS_f ($P < 0.0001$) and NS_w ($P < 0.0001$) were significantly different between bunk managements. Animals visited the feed bunk 6.91 more times in a day under ad libitum bunk management compared to slick. The results from the current study align with those from Schwartzkopf-Genswein et al. (2002) which found that animals visited the feed bunk more frequently ($P < 0.0001$) during ad libitum access compared to restricted feed access. The reason animals visited the bunk less with a slick protocol is likely because feed bunks could be empty for some time before the next meal compared to an ad libitum bunk management where feed is always available. Although animals always had ad libitum access to water, under ad libitum feed bunk management, animals visited the water bunk 1.44 more times per day compared to the slick feed bunk management. Dry matter intake and DWI have moderately positive phenotypic and genetic correlations, 0.38 and 0.34 (Ahlberg et al. 2019). Perhaps because animals were already standing up to eat at the feed bunk, animals also choose to visit the water bunk to drink before or after the meal.

The mean IR_f was not significantly different between seasons ($P = 0.2704$). However, the mean IR_w was significantly different between seasons ($P = 0.0012$). Animals drank 10.2 g per second faster in the winter compared to the summer. The reason for this is not known, although the water temperature due to differences in ambient temperature between summer and winter could have played a role in this (Andersson, 1985; Purwanto et al. 1996). The mean IR_f was significantly different between bunk managements ($P < 0.0001$). With a slick bunk management, animals ate 0.42 grams per second faster compared to ad libitum bunk management. Gonyou and Stricklin (1981) reported that cattle with restricted access to feed spent less time per day at the feed bunk per day but compensated for feed intake levels by increasing rate of feed consumption. Schwartzkopf-Genswein et al. (2002) reported faster eating rates by cattle under restricted feed compared to ad libitum bunk management. Erickson et al. (2003) reported that steers with a clean bunk management consumed feed at a faster rate compared to steers with ad libitum bunk management. When animals are managed with a slick bunk compared to ad libitum where feed is always available, animals may be hungrier and more eager to eat quickly when feed is delivered. The mean IR_w was significantly different between bunk managements ($P < 0.0001$). Animals under a slick feed bunk management drank 21.3 grams per second faster than animals under ad libitum feed bunk management. Although animals had ad libitum access to water, perhaps animals with a slick feed bunk management were in a resource limited mindset from limited access to feed that carried over to water behaviors causing animals to also be more eager to drink and therefore have faster IR_w.

There was not a statistical difference for the mean TS_f between seasons ($P = 0.2821$). This differs from the results from Mujibi et al. (2010) which found that visit duration (min/d) was significantly different between fall/winter and winter/spring groups. Although visit duration

(min/d) is a slightly different trait from TS_f (s/visit) and the seasons evaluated in Mujibi et al. (2010) are different which could be why the current study found different results. The mean TS_w was tended to be different between seasons ($P = 0.0851$). Animals spent 6.1 seconds longer during one drinking session in the summer compared to the winter. The amount of time spent at the water bunk during one session could be impacted by the IR_w. Animals had a faster IR_w in the winter compared to the summer, thus the total time spent at the water bunk during one session in the winter would be lower compared to the summer to consume the same amount of water. There was a difference in mean TS_f ($P = 0.0140$) and TS_w ($P = 0.0409$) between bunk managements. With ad libitum feed bunk management, animals spent 16.3 seconds longer at the feed bunk and 7.1 seconds longer at the water bunk during one session. For a similar trait, bunk attendance duration (min/d), Schwartzkopf-Genswein et al. (2002) found that animals attended the feed bunk for longer periods of time with ad libitum feed management compared to a restricted feeding management.

The mean SS_f was significantly different between seasons ($P = 0.0127$). In the summer, animals ate 0.018 kg more per session compared to the winter. As mentioned above, animals visited the feed bunk fewer times in the summer compared to the winter. Thus, animals may have eaten more in one session to compensate for having fewer sessions. There was no difference ($P = 0.2578$) between mean SS_w for different seasons. However, there was a difference in means for both SS_f ($P < 0.0001$) and SS_w ($P < 0.0001$) between bunk managements. Animals ate 0.06 kg more feed and drank 0.98 kg more water in one session under slick feed bunk management. Compared to always having feed available, animals under a slick feed bunk management could have eaten more during one session as they have a scarcity mindset. In other words, perhaps they

ate more as insurance because feed was not always readily available. That mindset could also be applicable to water intake as well.

There was a difference in means for SI_f between seasons ($P = 0.0074$). There was a 1.9 minute longer interval between sessions in the summer compared to the winter. Due to the higher ambient temperatures in the summer, animals may have shifted feeding visits to cooler parts of the day like early morning and late evening (Ray and Roubicek, 1971; Brown-Brandl et al. 2005) which could explain why the SI_f is longer in the summer. The mean SI_w was statistically different between seasons ($P < 0.0001$). In contrast to SI_f, the mean SI_w in the summer was 25.5 minutes shorter compared to the winter. According to Arias and Mader (2011) cattle finished in the summer consume 87.3% more water than cattle finished in the winter and ambient temperature is one of the primary factors that influences DWI. This could help explain the reason why animals wait a shorter amount of time between visits to the water bunk in the summer.

The mean SI_f was different between bunk managements ($P < 0.0001$). Cattle fed with ad libitum bunk management waited 4.1 minutes longer between visits to the feed bunk than cattle with slick bunk management. Animals with a slick bunk management may have waited a shorter amount of time between visits because the feed was limited thus, they checked the bunk more frequently to see when the next time feed would be available. The mean SI_w was different between bunk managements ($P = 0.0111$). Cattle with ad libitum access to feed waited 9.77 minutes longer between visits to the water bunk compared to animals with a slick feed bunk management. Although the reason is not known, perhaps cattle that had a slick feed bunk management were already standing to check the feed bunk more often, so they also chose to visit the water more often.

There was not a statistical difference in mean DMI between seasons ($P = 0.4980$). The mean DWI was statistically different between seasons ($P < 0.0001$). Animals drank 8.9 kg more water per day in the summer compared to the winter. This is consistent with the results from Ahlberg et al. (2018) that used a subset of the animals from the current study and found that in the summer months steers had significantly ($P < 0.0001$) higher water intake (as a percent of body weight) than the winter. Hoffman and Self (1973) found that animals had greater water consumption in the summer (30.7 L/d) compared to the winter (19.2 L/d). Arias and Mader (2011) reported that cattle finished in the summer consumed 87.3% more water compared to cattle finished in the winter. In the summer, the water demand for cattle is higher due to animal's efforts to reduce heat load by evaporative cooling (Morrison, 1983) which could explain why DWI was higher in the summer for the current study.

The mean DMI was statistically different between bunk managements ($P < 0.0001$). Cattle ate an average of 0.38 kg more feed per day with an ad libitum feed management compared to slick. This could be expected as animals had unrestricted access to feed at all times. Erickson et al. (2003) reported that steers with ad libitum bunk management had greater DMI ($P < 0.01$) than steers with a clean bunk management. There was a difference in mean DWI between bunk managements ($P = 0.0313$). Steers that were fed with an ad libitum feed bunk management drank 1.69 kg more water per day compared to steers fed with a slick bunk management. Ahlberg et al. (2018) reported that steers with ad libitum access to feed drank more water ($P < 0.0001$) than steers with a slick bunk management using a subset of animals from the current study. Conversely, Mader and Davis (2004) used pen water intakes allocated to individuals and reported no difference in water intake between ad libitum or slick feed bunk managements.

Variance Components and Heritability Estimates

The additive direct genetic, residual, and phenotypic variances for the GBLUP analyses as well as heritabilities and standard errors are reported in Table 3.4 for feed and water intake behavior traits, DMI, and DWI. The reported variance components for feed and water intake behavior traits were obtained from the bivariate analysis. The reported variance components for DMI and DWI are the mean from each respective bivariate analysis.

Ahlberg et al. (2019), using a subset of the animals from the current study, estimated the variance components for DMI that were slightly larger to those of the current study. The heritability estimate for DMI was 0.57 ± 0.11-0.12. Nkrumah et al. (2007) reported a heritability of 0.54 ± 0.15 for DMI using feedlot steers in Alberta, Canada, which is very similar to the estimate in the current study. In a large meta-analysis, Berry and Crowley (2013) reported a wide range for heritability of DMI from 0.14 to 0.70 and the estimate from the current study falls within that range. Berry and Crowley (2013) reported the pooled heritability from all studies in the meta-analysis was 0.40. Polizel et al. (2018) reported a heritability estimate for DMI from Nellore feedlot cattle to be 0.46 ± 0.09 and similarly Rolfe et al. (2011) reported a heritability for DMI of 0.40 ± 0.02. Purebred Angus and Charolais steers had a DMI heritability of 0.39 ± 0.10 and 0.54 ± 0.13, respectively (Mao et al. 2013). Ahlberg et al. (2019), using a subset of animals from the current study, reported a higher heritability for DMI than the current study (0.67 ± 0.04). While there are an ample number of heritability estimates for DMI available in literature, the heritability from the current study fits within the range previously published.

Robinson and Oddy (2004) reported variance components for three feed intake behavior traits: time spent eating (min/d), number of feeding sessions (no./d), and eating rate (g/min). Their calculation of number of feeding sessions was similar to NS_f in this study and they

reported smaller phenotypic and genetic variance estimates for NS_f than the estimates in the current study (Robinson and Oddy, 2004). This is perhaps because of a difference in study populations because Robinson and Oddy (2004) used tropically adapted breeds (Brahman, Belmont Red and Santa Gertrudis) in addition to temperate breeds like Angus and Hereford. The heritability estimate for NS_f in the current study was 0.50 ± 0.12. This is similar to the estimate for number of eating sessions (0.44 ± 0.07) published by Robinson and Oddy (2004). Nkrumah et al. (2007) reported a slightly lower heritability estimate of 0.38 ± 0.13 for a similar trait called feeding frequency (no. events/d).

The current study reports IR_f (g/s), while Robinson and Oddy (2004) reported a similar trait eating rate (g/min). The phenotypic and genetic variances of eating rate (g/min) estimated were much larger compared to the estimates from the current study. This is likely due to the difference in units used for time because when IR_f (g/s) is converted to grams per minute then the phenotypic variance is much closer to the estimate reported by Robinson and Oddy (2004) for eating rate. For IR_f, the estimated heritability was 0.63 ± 0.12. This estimate is slightly higher than the only other estimate available in literature from Robinson and Oddy (2004) for the trait eating rate (g/min; 0.51 ± 0.06).

Time spent eating (min/d) was calculated by Robinson and Oddy (2004) as the daily total minutes spent eating, while the current study evaluated TS_f, which was calculated as the daily average time (s) spent eating per eating session. Robinson and Oddy (2004) reported smaller variance component estimates than the current study. In the current study, TS_f has a heritability estimate of 0.35 ± 0.12. This is similar to the trait time spent eating with a heritability estimate of 0.36 ± 0.05 published by Robinson and Oddy (2004). Nkrumah et al. (2007) reported a lower heritability estimate of 0.28 ± 0.12 for feeding duration (min/d). While feeding duration (min/d)

from Nkrumah et al. (2007) and time spent eating from (Robinson and Oddy, 2004) were calculated differently than TS_f in the current study, they are similar traits with similar heritability estimates. The traits SS_f and SI_f have heritability estimates of 0.40 ± 0.13 and 0.60 ± 0.12, respectively. Unfortunately, there are no published heritability estimates in literature for traits similar to these for beef cattle.

The heritability estimates for NS_w, IR_w, SI_w, TS_w, and SS_w were 0.56 ± 0.11, 0.88 ± 0.07, 0.80 ± 0.10, 0.54 ± 0.12, and 0.70 ± 0.11, respectively. Although this is a fairly small population sample and replication should be done in other populations, these estimates are all highly heritable, suggesting that water intake behaviors would respond well to selection in cattle. Heritability estimates for IR_w and SI_w were very high. Currently, there are no other heritability estimates in published literature for drinking behavior. However, there are a few heritability estimates published for water intake. The current study estimates the heritability of water intake to be 0.44 ± 0.11. Ahlberg et al. (2019) reported variance components for DWI that were slightly smaller than the current study and a heritability estimate of 0.39 ± 0.07. The animals used by Ahlberg et al. (2019) were a subset of the animals used in the current study. However, a similar heritability estimate, 0.37 ± 0.04 for water intake was also reported by Pereira et al. (2021). Although heritability estimates for water intake in beef cattle are limited, several heritability estimates for water intake have been reported in mice. Bachmanov et al. (2002) and Ramirez and Fuller (1976) reported heritability estimates for water intake of 0.69 and 0.44, respectively. More research is needed in this area and in disparate populations to make additional comparisons.

Phenotypic Correlations

Phenotypic correlations between feed intake behavior traits are shown in the lower triangular portion of Table 3.5. The majority of the phenotypic correlations between feed intake

behavior traits were high and significantly different from zero. Most notable are the high negative correlations NS_f had with TS_f, SS_f, and SI_f (-0.71, -0.83, and -0.75, respectively). When an animal visits the feed bunk more frequently; the length of each visit tends to be shorter, and they tend to eat a smaller amount in each visit. The amount of time between visits also tends to be shorter. However, Robinson and Oddy (2004) published a correlation between number of eating sessions (no./d) and time spent eating (min/d) that was much lower (-0.01 ± 0.04) than this study. Robinson and Oddy (2004) used time spent eating as the total minutes an animal was at a feed bunk in one day whereas the current study used the trait TS_f, the average seconds for one visit to the feed bunk. Nkrumah et al. (2007) reported a correlation of 0.15 between feeding frequency (events/d) and feeding duration (min/day). This is similar to the traits NS_f and TS_f in the current study, which had a very different correlation in the current study (-0.71). However, Nkrumah et al. (2007) calculated feeding duration as total minutes at the feed bunk per day whereas the current study calculated TS_f as seconds at the feed bunk per visit. In the current study, there was no correlation between NS_f and IR_f (0.01). However, Robinson and Oddy (2004) reported a phenotypic correlation of 0.14 between number of sessions (no./d) and eating rate (g/min). The current study used different units for IR_f (g/s), however their estimate reported is similar to that from the current study.

Intake Rate for feed had a moderate negative correlation with TS_f (-0.49). It is logical that animals that eat slower will spend more time at the feed bunk and animals that eat faster will spend less time at the feed bunk. Robinson and Oddy (2004) reported a correlation of -0.77 between eating rate (g/min) and time spent eating (min/d). Despite the discrepancy between how Robinson and Oddy (2004) calculated time spent eating compared to TS_f in the current study, the correlations are similar. Time per Session had a correlation of 0.82 with SS_f, so animals that

spent more time at the feed bunk during one session tended to, eat more. Time per Session was also highly positively correlated with SI_f (0.74). Thus, animals that spend longer eating during one session tended to also spend more time between visits to the feed bunk. Session Interval and SS_f had a highly positive correlation of 0.75, meaning that animals that had larger amounts of time between visits to the feed bunk also tended to eat more in one session when they did visit. Animals that spent a shorter amount of time between visits tended to eat less when they visited the feed bunk.

It is also interesting to investigate if feeding behaviors have any effect on DMI. Phenotypic correlations between DMI and IR_f, and DMI and TS_f, were not significantly different from zero (-0.002 and 0.04, respectively). The phenotypic correlation between DMI and SS_f was small (0.10), although significant at $P < 0.05$. The phenotypic correlation between DMI and NS_f was 0.28 suggesting that increased eating sessions in a day would tend to also increase DMI. However, Nkrumah et al. (2007) reported the phenotypic correlation between DMI and daily feeding frequency, a similar trait to NS_f, to be -0.21. Montanholi et al. (2010) found the correlation between DMI and number of meals per day to be 0.16. Robinson and Oddy (2004) reported a phenotypic correlation between feed intake (kg/d) and number of eating sessions to be 0.18 ± 0.20. The correlations reported by Montanholi et al. (2010) and Robinson and Oddy (2004) are similar to the correlation from the current study. The phenotypic correlation between DMI and SI_f was -0.23 meaning that animals that wait longer between eating sessions tend to have a lower DMI.

The phenotypic correlations between water intake behavior traits are shown in the lower triangular portion of Table 3.6. Many of the phenotypic correlations between water intake behavior traits had moderate to high negative correlations and were significantly different from

zero. Number of Sessions for water had negative correlations with IR_w, SS_w, and SI_w of -0.31, -0.54, and -0.74, respectively. Animals that visited the water bunk more frequently tended to; drink at a slower rate, consume less water in one visit, and spend less time between visits to the water bunk. This is similar to the direction and magnitude of correlations between NS_f and SS_f as well as NS_f and SI_f. Intake Rate was negatively correlated with TS_w (-0.65) and positively correlated with SS_w (0.46). Thus, animals that drink at a faster rate tended to spend less time at the water bunk during each visit and drink more water in one session. The phenotypic correlation between SS_w and SI_w was 0.54 meaning that animals that drink more during one session tended to also spend more time between visits to the water bunk. Time per session had a low positive correlation with SS_w and SI_w, 0.21 and 0.16, respectively. Unfortunately, there are no published correlations between water intake behavior traits for comparison so only the results from this study will be discussed.

Daily water intake was positively correlated with NS_w and SS_w, 0.52 and 0.36, respectively. Animals that have a greater number of drinking sessions in a day and drink more during one drinking session $_{tend}$ to have greater DWI. Daily water intake was negatively correlated with SI_w (-0.30) meaning that animals that waited longer between visits to the water bunk tend to drink less water in a day.

Genetic Correlations

The genetic correlations between DMI and feed intake behaviors are reported in Table 3.5. Daily DMI had a moderate positive correlation with NS_f (0.47 ± 0.15), which mirrored the phenotypic correlation. Therefore, if there is selection to increase DMI, it would be expected that NS_f would also increase. Nkrumah et al. (2007) reported a much different genetic correlation between DMI and daily feeding frequency (-0.74 ± 0.15). A genetic correlation estimate that is

closer to the current study was reported by Robinson and Oddy (2004) for feed intake (kg/d) and number of eating sessions per day (0.16 ± 0.10).

The genetic correlation between DMI and IR_f is 0.49 ± 0.16, which is different than the phenotypic correlation that was close to 0. Robinson and Oddy (2004) reported a similar genetic correlation for feed intake and eating rate (g/min) of 0.33 ± 0.10. We would expect that IR_f would increase if there is selection to increase DMI. Daily DMI had a negative, moderate genetic correlation with TS_f, SS_f, and SI_f (-0.64 ± 0.34, -0.40 ± 0.29, and -0.59 ± 0.14, respectively). These antagonistic relationships with DMI means that as DMI is selected to increase, the time per eating session, size of meal, and amount of time between visits to the feed bunk would decrease. This relationship is logical given the relationships with other feeding behaviors. As DMI is selected to increase, we would expect to also observe more visits to the feed bunk per day where the meal is smaller, therefore the animal eats quicker and more frequently.

The genetic correlations between DWI and water intake behaviors are reported in Table 3.6. Daily water intake and NS_w have a genetic correlation of 0.48 ± 0.15, which is similar to the phenotypic correlation. This correlation is also very similar to the relationship between DMI and NS_f. We would expect NS_w to increase if we selected for increased DWI due to this positive correlation. Intake Rate of water and TS_w had genetic correlations with DWI that were not different from 0 (0.06 ± 0.15 and 0.03 ± 0.20, respectively). Therefore, selecting for DWI should have no impact on IR_w and TS_w. Daily water intake and SS_w had a low positive genetic correlation of 0.13 ± 0.17. Interestingly, this correlation is positive while the corresponding correlation for feed was negative. If DWI is selected for, the amount of water drank in one session would be expected to also increase, however, DMI had an antagonistic relationship with

the among of feed eaten in one session and would therefore be expected to decrease. The correlation between DWI and SI_w was -0.37 ± 0.14 which mimics the phenotypic correlation.

Genome-Wide Association Study

Significant SNPs were identified for all feed and water behavior traits. Significant SNPs are reported in Table 3.7 for feed traits and Table 3.8 for water traits. Manhattan plots are provided in Figure 3.1 for feed traits and Figure 3.2 for water traits. For each trait, previously reported QTL associated with feed or water intake behavior attributes were identified near the significant SNP (Table 3.9). Previously reported QTL were considered near the significant SNP if the QTL was within the QTL region (250 kilobases upstream or downstream of the significant SNP). All genes found within the QTL region (± 250 kilobases of the significant SNP) are outlined in Table A.1 through Table A.12. Genes discussed as potential candidate genes for feed traits are listed in Table 3.10 and Table 3.11 for water traits

Feed Traits

For NS_f, three SNPs were significant including rs110698940, rs109466582, and rs134486905 (Table 3.7). Forty-eight genes resided within the QTL regions for NS_f (Table A.1). The SNP, rs109466582, was in proximity to the gene ADCY4 (Adenylate Cyclase 4) which encodes a member of the adenylate cyclase family. Adenylate cyclase is an enzyme that catalyzes the conversion of ATP to cyclic AMP. Cyclic AMP is a second messenger with a variety of functions including intracellular signal transduction and activation of protein kinases and the associated kinase functions in several biochemical processes such as lipid, glycogen, and sugar metabolism (The UniProt Consortium, 2019). Adenylate cyclase 4 as well as adenylate cyclases 2 and 3 have been found to be expressed in the olfactory cilia in mice (Wong et al.,

2000). Olfaction is a major sense involved in food evaluation (Do et al., 2014) and therefore could influence the number of visits an animal makes to the feed bunk.

The SNP rs109466582 was also near the gene FITM1 (Fat Storage Inducing Transmembrane Protein 1) which plays an important role in fat storage and lipid metabolism. FITM1's gene product is an integral component of the endoplasmic reticulum membrane and plays an important role in the formation of lipid droplets, lipid droplet organization, and lipid storage. Lipid droplets are storage organelles principal to lipid metabolism (Kadereit et al., 2007). Thus, FITM1 is a gene candidate due to its roles in lipid and energy homeostasis.

Previously reported QTL by McClure et al. (2010) that overlap with rs109466582 are associated with body weight, body height and body weight at weaning in Angus cattle. The size of an animal has some influence on that animal's nutritional requirements (National Research Council, 2016) and therefore the feed intake of that animal. Previously reported QTL that overlap with rs110698940 are associated with residual feed intake (Sherman et al. 2009) and weaning body weight (McClure et al. 2010). Previously reported QTL that overlap with rs134486905 are associated with body weight and body weight gain (Snelling et al. 2010). In the current study, the genetic correlation between NS_f and DMI was 0.47 ± 0.15, if DMI is selected for, there would also be an expected response on NS_f . Thus, NS_f could be associated with growth characteristics such as weight through the mutual association with feed intake (Hicks et al. 1990; Robinson and Oddy, 2004).

Ten significant SNPs were identified for IR_f (Table 3.7). For IR_f, 37 genes were within the QTL region (Table A.2). Of the 37 total, the rs135671861 QTL region overlapped with 16 gene candidates. Five of the 16 genes were related to olfactory receptors, LOC101905743, LOC524304, LOC615808, OR9Q2, and LOC107133203. Olfactory receptors are responsible for

the perception of odor. Olfaction influences food choice and food consumption because it is one of the major sensory modalities that contributes to food evaluation (Do et al., 2014). Previous studies have reported the association of olfaction with feed efficiency (Takeda et al., 2019; Zhou et al., 2018), DMI (Olivieri et al., 2016), and residual feed intake (Saatchi et al. 2014). Another variant identified in this study, rs41732798, was near two genes (NKAIN3 and YTHDF3) previously associated with growth or body size characteristics. The NKAIN3 (Sodium/Potassium Transporting ATPase Interacting 3) gene is involved in the regulation of sodium ion transport, imports metabolites necessary for cell metabolism, and maintains cell potential. In addition to its roles in cell metabolism, Marete et al. (2018) discovered its association with insulin-like growth factor 1 (IGF1) level. Insulin-like growth factor 1 level is similar to growth hormone in that levels are low during infancy and increase during development, eventually declining during adulthood. It is also documented that IGF1 is linked to reproductive and growth traits (Fortes et al., 2012; Yilmaz et al., 2004; Yilmaz et al., 2005). Feed intake and growth have an obvious association, therefore NKAIN3 is a gene candidate as it is related to both cell metabolism and growth. The final gene with an obvious association with IR_f was YTHDF3 (YTH N6-Methyladenosine RNA Binding Protein 3) which has a vast number of biological functions; specifically, it recognizes and binds N6-methyladenosine (m6A)-containing RNAs and regulates their stability. Bhuiyan et al. (2018) reported that YTHDF3 contributes to carcass weight. Cattle that have a greater dressed carcass weight likely have had greater feed intake, which is related to IR_f. Quantitative trait loci regions associated with body weight (Mizoshita et al. 2004; Kneeland et al. 2004), average daily gain (Mizoshita et al. 2004), and carcass weight (McClure et al. 2010) were near rs41732798 (Table 3.9). These traits are all related to growth and feed efficiency, which could be connected to how quickly an animal eats or IR_f.

Eight significant SNPs were identified for TS_f (Table 3.7). Of the 35 genes within these QTL regions, 28 were a members of the olfactory receptor family and were near rs135936657 (Table 3.10). Olfactory receptors function by combining with the odorant in the nose and transmitting the signal to the brain which triggers the perception of smell. Takeda et al. (2019) identified genes involved in olfactory transduction as candidate genes for residual feed intake and residual body weight gain. The olfactory receptor gene family is the largest in the bovine genome (Lee et al. 2013). Takeda et al. (2019) proposed that olfaction may have evolved in cattle to allow them to seek better quality grass and avoid toxic plants, so perhaps that is why olfactory receptors are involved in appetite regulation and feed efficiency. The perception of smell is an important component of food choice and consumption (Do et al., 2014) and perhaps could influence how long cattle spend eating during one meal.

Interestingly, the significant SNP, rs134486905 for TS_f was also identified for NS_f. A previously reported QTL region by Snelling et al. (2010) for body weight gain was near rs134486905. Thus, rs134486905 could be a likely gene candidate as it was identified for NS_f and TS_f and also has an association with body weight gain.

For SS_f, eight significant SNPs were identified (Table 3.7). Sherman et al. (2010) identified QTL regions associated with dry matter intake and residual feed intake near rs109939302 (Table 3.9). Dry matter intake and residual feed intake are measures of how much feed an animal consumes, which could be influenced by the amount of feed consumed in one eating session, namely SS_f. Previously identified QTL regions associated with carcass weight and body weight were found near rs29010859 and rs42867118 (McClure et al. 2010). The amount of feed consumed by an animal has an influence on the weight of that animal (Berry and Crowley, 2012). In the current study, DMI and SS_f had a genetic correlation of -0.40 ± 0.29. So,

because DMI is related to both SS_f and body weight, perhaps there is an association between SS_f and body weight through the mutual relationship with DMI.

The significant SNP rs42867118 was in proximity to HSD17B3 (Hydroxysteroid 17-Beta Dehydrogenase 3) which is a gene that is primarily expressed in the testis and catalyzes the conversion of androstenedione to testosterone. HSD17B3 is involved in male genitalia development and testosterone biosynthetic processes through testosterone 17-beta-deydrogenase activity. Castration of male cattle is a common practice with behavioral benefits such as reduced aggression and sexual activity due to reduced testosterone levels (Thomson et al. 2017). Puzio et al. (2019) studied the effect of gender (bull vs steer) on feeding behavior. Feed intake per meal, similar to SS_f in the current study, was lower in steers than bulls by 9.7% (Puzio et al., 2019). The influence gender (bull vs steer) had on feed intake per meal makes HSD17B3 a gene candidate for SS_f due to the gene's role in male hormones.

At a $-\log_{10}$ p-value of 4.5, SI_f, did not have any significant SNPs. However, five SNPs could be considered suggestive at a $-\log_{10}$ p-value that exceeded 4 including rs42032214, rs109190582, rs109876422, rs109593147, and rs132720650. PPP1R3E (Protein phosphatase 1 regulatory subunit 3E) was in proximity to rs109190582 and acts as a glycogen-targeting subunit for protein phosphatase 1. The functions of PPP1R3E include glycogen binding and the regulation of glycogen biosynthetic processes meaning it aids in modulating the frequency, rate or extent of the chemical reactions and pathways resulting in the formation of glycogen. When there is excess energy in the form of glucose, mammals can combine glucose units into a complex sugar called glycogen which is then stored primarily in the muscle cells as an energy reserve. Feedlot cattle are often fed to caloric excess, therefore glucose homeostasis is an important metabolic process and could be influenced by how frequently an animal is eating,

namely SI_f in the current study. Another gene involved in metabolism found in proximity to rs109190582 was LRP10 (LDL receptor related protein 10). LRP10's gene product is an integral component of the plasma membrane with a role in lipid transport, specifically low-density lipoprotein particle receptor activity. Low-density lipoproteins are responsible for binding to cholesterol and moving it through the bloodstream to the cells in the body that need it. Lipid metabolism is important for maintaining an energy balance and could possibly be related to a feed intake behavior such as SI_f.

Five QTL regions with an association to feed intake or growth traits were near rs42032214 (Table 3.9). McClure et al. (2010) discovered QTL regions associated with body weight and body weight at weaning. The QTL regions related to feed intake were average daily gain (Akanno et al. 2018), residual feed intake (Seabury et al. 2017), and feed conversion ratio (Santana et al. 2016), all traits that could potentially be impacted by the IR_f of an animal.

Five significant SNPs were identified for DMI (Table 3.7). The significant SNP rs42644402 was in proximity to the gene CNR1 (Cannabinoid receptor 1). CNR1's gene product is a cellular component of synapses that use glutamate, an excitatory neurotransmitter. CNR1 functions in a variety of synaptic signaling processes that influence glucose homeostasis. Glucose homeostasis is an important metabolic process that could be influenced by the feed intake of an animal. Alexander et al. (2007) reported a QTL region near rs42644402 related to average daily gain. Average daily gain and DMI are moderately correlated genetically and phenotypically (Polizel et al. 2018). Other previously reported QTL regions near rs42644402 are related to retail product yield (Casas et al. 2003) and general disease susceptibility (Holmberg et al. 2004), which also could be influenced by DMI.

Other genes identified within the QTL region of significant SNPs did not have an obvious association or relationship with DMI. However, several QTL related to feed intake measures were previously reported near significant SNPs for DMI. Body weight gain and average daily gain QTL regions identified by Snelling et al. 2010 and Seabury et al. 2017 were near rs42865604. Sherman et al. (2009) identified a QTL region for residual feed intake that was near rs43076526.

Water traits

For NS_w, four significant SNPs were identified including rs136215737, rs133766990, rs110460578, and rs136946884. Of the four SNPs identified, only two (rs136946884 and rs136215737) had genes within the QTL region (Table 3.11). The variant, rs136946884 was in the vicinity of the gene CD226 Molecule. This gene functions in immunity through a vast number of biological processes through positive regulation of mast cell activation, interferon-gamma production, immunoglobulin mediated immune response and natural killer cell cytokine production (UniProt Consortium, 2019). Water intake can be used as an indicator of animal health and a sufficient supply of water is critical to maintain animal health (Golher et al. 2021). Perhaps sick or immune compromised animals will be less likely to get up and move to a water bunk, impacting the number of visits to the water bunk daily.

Eight significant SNPs were identified for IR_w (Table 3.8). Five of the 17 genes identified are uncharacterized by NCBI for the bovine genome (Table A.8). One SNP, BovineHD4100008521, was near MAP4K4 (Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4). This gene encodes for a member of the serine/threonine protein kinase family and activates MAPK8 (mitogen-activated protein kinase 8) as a function in the MAPK cascade. This gene has also been shown to mediate the TNF-alpha signaling pathway, which is a signaling

pathway for the pro-inflammatory cytokine TNF-alpha. TNF-alpha is produced in response to inflammation, infection, and other environmental stresses. Water intake is influenced by a variety of factors, environmental stressors being one (Ahlberg et al., 2018), so perhaps there is a relationship between MAPK8 and IR_w.

Quantitative trait loci previously reported near rs132772434 are associated with average daily gain (Zhang et al. 2020), residual feed intake (Sherman et al. 2009), and dry matter intake (Tetens et al. 2014). Dry matter intake and water intake have a genetic correlation of 0.34 ± 0.27 (Ahlberg et al. 2019), which could explain the association of previously reported QTLs with IR_w.

Two significant SNPs were identified for TS_w: rs110942558 and rs42155131 (Table 3.8). The SNP rs110942558 was near the genes CD5 and CD6. These genes both encode for proteins that are members of the scavenger receptor cysteine-rich superfamily and are mainly associated with the immune system through various functions involving T cells. Sowell et al. (1999) reported that healthy steers spent more time and had more feeding bouts during the day than morbid steers. Presumably, health and the immune system would have an effect on water intake behavior as well.

Three SNP were significant for SS_w: rs109807965, rs133469167, and rs41634083. (Table 3.8). Genes within the QTL region for these SNPs are listed in Table A.9. The SNP, rs109807965 was in the vicinity of ADCY8 (Adenylate Cyclase 8) which catalyzes the formation of cyclic AMP from ATP. ADCY8 is involved in several biological processes according to the UniProt Consortium (2019) such as the glucose mediated signaling pathway, neuroinflammatory response, positive regulation of insulin secretion involved in cellular response to glucose stimulus, regulation of cellular response to stress, long-term memory, and locomotory behavior. Locomotory behavior is defined as the specific movement from place to place of an organism in

response to external or internal stimuli (The UniProt Consortium, 2019). Interestingly, long-term memory and locomotory behavior are two biological processes that would impact an animal's decision to visit a water bunk and possibly water intake behavior such as SS_w.

At a $-\log_{10}$ p-value = 4.5, SI_w, did not have any significant SNPs. The top five SNP had a $-\log_{10}$ p-value between 3.04 and 3.18 (Table 3.8). Of these SNP, only two SNP (rs41729388 and rs41660319) had identified genes within the QTL region (Table 3.11). The gene identified near rs41660319 has not been characterized by NCBI for the bovine genome. The variant. rs41729388, was near two genes: one functions in mitochondrial ribosomes and PEX2 (Peroxin-2). Peroxin-2 is involved in the biogenesis of peroxisomes, which contain enzymes involved in a variety of metabolic reactions. Peroxin-2 is associated with fatty-acid beta-oxidation and very long-chain fatty acid metabolic processes.

Four SNPs were significant for DWI: rs109310532, rs42951507, rs41595591, and rs134583473 (Table 3.8). The SNP rs109310532 was near five genes for olfactory receptors (Table 3.11). Olfaction is an important sense used in food evaluation (Do et al., 2014), so perhaps olfaction is also used in water evaluation and could influence the amount of water consumed. A previously reported QTL region for social separation vocalization was near rs109310532 as well as rs42951507 (Gutierrez-Gill et al. 2008). It is interesting that social separation vocalization had QTL regions near two SNPs identified for DWI on different chromosomes. Perhaps group dynamics or behaviors related to social hierarchy have an influence on DWI.

Previously reported QTL regions near rs41595591were related to feed efficiency, residual gain, residual feed intake (Brunes et al. 2020) and feed conversion ratio (Sherman et al.

2009). Residual feed intake and other feed intake measures are phenotypically and genetically correlated with DWI (Ahlberg et al. 2019).

Conclusion

Feed and water usage has been studied in pervious literature, however there is a gap in knowledge about animal behaviors surrounding feed and water intake. Standardized feeding and drinking behavior phenotypes have not been established. The current study defined five traits: number of sessions (no/d), intake rate (g/s), session size (kg), time per session (s), and session interval (min). Behavior traits are heritable ranging from 0.35 to 0.63 for feeding behaviors and 0.54 to 0.88 for drinking behaviors. The heritability of DMI and DWI were 0.57 and 0.44, respectively. The behavior traits had varying phenotypic correlations. The genetic correlations between DMI and feeding behaviors were moderate to high with NS_f and IR_f being positive (0.47 and 0.49, respectively) and SS_f, SI_f, and TS_f being negative (-0.40, -0.59, and -0.64, respectively). The genetic correlations between DWI and drinking behaviors were low to moderate for NS_w, SS_w, and SI_w (0.48, 0.13, and -0.37). Genetic correlations between DWI and TS_w and IR_w were not different from zero.

Several SNPs were identified in this study for the feed and water intake behaviors. Genes and previously reported QTL near SNPs were evaluated. Some genes had functions that could be associated with feed and water intake behaviors, while others did not have a known association. The results from the current study paired with previous literature suggest that feeding and drinking behaviors are controlled by genetic factors. Additional research should be done to confirm these results and evaluate feeding and drinking behavior's role in improving feed and water efficiency.

Table 3.1-Trial dates, number of animals, season, and bunk management by group.

Group	n	Season	Bunk Management
1	117	Summer	Slick
2	116	Winter	Slick
3	118	Summer	Slick
4	105	Summer	Ad libitum
5	123	Winter	Ad libitum
6	120	Summer	Ad libitum
7	100	Winter	Ad libitum

Table 3.2- Covariates that improved model fit determined by forward selection and were included in the model in addition to the base model. Base model for the genetic model included pen nested within group and three breed composition (Bos indicus, continental, and dairy). Base model for the phenotypic model included season and bunk management.

Trait	Average Daily Gain	Ribeye Area	Back fat	Initial body weight	Marbling
Number of Sessions, Feed	X	X			
Intake Rate, Feed					
Session Interval, Feed	X	X	X		
Session Size, Feed	X			X	
Time per Session, Feed	X		X		
Daily Dry Matter Intake	X		X	X	
Number of Sessions, Water			X		
Intake Rate, Water	X		X		
Session Interval, Water	X	X	X	X	
Session Size, Water	X				
Time per Session, Water	X		X	X	
Daily Water Intake	X		X	X	

Table 3.3- Summary statistics for feed and water intake behavior traits

	Trait	n	Min	Max	Mean	Standard Deviation
Feed	Number of Sessions, no/d	824	11.53	82.53	39.21	12.11
	Intake Rate, g/s	824	0.85	4.25	1.67	0.44
	Time per Session, s	824	43.88	902.27	203.07	79.36
	Session Size, kg	824	0.10	0.89	0.31	0.10
	Session Interval, min	824	9.09	62.67	21.50	8.14
	Dry Matter Intake, kg	824	6.00	16.17	10.73	1.47
Water	Number of Sessions, no/d	786	2.72	18.66	6.399	2.06
	Intake Rate, g/s	786	9.55	256.11	78.00	34.26
	Time per Session, s	786	22.74	373.25	101.18	37.41
	Session Size, kg	786	2.88	15.29	6.50	1.85
	Session Interval, min	786	61.79	295.47	155.22	39.77
	Daily Water Intake, kg	786	15.37	102.39	37.50	10.50

Table 3.4- Variance Components for feed and water intake behavior traits from bivariate analysis. Variance components for daily dry matter intake (DMI) and daily water intake (DWI) calculated as the average from each bivariate.

	Trait	n	Residual Variance	Genetic Variance	Phenotypic Variance	Heritability± Standard Error
Feed	Number of Sessions, no/d	718	43.70	45.64	89.34	0.50 ± 0.12
	Intake Rate, g/s	791	0.0355	0.062	0.098	0.63 ± 0.12
	Session Interval, min	676	15.83	24.39	40.23	0.60 ± 0.12
	Session Size, kg	791	0.00356	.00243	0.00599	0.40 ± 0.13
	Time per Session, s	676	2745.4	1550.3	4295.7	0.35 ± 0.12
	DMI, kg	676	0.3022	0.401	0.703	0.57 ± 0.11*
Water	Number of Sessions, no/d	639	1.24	1.65	2.89	0.56 ± 0.11
	Intake Rate, g/s	639	112.68	843.84	956.52	0.88 ± 0.07
	Session Interval, min	639	258.38	1074.5	1332.88	0.80 ± 0.10
	Session Size, kg	753	0.5762	1.391	1.968	0.70 ± 0.11
	Time per Session, s	639	524.16	639.40	1163.56	0.54 ± 0.12
	DWI, kg	639	24.48	19.65	44.13	0.44 ± 0.11**

* Range of standard error 0.117 - 0.120.
** Range of standard error 0.1163 - 0.1166.

Table 3.5- Phenotypic (below diagonal) and genetic (above diagonal) correlations between feed intake behavior traits.

	Daily Dry Matter Intake, kg	Number of Sessions, no./d	Intake Rate, g/s	Time per Session, s	Session Size, kg	Session Interval, min
Daily Dry Matter Intake, kg		0.47 ± 0.15	0.49 ± 0.16	-0.64 ± 0.34	-0.40 ± 0.29	-0.59 ± 0.14
Number of Sessions, no./d	0.28**					
Intake Rate, g/s	-0.0029	0.015				
Time per Session, s	0.04	-0.71**	-0.49**			
Session Size, kg	0.10*	-0.83**	0.0077	0.82**		
Session Interval, min	-0.23**	-0.75**	-0.18**	0.74**	0.75**	

*Correlations are significantly different from zero at * $P < 0.05$ or ** $P < 0.0001$.

Table 3.6- Phenotypic (below diagonal) and genetic (above diagonal) correlations between water intake behavior traits.

	Daily Water Intake, kg	Number of Sessions, no./d	Intake Rate, g/s	Time per Session, s	Session Size, kg	Session Interval, min
Daily Water Intake, kg		0.48 ± 0.15	0.06 ± 0.15	0.03 ± 0.20	0.13 ± 0.17	-0.37 ± 0.14
Number of Sessions, no./d	0.52**					
Intake Rate, g/s	0.09*	-0.31**				
Time per Session, s	0.17**	-0.04	-0.65**			
Session Size, kg	0.36**	-0.54**	0.46**	0.21**		
Session Interval, min	-0.30**	-0.74**	0.20**	0.16**	0.54**	

*Correlations are significantly different from zero at * $P < 0.05$ or ** $P < 0.0001$.

Table 3.7- Genomic regions identified by genome-wide association study contributing significantly to feed intake behavior traits and dry matter intake.

Trait	SNP name	rsID	Chromosome	Position	-log$_{10}$ p-value
Intake Rate (g/s)	BovineHD0800015068	rs110762691	8	50057408	5.693
	ARS-USDA-AGIL-chr10-37393241-000186	No rsID	10	37276549	5.041
	Hapmap34036-BES10_Contig636_1251	rs41732798	14	27952572	4.880
	BovineHD1500024034	rs135671861	15	81138619	4.914
	BovineHD1700013389	rs4248061817	17	46716862	5.854
	BovineHD1700013403	rs42906672	17	46739443	5.503
	BovineHD1700013409	rs42906269	17	46765070	5.399
	BTA-93763-no-rs	rs41667334	17	46785750	5.449
	BTB-00876819	rs42035549	24	2953070	4.682
	BovineHD2800011375	rs42148856	28	40568738	4.962
Session Interval (min)	ARS-BFGL-NGS-67261	rs109876422	2	3245057	4.057
	BovineHD0200000837	rs109593147	2	3193020	4.151
	BovineHD0200000883	rs132720650	2	3338155	4.349
	ARS-BFGL-NGS-54655	rs109190582	10	21841817	4.043
	ARS-BFGL-NGS-24783	rs42032214	12	65289522	4.380
Session Size (kg)	BovineHD0100027341	rs109939302	1	95417319	4.808
	Hapmap53387-rs29010859	rs29010859	3	13768233	5.495
	BovineHD0800025093	rs42867118	8	82934733	4.564
	ARS-BFGL-NGS-19199	rs110052485	10	57415611	5.463
	BovineHD1000016992	rs42194612	10	57107399	4.653
	BovineHD1600022857	rs42425081	16	76612375	4.743
	BovineHD1600022863	rs133983237	16	76632577	4.855
	BovineHD1600022873	rs110384764	16	76653998	4.535
Number of Sessions (no/d)	ARS-BFGL-NGS-44623	rs109466582	10	20834705	4.529
	BovineHD1000016131	rs134486905	10	54343151	5.353
	ARS-BFGL-NGS-119388	rs110698940	19	30952848	4.969
Time per Session (s)	BovineHD0900025025	rs42026914	9	87634273	4.642
	BovineHD0900025029	rs42026908	9	87645193	4.676
	Hapmap58334	rs29012728	9	87701436	4.716
	BovineHD1000016131	rs134486905	10	r54343151	5.424
	BovineHD1000016992	rs42194612	10	57107399	5.006
	BovineHD1500023539	rs135936657	15	79666048	5.185
	BovineHD1500023548	rs135228097	15	79681685	4.966
	BovineHD2600000141	rs42953730	26	1241221	6.007
Dry Matter Intake (kg)	BovineHD0700008050	rs43076526	7	27252564	4.713
	BovineHD0800017078	rs110102846	8	56438327	4.572
	BovineHD0800017083	rs109372479	8	56452230	5.143
	BovineHD0900017249	rs42644402	9	61913789	5.106
	BovineHD2000020297	rs42865604	20	69425225	4.798

Table 3.8- Genomic regions identified by genome-wide association study contributing significantly to water intake behavior traits.

Trait	SNP name	rsID	Chromosome	Position	$-\log_{10}$ p-value
Intake Rate (g/s)	BovineHD0200015569	rs133017083	2	54721378	4.60828
	BovineHD0200025079	rs135885260	2	87929185	4.86721
	BovineHD0200025093	rs135384440	2	87981727	4.71106
	BovineHD0800028898	rs42225372	8	96201693	4.640665
	BovineHD4100008521	No rsID	11	6760989	4.51176
	BovineHD2000011769	rs136922488	20	40926479	5.180005
	BovineHD2500011433	rs132772434	25	40056270	4.56698
	ARS-BFGL-NGS-115197	rs110017100	25	39767015	4.85750
Session Interval (min)	BovineHD0800011192	rs133766990	8	37352437	3.04817
	BovineHD0800017612	rs43560365	8	58531522	3.14675
	BovineHD1400012108	rs41729388	14	40398857	3.18897
	BovineHD2400001830	rs110460578	24	6532273	3.17547
	BTA-87355-no-rs	rs41660319	26	27000142	3.05654
Session Size (kg)	BovineHD1400003099	rs109807965	14	9876129	4.89670
	Hapmap50662-BTA-33967	rs41634083	13	13132195	4.93443
	BovineHD2400003540	rs133469167	24	12240445	4.61813
Number of Sessions (no/d)	BovineHD0700014659	rs136215737	7	48783256	4.50026
	BovineHD0800011192	rs133766990	8	37352437	4.59334
	BovineHD2400001830	rs110460578	24	6532273	4.51842
	BovineHD2400002130	rs136946884	24	7336349	4.89128
Time per Session (s)	ARS-BFGL-NGS-28913	rs42155131	28	42608083	4.56519
	ARS-BFGL-NGS-104120	rs110942558	29	37411101	4.70648
Daily Water Intake (kg)	BovineHD1000007082	rs109310532	10	22062093	4.933939
	BovineHD1000007093	rs134583473	10	22115454	4.827071
	Hapmap50507-BTA-94221	Rs41595591	24	54187542	4.982513
	BTB-01841682	rs42951507	26	12274011	5.914186

Table 3.9- Previously reported QTL regions which overlapped QTL in this study identified for feed or water intake attributes. If no QTL were determined to be associated with feed or water intake behaviors, then no QTL were listed in the table.

	Trait	rsID	Chromosome: Position	QTL trait	Source
Feed	Number of Sessions	rs109466582	10:20834705	Body weight	McClure et al. (2010)
				Carcass weight	Casas et al. (2003)
				Body weight (weaning)	McClure et al. (2010)
				Body height	Snelling et al. (2010)
		rs134486905	10:54343151	Body weight	Snelling et al. (2010)
				Body weight gain	Sherman et al. (2009)
		rs110698940	19:30952848	Residual feed intake	McClure et al. (2010)
				Body weight (weaning)	Sherman et al. (2009)
	Intake Rate	rs42480618	17:46716862	Residual feed intake	McClure et al. (2010)
				Body weight	Brunes et al. (2020)
				Feed efficiency	McClure et al. (2010)
				Carcass weight	McClure et al. (2010)
		ARS-USDA-AGIL-chr10-37393241-000186	10:37276549	Body weight	McClure et al. (2010)
				Body weight (weaning)	McClure et al. (2010)
		rs42148856	28:40568738	Body weight (weaning)	McClure et al. (2010)
		rs135671861	15:81138619	Carcass weight	Seabury et al. (2017)
				Residual feed intake	Maltecca et al. (2008)
		rs41732798	14:27952572	Body weight	Mizoshita et al. (2004)
				Average daily gain	Srikanth et al. (2020)
				Carcass weight	Fortes et al. (2012)
				Insulin-like growth factor 1 level	McClure et al. (2010)

		rs42035549	24:2953070	Carcass weight	Snelling et al. (2010)
				Body weight	Snelling et al. (2010)
				Body weight gain	Crispim et al. (2015)
				Maturity rate	Snelling et al. (2010)
	Time per Session	rs134486905	10:54343151	Body weight	Snelling et al. (2010)
				Body weight gain	McClure et al. (2010)
		rs135936657	15:79666048	Body weight	Michenet et al. (2016)
		rs29012728	9:87701436	Body weight (weaning)	Doran et al. (2014)
				Carcass weight	Sherman et al. (2009)
	Session Size	rs29010859	3:13768233	Residual feed intake	McClure et al. (2010)
				Body weight (weaning)	McClure et al. (2010)
				Body weight	McClure et al. (2010)
				Carcass weight	Sherman et al. (2009)
		rs109939302	1:95417319	Residual feed intake	Sherman et al. (2009)
				Dry matter intake	McClure et al. (2010)
		rs42867118	8:82934733	Body weight	McClure et al. (2010)
				Carcass weight	McClure et al. (2010)
	Session Interval	rs42032214	12:65289522	Body weight	McClure et al. (2010)
				Body weight (weaning)	Akanno et al. (2018)
				Average daily gain	Seabury et al. (2017)
				Residual feed intake	Santana et al. (2016)
				Feed conversion ratio	Casas et al. (2003)
		rs109190582	10:21841817	Carcass weight	McClure et al. (2010)
				Body weight	McClure et al. (2010)

				Body weight (weaning)	McClure et al. (2010)
	Dry Matter Intake	rs43076526	7:27252564	Residual feed intake	Sherman et al. (2009)
				Body height	McClure et al. (2010)
				Social separation-vocalization	Gutierrez-Gill et al. (2008)
		rs110102846	8:56438327	Body weight	McClure et al. (2010)
				Carcass weight	McClure et al. (2010)
				Body weight (weaning)	Michenet et al. (2016)
				Gastrointestinal nematode burden	Kim et al. (2013)
		rs109372479	8:56452230	Body weight	McClure et al. (2010)
				Body height	McClure et al. (2010)
				Carcass weight	McClure et al. (2010)
				Body weight (weaning)	Michenet et al. 2016
				Gastrointestinal nematode burden	Kim et al. (2013)
		rs42644402	9:61913789	Average daily gain	Alexander et al. (2007)
				General disease susceptibility	Holmberg et al. (2004)
				Retail product yield	Casas et al. (2003)
		rs42865604	20:69425225	Body weight gain	Snelling et al. (2010)
				Average daily gain	Seabury et al. (2017)
				Maturity rate	Crispim et al. (2015)
Water	Number of Sessions	rs136946884	24:7336349	Carcass weight	McClure et al. (2010)
				Body weight gain	Snelling et al. (2010)
		rs13621573	7:48783256	Body weight	Hanotte et al. (2003)
				Body weight (weaning)	McClure et al. (2010)

				Body weight gain	Snelling et al. (2010)
	Intake Rate	rs136922488	20:40926479	Body weight	McClure et al. (2010)
				Body weight (weaning)	Michenet et al. (2016)
		rs135885260	2:87929185	Body weight	McClure et al. (2010)
				Carcass weight	McClure et al. (2010)
		rs110017100	25:39767015	Body weight	Snelling et al. (2010)
				Residual feed intake	Sherman et al. (2009)
		rs132772434	25:40056270	Average daily gain	Zhang et al. (2020)
				Residual feed intake	Sherman et al. (2009)
				Body weight	Snelling et al. (2010)
				Dry matter intake	Tetens et al. (2014)
		BovineHD4100008521	11:6760989	Body weight	McClure et al. (2010)
				Residual feed intake	Sherman et al. (2009)
				Dry matter intake	Seabury et al. (2017)
				Body weight gain	Snelling et al. (2010)
	Time per Session	rs110942558	29:37411101	Body weight	McClure et al. (2010)
				Carcass weight	McClure et al. (2010)
				Body weight gain	Snelling et al. (2010)
				Body weight (weaning)	Casas et al. (2003)
				Flight from feeder	Gutierrez-Gill et al. (2008)
		rs42155131	28:42608083	Body weight (weaning)	McClure et al. (2010)
				Body weight	Peters et al. (2012)
	Session Size	rs41634083	13:13132195	Body weight (weaning)	McClure et al. (2010)
		rs109807965	14:9876129	Body weight	McClure et al. (2010)

				Body weight (weaning)	Lu et al. (2013)
		rs133469167	24:12240445	Body weight gain	Snelling et al. (2010)
				Body weight	Snelling et al. (2010)
				Carcass weight	McClure et al. (2010)
	Session Interval	rs41729388	14:40398857	Body weight	Maltecca et al. (2008)
				Body weight (weaning)	McClure et al. (2010)
				Average daily gain	Kneeland et al. (2004)
				Carcass weight	Mizoshita et al. (2004)
		rs41660319	26:27000142	Body weight	McClure et al. (2010)
				Body weight (weaning)	McClure et al. (2010)
	Daily Water Intake	rs109310532	10:22062093	Carcass weight	Casas et al. (2003)
				Body weight	McClure et al. (2010)
				Body weight (weaning)	McClure et al. (2010)
				Body height	McClure et al. (2010)
				Social separation-vocalization	Gutierrez-Gill et al. (2008)
		rs42951507	26:12274011	Body height	McClure et al. (2010)
				Body weight	Hanotte et al. (2003)
				Retail product yield	Casas et al. (2003)
				Social separation-vocalization	Gutierrez-Gill et al. (2008)
				Immunoglobulin G level	Leach et al. (2010)
		rs41595591	24:54187542	Feed conversion ratio	Sherman et al. (2009)
				Feed efficiency	Brunes et al. (2020)
				Residual gain	Brunes et al. (2020)

				Residual feed intake	Brunes et al. (2020)
				Body weight	Akanno et al. (2015)

Table 3.10- Significant Single Nucleotide Polymorphism (SNP) associated with feed traits with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP.

Trait	rs ID	Chromosome: Position	Gene Name	Description
Number of Sessions, no/d	rs109466582	10: 20834705	ADCY4	Adenylate Cyclase 4
			FITM1	Fat Storage Inducing Transmembrane Protein 1
Intake Rate, g/s	rs13567186	15:81138619	LOC101905743	olfactory receptor family 6 subfamily Q member 1
			LOC524304	olfactory receptor family 9 subfamily I member 17
			LOC615808	olfactory receptor family 9 subfamily I member 2
			OR9Q2	Olfactory Receptor Family 9 Subfamily Q Member 2
			LOC107133203	olfactory receptor family 1 subfamily S member 8
	rs41732798	14:27952572	NKAIN3	(Sodium/Potassium Transporting ATPase Interacting 3
			YTHDF3	YTH N6-Methyladenosine RNA Binding Protein 3
Time per Session, s	rs135936657	15: 79666048	LOC522775	olfactory receptor family 8 subfamily J member 2
			LOC100298605	olfactory receptor family 5 subfamily T member 23
			LOC104969845	olfactory receptor family 5 subfamily T member 2
			LOC510433	olfactory receptor family 8 subfamily H member 14
			LOC506891	olfactory receptor family 9 subfamily G member 4
			LOC509594	olfactory receptor family 9 subfamily G member 10C
			LOC785896	olfactory receptor family 8 subfamily K member 63
			LOC785914	olfactory receptor family 8 subfamily K member 60
			LOC790274	olfactory receptor family 8 subfamily K member 5
			LOC786201	olfactory receptor family 8 subfamily K member 1B
			LOC100336901	olfactory receptor family 8 subfamily J member 17
			LOC100336916	olfactory receptor family 8 subfamily J member 15
			LOC100300446	olfactory receptor family 8 subfamily J member 3

			LOC100300488	olfactory receptor family 8 subfamily U member 1
			LOC100299725	olfactory receptor family 8 subfamily U member 9
			LOC100300575	olfactory receptor family 5 subfamily AL member 2
			LOC100299764	olfactory receptor family 5 subfamily AL member 1
			LOC100299808	olfactory receptor family 8 subfamily U member 3
			OR5M3	olfactory receptor family 5 subfamily M member 3
			LOC788130	olfactory receptor 5M3-like
			LOC618091	olfactory receptor family 5 subfamily M member 13D
			LOC782555	olfactory receptor family 5 subfamily M member 10
			LOC781287	olfactory receptor family 5 subfamily AP member 2
			OR5AR1	olfactory receptor family 5 subfamily AR member 1
			LOC615810	olfactory receptor family 2 subfamily AH member 1
			OR9G9	olfactory receptor family 9 subfamily G member 9
			LOC509124	olfactory receptor family 9 subfamily G member 4D
Session Size, kg	rs42867118	8:82934733	HSD17B3	Hydroxysteroid 17-Beta Dehydrogenase 3
Session Interval, min	rs109190582	10:21841817	PPP1R3E	Protein phosphatase 1 regulatory subunit 3E
			LRP10	LDL receptor related protein 10
Dry Matter Intake, kg	rs42644402	9:61913789	CNR1	Cannabinoid receptor 1

Table 3.11- Significant Single Nucleotide Polymorphism (SNP) associated with water traits with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP.

Trait	rsID or SNP ID	Chromosome: Position	Gene Name	Description
Number of Sessions, no/d	rs136946884	24:7336349	CD226	CD226 Molecule
Intake Rate, g/s	BovineHD4100008521	11:6760989	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4	MAP4K4
Time per Session, s	rs110942558	29: 37411101	CD5	CD5 Molecule
			CD6	CD6 Molecule
Session Size, kg	rs109807965	14: 9876129	ADCY8	Adenylate Cyclase 8
Session Interval, min	rs41660319	26:27000142	LOC107131335	Uncharacterized
	rs41729388	14: 40398857	PEX2	Peroxin-2
			LOC101904449	39S ribosomal protein L33, mitochondrial
Daily Water Intake, kg	rs109310532	10:22062093	LOC615014	olfactory receptor family 6 subfamily E member 1D
			LOC619067	olfactory receptor family 6 subfamily E member 1B
			LOC104973083	olfactory receptor family 6 subfamily E member 1C
			LOC104973084	olfactory receptor family 6 subfamily E member 1
			LOC615040	olfactory receptor family 6 subfamily E member 1E

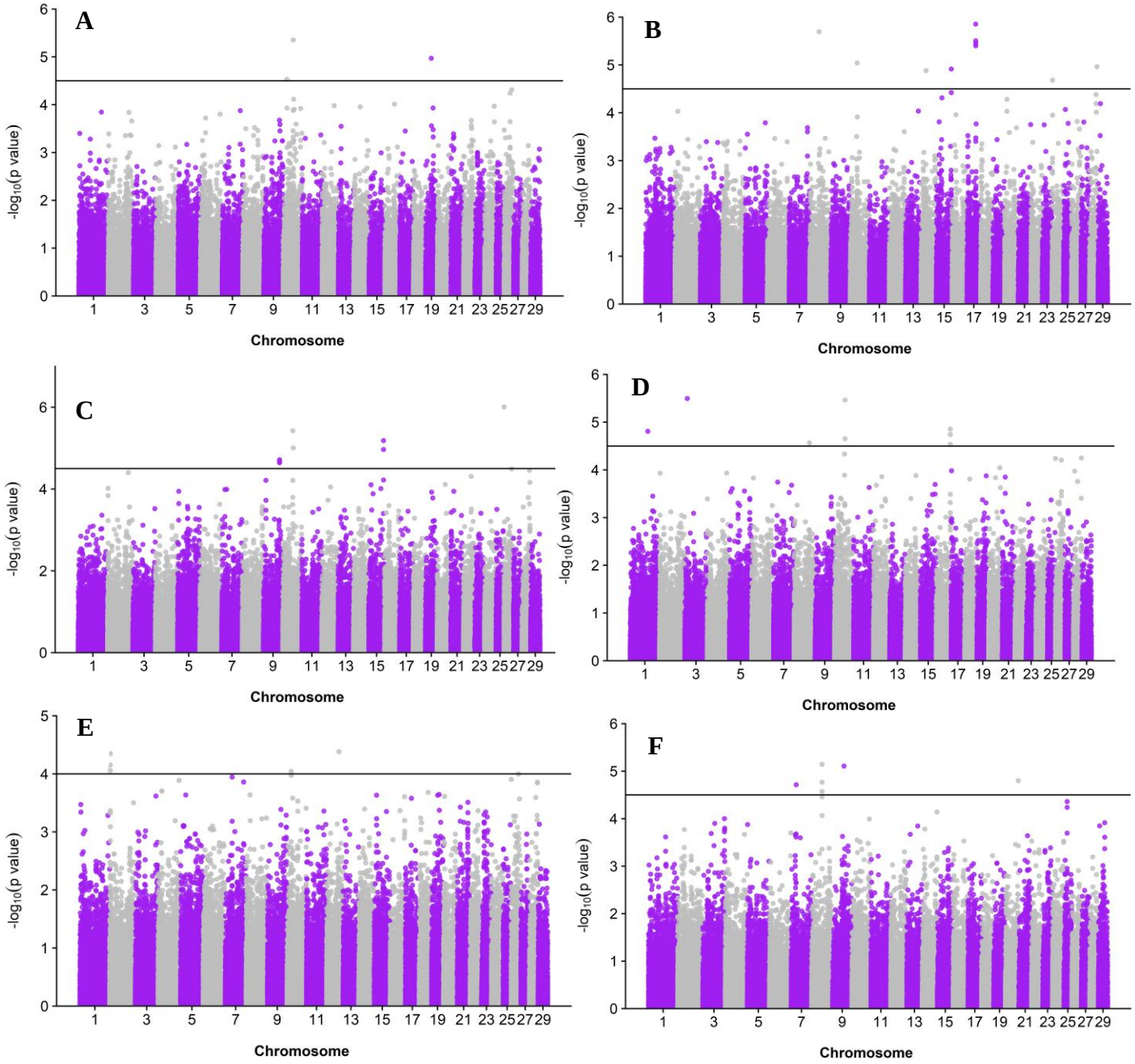

Figure 3.1- Manhattan plot showing the result of univariate genome-wide association mapping with a significance threshold of 4.5 for feed intake traits. A- Number of Sessions (no/d), Panel B- Intake rate (g/s), Panel C- Time per session (s), Panel D- Session Size (kg), Panel E- top 5 SNPs for Session Interval (min), Panel F- Daily feed intake (kg).

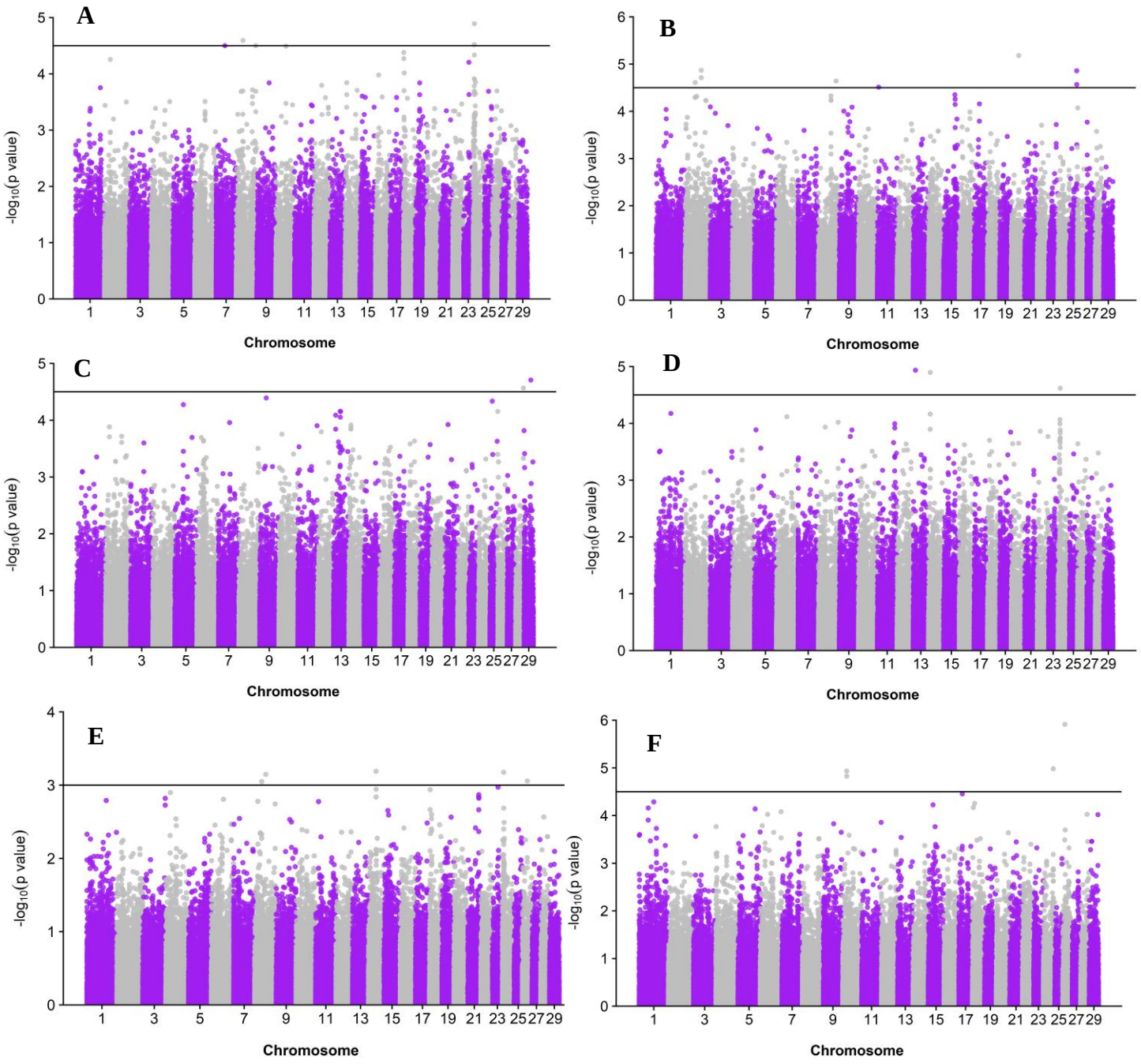

Figure 3.2- Manhattan plot showing the result of univariate genome-wide association mapping with a significance threshold of 4.5 for water intake traits. Panel A- Number of Sessions (no/d), Panel B- Intake rate (g/s), Panel C- Time per session (s), Panel D- Session Size (kg), Panel E- top 5 SNPs for Session Interval (min), Panel F- Daily water intake (kg).

References

Chapter 1

Alemu, A. W., D. Vyas, G. Manafiazar, J. A. Basarab, and K. A. Beauchemin. 2017. Enteric methane emissions from low- and high-residual feed intake heifers measured using GreenFeed and respiration chamber techniques. J. Anim. Sci. 95:3727-3737. doi: 10:2527/jas2017.1501.

Appuhamy, J. A. D. R. N., J. France, and E. Kebreab. 2016. Models for predicting enteric methane emissions from dairy cows in North America, Europe, and Australia and New Zealand. Glob. Change Biol. 22: 3039-3056. doi: 10.1111/gcb.13339.

Arthur, P.F., I.M. Barchia, C. Weber, T. Bird-Gardiner, K.A. Donoghue, R.M. Herd and R.S. Hegarty. 2017. Optimizing test procedures for estimating daily methane and carbon dioxide emissions in cattle using short-term breath measures. J. Anim. Sci. 95:645-656. doi: 10.2527/jas2016.0700.

Arthur, P.F., T. Bird-Gardiner, I.M. Barchia, K.A. Donoghue, and R.M. Herd. 2018. Relationships among carbon dioxide, feed intake, and feed efficiency traits in ad libitum fed beef cattle. J. Anim. Sci. 96:4859-4867. doi:10.1093/jas/sky308.

Bannink, A., M. W. van Schijndel, J. Dijkstra. 2011. A model of enteric fermentation in dairy cows to estimate methane emission for the Dutch National Inventory Report using the IPCC Tier 3 approach. Anim. Feed Sci. Technol. 166-167:603-618. doi:10.1016/j.anifeedsci.2011.04.043.

Beauchemin, K. A., H. H. Janzen, S. M. Little, T. A. McAllister, and S. M. McGinn. 2010. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. Agric. Syst. 103:371-379. doi: 10.1016/j.agsy.2010..03.008.

Berndt, A., T. M. Boland, M. H. Deighton, J. I. Gere, C. Grainger, R. S. Hegarty, A. D. Iwaasa, J. P. Koolaard, K. R. Lassey, D. Luo, R. J. Martin, C. Martin, P. J. Moate, G. Molano, C. Pinares-Patiño, B. E. Ribaux, N. M. Swainson, G. C. Waghorn, and S. R. O. Williams. 2014. Guidelines for use of sulphur hexafluoride (SF 6) tracer technique to measure enteric methane emissions from ruminants. Pages 166. M. G. Lambert, ed. New Zealand Agricultural Greenhouse Gas Research Centre, New Zealand.

Bickell, S.L., D.K. Revell, A.F. Toovey, and P.E. Vercoe. 2014. Feed intake of sheep when allowed ad libitum access to feed in methane respiration chambers. J. Anim. Sci. 92:2259-2264. doi: 10.2527/jas2013-7192.

Blaxter, K.L., and J.L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. Br. J. Nutr. 19(1):511-522. doi: 10.1079/BJN19650046.

Capper, J. L. 2011. The environmental impact of beef production in the United States: 1977 compared with 2007. J. Anim. Sci. 89:4249-4261. doi: 10.2527/jas.2010-3784.

CLEAR Center. 2020. The biogenic carbon cycle and cattle. https://clear.ucdavis.edu/explainers/biogenic-carbon-cycle-and-cattle. (Accessed 21 June 2021.)

Czerkawski, J.W., K.L. Blaxter, and F.W. Wainman. 1966. The metabolism of oleic, linolenic acids by sheep with reference to their effects on methane production. Br. J. Nutr. 20:349-362. doi:10.1079/BJN19660035.

de Haas, Y., J. J. Windig, M. P. Calus, J. Dijkstra, M. de Haan, A. Bannink, and R. F. Veerkamp. 2011. Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. J. Dairy Sci. 94:6122-6134. doi: 10.3168/jds.2011-4439.

Dijkstra, J., S. M. van Zijderveld, J. A. Apajalahti, A. Bannink, W. J. J. Gerrits, J. R. Newbold, H. B. Perdok, and H. Berends. 2011. Relationships between methane production and milk fatty acid profiles in dairy cattle. Anim. Feed Sci. Technol. 166:590-595.

Donoghue, K. A., T. Bird-Gardiner, P. F. Arthur, R. M. Herd, and R. F. Hegarty. 2016. Genetic and phenotypic variance and covariance components for methane emission and postweaning traits in Angus cattle. J. Anim. Sci. 94:1438-1445. doi: 10.2527/jas2015-0065.

Dorich, C.D., R.K. Varner, A.B.D. Pereira, R. Martineau, K.J. Soder, and A.F. Brito. 2014. Short communication: Use of a portable, automated, open-circuit gas quantification system and the sulfur hexafluoride tracer technique for measuring enteric methane emissions in Holstein cows fed ad libitum or restricted. J. Dairy. Sci. 98:2672-2681.

FAOSTAT. 2019. Enteric Fermentation Data. http://www.fao.org/faostat/en/#data/GE. (Accessed 20 August 2021).

Gardiner, T. D., M. D. Coleman, F. Innocenti, J. Tompkins, A. Connor, P. C. Garnsworthy, J. M. Moorby, C. K. Reynolds, A. Waterhouse, and D. Wills. 2015. Determination of the absolute accuracy of UK chamber facilities used in measuring methane emissions from livestock. Measurement. 66:272–279.

Gunter, S.A., J.A. Bradford, and C.A. Moffet. 2017. Effects of mass airflow rate through an open-circuit gas quantification system when measuring carbon emissions. J. Anim. Sci. 95:475-484.

Gunter, S.A., PAS, ACAN, and J.A. Bradford. 2017b. Technical Note: Effect of bait delivery interval in an automated head-chamber system on respiration gas estimates when cattle are grazing rangeland. Prof. Anim. Sci. 33:490-497.

Gunter, S.A., and M.R. Beck. 2018. Measuring the respiratory gas exchange by grazing cattle using an automated open-circuit gas quantification system. Transl. Anim. Sci. 2:11-18. doi: 10.1093/tas/txx009.

Hales, K.E., and N.A. Cole. 2017. Hourly methane production in finishing steers fed at different levels of dry matter intake. J. Anim. Sci. 95:2089-2096. doi:10.2527/jas2016.1023

Hammond, K., D. J. Humphries, L. A. Crompton, P. Kirton, C. Green, and C. K. Reynolds. 2013. Methane (GreenFeed) compared emissions from growing dairy heifers estimated using an automated head chamber to respiration chambers or SF6 techniques. Adv. Anim. Biosci. 4:447.

Hammond, K. J., D. J. Humphries, L. A. Crompton, C. Green and C. K. Reynolds. 2015. Methane emissions from cattle: Estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulphur hexafluoride tracer. Anim. Feed. Sci. Technol. 203:41-52.

Hammond, K. J., L. A. Crompton, A. Bannink, J. Dijkstra, D. R. Yanez-Ruiz, P. O'Kiely, E. Kebreab, M. A. Eugene, Z. Yu, K. J. Shingfield, A. Schwarm, A. N. Hristov, and C. K. Reynolds. 2016. Review of current in vivo measurement techniques for quantifying enteric methane emission from ruminants. Anim. Feed Sci. Technol. 219:13–30

Hayes, B.J., K.A. Donoghue, C.M. Reich, B.A. Mason, T. Bird-Gardiner, R.M. Herd, and P.F. Arthur. 2016. Genomic heritabilities and genomic estimated breeding values for methane traits in Angus cattle. J. Anim. Sci. 94:902-908. doi: 10.2527/jas2015-0078.

Hegarty, R. S. 2013. Applicability of short-term emission measurements for on-farm quantification of enteric methane. Animal. 7:401-408. doi:10.1017/S1751731113000839

Herd, R. M., P. F. Arthur, K. A. Donoghue, S. H. Bird, T. Bird-Gardiner, and R. S. Hegarty. 2014. Measures of methane production and their phenotypic relationships with dry matter intake, growth, and body composition traits in beef cattle. J. Anim. Sci. 92: 5267-5274. doi: 10.2527/jas2014-8273.

Herd, R. M., J. L. Velazco, P. F. Arthur, and R. F. Hegarty. 2016. Associations among methane emission traits measured in the feedlot and in respiration chambers in Angus cattle bred to vary in feed efficiency. J. Anim. Sci. 94:4882-4891. doi: 10.2527/jas2016-0613.

Herd, R.M., P.F. Arthur, R.S. Hegarty, T. Bird-Gardiner, K.A. Donoghue, and J.I. Velazco. 2020. Predicting metabolizable energy intake by free-ranging cattle using multiple short-/term breath samples and applied to a pasture case-study. Anim. Prod. Sci. 61:381-389. doi: 10.1071/AN20162.

Hill, J., C. McSweeney, A. G. Wright, G. Bishop-Hurley, and K. Kalantar-zadeh. 2016. Measuring methane production from ruminants. Trends Biotechnol. 34:26-35. doi: 10.1016/j.tibtech.2015.10.004.

Hristov, A.N., J. Oh, F. Giallongo, T. Frederick, H. Weeks, P. R. Zimmerman, M. T. Harper, R. A. Hristova, R. S. Zimmerman, and A. F. Branco. 2015. The use of an automated system (GreenFeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. J. Vis. Exp. 103:e52904. doi: 10.3791/52904.

Hristov, A.N., E. Kebreab, M. Niu, J. Oh, A. Bannink, A.R. Bayat, T.M. Boland, A.F. Brito, D.P. Casper, L.A. Crompton, J. Dijkstra, M. Eugène, P.C. Garnsworthy, N. Haque, A.L.F. Hellwing, P. Huhtanen, M. Kreuzer, B. Kuhla, P. Lund, J. Madsen, C. Martin, P.J. Moate, S. Muetzel, C. Muñoz, N. Peiren, J.M. Powell, C.K. Reynolds, A. Schwarm, K.J. Shingfield, T.M. Storlien, M.R. Weisbjerg, D.R. Yáñez-Ruiz, and Z. Yu. 2018.

Symposium review: uncertainties in enteric methane inventories measurement techniques, and prediction models. J. Dairy. Sci. 101:6655-6674. doi: 10.3168/jds.2017-13536.

Huhtanen, P., M. Ramin, and A. N. Hristov. 2019. Enteric methane emission can be reliably measured by the GreenFeed monitoring unit. Livest. Sci. 222:31-40. doi: 10.1016/j.livsci.2019.01.017.

Hunerberg, M., S.M. McGinn, K.A. Beauchemin, T. Entz, E.K. Okine, O.M. Harstad, and T.A. McAllister. 2015. Impact of ruminal pH on enteric methane emissions. J. Anim. Sci. 93:1760-1766.

Janssen, P.H. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim. Feed Sci. Technol. 160, 1–22

Johnson, D.E., and G.M. Ward. 1996. Estimates of animal methane emissions. Environ Monit Assess. 42(1-2):133-41. doi: 10.1007/BF00394046.

Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492. doi:1995.7382483x.

Jonker, A., G. Molano, C. Antwi, and G. C. Waghorn. 2016. Enteric methane and carbon dioxide emissions measured using respiration chambers, the sulfur hexafluoride tracer technique, and a GreenFeed head-chamber system from beef heifers fed alfalfa silage at three allowances and four feeding frequencies. J. Anim. Sci. 94:4326-4337. doi:10.2527/jas2016-0646.

Kandel, P. B., M. L. Vanrobays, A. Vanlierde, F. Dehareng, E. Froidmont, N. Gengler, and H. Soyeurt. 2017. Genetic parameters of mid-infrared methane predictions and their

relationships with milk production traits in Holstein cattle. J. Dairy Sci. 100:5578-5591. doi: 10.3168/jds.2016-11954.

Kebreab, E., L. Tedeschi, J. Dijkstra, J. Ellis, A. Bannink, and J. France. 2016. Modeling greenhouse gas emissions from enteric fermentation. In: Synthesis and Modeling of Greenhouse Gas Emissions and Carbon Storage in Agricultural and Forest Systems to Guide Mitigation and Adaption. 6:173-195. doi: 10.2134/advagricsystmodel6.2013.0006.

Lakamp, A. D. 2021. Genetic influences on predicted methane production and natural resource allocation of beef cattle in the Great Plains. [Masters Thesis, Kansas State University]. K-REx.

Lassen, J., and P. Løvendahl. 2015. Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. J. Dairy Sci. 99:1959-1967. doi: 10.3168/jds.2015-10012.

Manzanilla-Pech, C. I. V., Y. De Haas, B. J. Hayes, R. F. Veerkamp, M. Khansefid, K. A. Donoghue, P. F. Arthur, and J. E. Pryce. 2016. Genomewide association study of methane emissions in Angus beef cattle with validation in dairy cattle. J. Anim. Sci. 94:4151-4166. doi: 10.2527/jas2016-0431.

McGinn, S.M., K.A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, yeast, and fumaric acid. J. Anim. Sci. 82:3346-3356. doi: 10.2527/2004.82113346x.

McGinn, S. M., J. Coulombe, and K. A. Beauchemin. 2021. Technical note: validation of the GreenFeed system for measuring enteric gas emissions from cattle. J. Anim. Sci. 99:1-6. doi: 10.1093/jas/skab046.

McGovern, E., M. McGee, C.J. Byrne, D.A. Kenny, A.K. Kelly and S.M. Waters. 2020. Investigation into the effect of divergent feed efficiency phenotype on the bovine rumen microbiota across diet and breed. Nature. 10:15317. doi:10.1038/s41598-020-71458-0.

Mills, J. A. N., E. Kebreab, C. M. Yates, L. A. Crompton, S. B. Cammell, M. S. Dhanoa, R. E. Agnew, and J. France. 2003. Alternative approaches to predicting methane emissions from dairy cows. J. Anim. Sci. 81:3141–3150.

Moe, P. W., and H. F. Tyrrell. 1979. Methane production in dairy cows. J. Dairy Sci. 62:1583–1586. doi:10.3168/jds.S0022-0302(79)83465-7.

Muñoz, C., T. Yan, D. A. Wills, S. Murray, and A. W. Gordon. 2012. Comparison of the sulphur hexafluoride tracer and respiration chamber techniques for estimating methane emissions and correction for rectum methane output from dairy cows. J. Dairy Sci. 95:3139–3148.

Murray, R. M., A. M. Bryant, and R. A. Leng. 1975. Rates of production of methane in the rumen and large intestine of sheep. Br. J. Nutr. 36:1–14.

Myhre, G., D. Shindell, F.M. Bréon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura and H. Zhang. 2013. Anthropogenic and natural radiative forcing. Supplementary material. In: Climate change 2013: The physical science basis. Contribution of working group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley.

Pinares-Patiño, C.S., S. M. Hickey, E. A. Young, K. G. Dodds, S. MacLean, G. Molano, E. Sandoval, H. Kjestrup, R. Harland, C. Hunt, N. K. Pickering, and J. C. McEwan. 2013.

Heritability estimates of methane emissions from sheep. Animal. 7:s2 316-321. doi:10.1017/S1751731113000864.

Robinson, D. L., J. P. Goopy, R. S. Hegarty, and P. E. Vercoe. 2010. Repeatability, animal and sire variation in 1-hr methane emissions and relationships with rumen volatile fatty acid concentrations. Abstract 712 in Proc. 9th World Congress in Genetics Applied to Livestock. German Society of Animal Science, Giessan, Germany.

Stackhouse-Lawson K. R., C. A. Rotz, J. W. Oltjen, and F. M. Mitloehner. 2012. Carbon footprint and ammonia emissions of California beef production. J. Anim. Sci. 90:4641-4655. doi: 10.2527/jas2011-4653.

Teye, F. K., E. Alkkiomaki, A. Simojoki, M. Pastell, and J. Ahokas. 2009. Instrumentation, measurement and performance of three air quality measurement systems for dairy buildings. Appl. Eng. Agric. 25:247–256.

Uemoto, Y., S. Ogawa, M. Satoh, H. Abe, and F. Terada. 2020. Development of prediction equation for methane-related traits in beef cattle under high concentrate diets. Anim. Sci. J. 91:e13341. doi: 10.1111/asj.13341.

U.S. EPA. 2021. Inventory of U.S. greenhouse gas emissions and sinks. https://www.epa.gov/sites/production/files/2021-04/documents/us-ghg-inventory-2021-main-text.pdf. (Accessed 28 July 2021.)

van der Honing, Y., B.J. Wieman, A. Steg, and B. van Donselaar. 1981. The effect of fat supplementation of concentrates on digestion and utilization of energy by productive dairy cows. Neth. J. agric. Sci. 29:79-92. doi: 10.18174/njas.v29i2.17009.

van Engelen, S., H. Bovenhuis, J. Dijkstra, J. A. M. van Arendonk, and M. H. P. W. Visker. 2015. Short communication: Genetic study of methane production predicted from milk fat composition in dairy cows. J. Dairy Sci. 98:8223-8226. doi: 10.3168/jds.2014-8989.

Van Kessel, J.S., J.B. Russell. 1996. The effect of pH on ruminal methanogenesis. FEMS Microbiol. Ecol. 20:205-210.

Velazco, J. I., G. Bremner, L. Li, K. Luijben, R. S. Hegarty, and H. Perdok. 2013. Short-term emission measurements in beef feedlot cattle to demonstrate enteric methane mitigation from dietary nitrate. Adv. Anim. Biosci. 4:279.

Waghorn, G., E. J. Garnet, C. S. Pinares, and S. Zimmerman. 2013. Implementation of GreenFeed for estimating methane in a dairy herd grazing pasture. Adv. Anim. Biosci. 4:436.

Waghorn, G.C., A. Jonker, and K.A. Macdonald. 2016. Measuring methane from grazing dairy cows using GreenFeed. Anim. Prod. Sci. 56:252-257.

Williams, S.R.O., P.J. Moate, M.C. Hannah, B.E. Ribaux, W.J. Wales, and R.J. Eckard. 2011. Background metters with the SF6 tracer method for estimating enteric methane emissions from dairy cows: A critical review of the SF6 procedure. Anim. Feed Sci. Technol. 170:265-276.

Zimmerman, P.R. 1993. System for measuring metabolic gas emissions from animals. US Patent 5,265,618

Chapter 2

Aguerre, M. J., M. A. Wattiaux, J. M. Powell, G. A. Broderick and C. Arndt. 2011. Effect of forage-to-concentrate ratio in dairy cows diets on emission of methane, carbon dioxide,

and ammonia, lactation performance, and manure excretion. J. Dairy. Sci. 94:3081-3093. doi: 10.3168/jds.2010-4011.

Allen, M. R., K. P. Shine, J. S. Fuglestvedt, R. J. Millar, M. Cain, D. J. Frame, and A. H. Macey. 2018. A solution to the misrepresentations of CO2-equivalent emissions of short-lived climate pollutants under ambitious mitigation. NPJ Clim. Atmos. Sci. 1:16. doi:10.1038/s41612-018-0026-8.

Arbre, M., Y. Rochette, J. Guyader, C. Lascoux, L. M. Gomez, M. Eugene, D. P. Morgavi, G. Renand, M. Doreau, and C. Martin. 2016. Repeatability of enteric methane determination from cattle using either the SF6 tracer technique or the GreenFeed system. Anim. Prod. Sci. 56:238-243. doi: 10.1071/AN15512

Arthur, P. F., I. M. Barchia, C. Weber, T. Bird-Gardiner, K. A. Donoghue, R. M. Herd, and R. S. Hegarty. 2017. Optimizing test procedures for estimating daily methane and carbon dioxide emissions in cattle using short-term breath measures. J. Anim. Sci. 95:645-656. doi: 10.2527/jas2016.0700.

Arthur, P. F., T. Bird-Gardiner, I. M. Barchia, K. A. Donoghue, and R. M. Herd. 2018. Relationships among carbon dioxide, feed intake, and feed efficiency traits in ad libitum fed beef cattle. J. Anim. Sci. 96:4859-4867. doi: 10.1093/jas/sky308.

Aubry, A. and T. Yan. 2015. Meta-analysis of calorimeter data to establish relationships between methane and carbon dioxide emissions or oxygen consumption for dairy cattle. Anim. Nutr. 1:128-134. doi: 10.1016/j.aninu.2015.08.015

Beauchemin K. A., and S. M. McGinn. 2005. Methane emissions from feedlot cattle fed barley and corn diets. J. Anim. Sci. 83:653-661. doi: 10.2527/2005.833653x

Beef Improvement Federation (BIF). 2021. Guidelines for uniform beef improvement program. BIF Guidelines Wiki. https://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement_Programs.

Blaxter, K. 1989. Energy metabolism in animals and man. Cambridge University Press, Cambridge.

Broucek, J. 2014. Production of methane emissions form ruminant husbandry: a review. J. Environ. Prot. Sci. 5:1482-1493. doi: 10.4236/jep.2014.515141.

Brouwer, E. 1965. Report of Sub-Committee on Constants and Factors. In: Blaxter, K.L., Ed., Proceedings of the 3rd Symposium on Energy Metabolism, Academic Press, London, 441-443.

Caetano, M., M. J. Wilkes, W. S. Pitchford, S. J. Lee, and P. I. Hynd. 2019. Effect of ensiled crimped grape marc on energy intake, performance and gas emissions of beef cattle. Anim. Feed Sci. Tech. 247:166-172. doi: 10.1016/j.anifeedsci.2018.10.007.

Donoghue, K. A., T. Bird-Gardiner, P. F. Arthur, R. M. Herd, and R. F. Hegarty. 2016. Genetic and phenotypic variance and covariance components for methane emission and postweaning traits in Angus cattle. J. Anim. Sci. 94:1438-1445. doi: 10.2527/jas2015-0065.

Dorich, C. D., R. K. Varner, A. B. D. Pereira, R. Martineau, K. J. Soder, and A. F. Brito. 2015. Short communication: Use of portable, automated, open-circuit gas quantification system and the sulfur hexafluoride tracer technique for measuring enteric methane emissions in Holstein cows fed ad libitum or restricted. J. Dairy Sci. 98:2676-2681. doi: 10.3168/jds.2014-8348.

George, M., G. Nader, and J. Dunbar. 2001. *Rangeland Management Series: Balancing Beef Cow Nutrient Requirements and Seasonal Forage Quality*. doi: 10.3733/ucanr.8021

Gregorini, P. 2012. Diurnal grazing pattern: its physiological basis and strategic management. Anim. Prod. Sci. 52:416-430. doi: 10.1071/AN11250.

Guarnido-Lopez, P., I. Ortigues-Marty, L. Salis, C. Chantelauze, A. Bes, P. Noziere, and G. Cantalapiedra-Hijar. 2022. Protein metabolism, body composition and oxygen consumption in young bulls divergent in residual feed intake offered two contrasting forage-based diets. Animal. 16:100558. doi: 10.1016/j.animal.2022.100558.

Gunter, S. A. and M. R. Beck. 2018. Measuring the respiratory gas exchange by grazing cattle using an automated open-circuit gas quantification system. Transl. Anim. Sci. 2:11-18. doi: 10.1093/tas/txx009.

Gunter, S. A. and J. A. Bradford. 2017. Technical note: Effect of bait delivery interval in an automated head-chamber system on respiration gas estimates when cattle are grazing rangeland. Prof. Anim. Sci. 33:490-497. doi: 10.15232/pas.2016-01593.

Hammond, K. J., D. J. Humphries, L. A. Crompton, C. Green, and C. K. Reynolds. 2015. Methane emissions from cattle: estimates from short-term measurements using a GreenFeed system compared with measurements obtained using respiration chambers or sulfur hexafluoride tracer. Anim. Feed Sci. Technol. 203:41-52. doi: 10.1016/j.anifeedsci.2015.02.008.

Huhtanen, P., E. H. Cabezas-Garcia, S. Utsumi and S. Zimmerman. 2015. Comparison of methods to determine methane emissions from dairy cows in farm conditions. J. Dairy Sci. 98:3394-3409. doi: 10.3168/jds.2014-9118.

Johnson, K.A., and D.E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492. doi:1995.7382483x.

Jonker, A., S. Muetzel, G. Molano and D. Pacheco. 2016. Effect of fresh pasture forage quality, feeding level, and supplementation on methane emissions from growing beef cattle. Anim. Prod. Sci. 56:1714-1721. doi: 10.1071/AN15022.

Kumar, S., S. V. Singh, P. Pandey, N. Kumar, and O. K. Hooda. 2016. Estimation of metabolic heat production and methane emission in Sahiwal and Karan Fries heifers under different feeding regimes. Vet. World. 9:496-500. doi: 10.14202/vetworld.2016.496-500.

Liu, Z., W. Powers, B. Oldick, J. Davidson and D. Meyer. 2012. Gas emissions from dairy cows fed typical diets of Midwest, south, and west regions of the United States. J. Environ. Qual. 41:1228-1237. doi: 10.2134/jeq2011.0435.

Manafiazar, G., S. Zimmerman, and J. A. Basarab. 2016. Repeatability and variability of short-term spot measurement of methane and carbon dioxide emissions from beef cattle using GreenFeed emissions monitoring system. Can. J. Anim. Sci. doi: 10.1139/cjas-2015-0190

Manzanilla-Pech, C. I. V., P. Lovendahl, D. Mansan Gordo, G. F. Difford, J. E. Pryce, F. Schenkel, S. Wegmann, F. Miglior, T. C. Chud, P. J. Moate, S. R. O. Willams, C. M. Richardson, P. Stothard, and J. Lassen. 2021. Breeding for reduced methane emission and feed-efficienct Holstein cows: An international response. J. Dairy. Sci. 104:8983-9001. doi: 0.3168/jds.2020-19889.

Manzanilla-Pech, C. I. V., Y. De Haas, B. J. Hayes, R. F. Veerkamp, M. Khansefid, K. A. Donoghue, P. F. Arthur, and J. E. Pryce. 2016. Genomewide association study of methane emissions in Angus beef cattle with validation in dairy cattle. J. Anim. Sci. 94:4151-4166. doi: 10.2527/jas2016-0431

McGeogh, E. J., P. O. Kiely, P. A. Foley, K. J. Hart, T. M. Boland, and D. A. Kenny. 2010. Methane emissions, feed intake, and performance of finishing beef cattle offered maize silages harvested at 4 different stages of maturity. J. Anim. Sci. 88:1479-1491. doi: 10.2527/jas.2009-2380.

McLean, J. A., and G. Tobin. 1987. Animal and Human Calorimetry. Cambridge Univ. Press, New York, NY.

Mitloehner, F. 2021. For methane, GWP100 not measuring up. https://clear.ucdavis.edu/blog/methane-gwp100-not-measuring. (Accessed 16 June 2021).

Muñoz, C., T. Yan, D. A. Wills, S. Murray, and A. W. Gordon. 2012. Comparison of the sulphur hexafluoride tracer and respiration chamber techniques for estimating methane emissions and correction for rectum methane output from dairy cows. J. Dairy Sci. 95:3139–3148.

Murray, R. M., A. M. Bryant, and R. A. Leng. 1975. Rates of production of methane in the rumen and large intestine of sheep. Br. J. Nutr. 36:1–14.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th Revised Edition (National Academy of Sciences Press, Washington, DC).

Nkrumah, J.D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior, with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153. doi: 10.2527/2006.841145x.

Pickett, A. T., E. Kegley, P.A. Beck, and S. A. Gunter. 2020. The effects of energy and lasalocid supplementation on forage intake and energetics of cattle grazing wheat pasture. J. Anim. Sci. 98(Supplement 2):11-12. doi: 10.1093/jas/skz397.026.

Pinares-Patino, C. S., P. D. D'Hour, J. P. Jouany, and C. Martin. 2007. Effects of stocking rate on methane and carbon dioxide emissions grom grazing cattle. Agric. Ecosyst. Environ. 121:30-46. doi: 10.1016/j.agee.2006.03.024.

Pinares-Patino, C. S., R. Baumont, and C. Martin. 2003. Methane emissions by Charolais cows grazing a monospecific pasture of timothy at four stages of maturity. Can. J. Anim. Sci. 83:769-777. doi: 10.4141/A03-034.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Renand, G. and D. Maupetit. 2015. Assessing individual differences in enteric methane emission among beef heifers using the GreenFeed Emission Monitoring system: effect of the length of testing period on precision. Anim. Prod. Sci. 56:218-223. doi: 10.1071/AN15429.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: whole body energy and nitrogen balance and visceral heat production. J. Nutr. 121:994-1003. doi: 10.1093/jn/121.7.994

Rischewski, J., A. Bielak, G. Nurnberg, M. Derno, and B. Kuhla. 2017. Rapid communication: ranking dairy cows for methane emissions measured using respiration chamber or GreenFeed techniques during early, peak, and late lactation. J. Anim. Sci. 95:3154-3159. doi: 10.2527/jas2017.1530.

Shreck, A. L., J. M. Zeltwanger, E. A. Bailey, J. S. Jennings, B. E. Meyer, and N. A. Cole. 2021. Effects of protein supplementation to steers consuming low-quality forages on greenhouse gas emissions. J. Anim. Sci. 99(7). doi: 10.1093/jas/skab147.

U.S. EPA. 2021. Inventory of U.S. greenhouse gas emissions and sinks. https://www.epa.gov/sites/production/files/2021-04/documents/us-ghg-inventory-2021-main-text.pdf. (Accessed 28 July 2021.)

Waghorn, G. C., A. Jonker, and K. A. Macdonald. 2016. Measuring methane from grazing dairy cows using GreenFeed. Anim. Prod. Sci. 56:252-257. doi: 10.2527/2005.833653x

West, J. W. 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86:2132-2144. doi: 10.3168/jds.S0022-0302(03)73803-X.

Chapter 3

Ahlberg, C. M., K. Allwardt, A. Broocks, K. Bruno, L. McPhillips, A. Taylor, C. R. Krehbiel, M. S. Calvo-Lorenzo, C. J. Richards, S. E. Place, U. DeSilva, D. L. VanOverbeke, R. G. Mateescu, L. A. Kuehn, R. L. Weaber, J. M. Bormann, M. M. Rolf. 2018. Environmental effects on water intake and water intake prediction in growing beef cattle. J. Anim. Sci. 96:4368-4384. doi: 10.1093/jas/sky267

Ahlberg, C. M., K. Allwardt, A. Broocks. K. Bruno, A. Taylor, L. Mcphillips, C. R. Krehbiel, M. Calvo-Lorezno, C. J. Richards, S. E. Place, U. Desilva, D. L. Vanoverbeke, R. G. Mateescu, L. A. Kuehn, R. Weaber, J. Bormann, and M. M. Rolf. 2019. Characterization of water intake and water efficiency in beef cattle. J. of Anim. Sci. 97:4770-4782.

Akanno, E. C., L. Chen, M. K. Abo-Ismail, J. J. Crowley, Z. Wang, C. Li, J. A. Basarab, M. D. MacNeil, and G. S. Plastow. 2018. Genome-wide association scan for heterotic quantitative trait loci in multi-breed and crossbred beef cattle. Genetics. 50:48. doi: 10.1186/s12711-018-0405-y.

Alexander, L. J., T. W. Geary, W. M. Snelling, and M. D. Macneil. 2007. Quantitative trait loci with additive effects on growth and carcass traits in a Wagyu-Limousin F2 population. Anim. Gent. 38:413-416. doi: 10.1111/j.1365-2052.2007.01616.x.

Allwardt, K., C. Ahlberg, A. Broocks, K. Bruno, A. Taylor, S. Place, C. Richards, C. Krehbiel, M. Calvo-Lorenzo, U. DeSilva, D. VanOverbeke, R. Mateescu, C. Goad, and M. M. Rolf. 2017. Technical note: validation of an automated system for monitoring and restricting water intake in group-housed beef steers. J. Anim. Sci. 95:4213-4219. doi:10.2527/jas2017.1593.

Andersson, M. 1985. Effects of drinking water temperatures on water intake and milk yield of tied-up dairy cows. Livest. Prod. Sci. 12:329-338. doi: 10.1016/0301-6226(85)90132-0.

Arias, R. A. and T. L. Mader. 2011. Environmental factors affecting daily water intake on cattle finished in feedlots. J. Anim. Sci. 89:245-251. doi:10.2527/jas.2010-3014

Beede, D. K. and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62:543-554. doi: 10.2527/jas1986.622543x.

Beef Improvement Federation (BIF). 2021. Guidelines for uniform beef improvement program. BIF Guidelines Wiki. https://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement_Programs.

Berry, D.P., and J. J. Crowley. 2012. Residual intake and body weight gain: a new measure of efficiency in growing cattle. J. Anim. Sci. 90:109-115. doi: 10.2527/jas.2011-4245.

Berry, D. P. and J. J. Crowley. 2013. Genetics of feed efficiency in dairy and beef cattle. J. Anim. Sci. 91:1594-1613. doi: 10.2527/jas.2012-5862.

Bhuiyan, M. S. A., D. Lim, M. Park, S. Lee, Y. Kim, C. Gondro, B. Park, and S. Lee. 2018. Functional partitioning of genomic variance and genome-wide association study for carcass traits in Korean Hanwoo cattle using imputed sequence level SNP data. Front. Genet. 9:217. doi: 10.3389/fgene.2018.00217.

Bovine HapMap Consortium. 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science. 324:528-532. doi: 10.1126/science.1167936

Brew, M. N., R. O. Myer, M. J. Hersom, J. N. Carter, M. A. Elzo, G. R. Hansen, and D. G. Riley. 2011. Water intake and factors affecting water intake of growing beef cattle. Livest. Sci. 140:297-300.

Brown-Brandl, T. M., R. A. Eigenberg, J. A. Nienaber, G. L. Hahn. 2005. Dynamic response indicators of heat stress in shaded and non-shaded feedlot cattle, part 1. Biosyst. Eng. 90:451-462. doi: 10.1016/j.biosystemseng.2004.12.006.

Brunes, L. C., F. Baldi, F. B. Lopes, R. B. Lobo, R. Espigolan, M. F. O. Costa, N. B. Stafuzza, and C. U. Magnabosco. 2020. Weighted single-step genome-wide association study and pathway analyses for feed efficiency traits in Nellore cattle. J. Anim. Breed. Genet. 138:23-44. doi: 10.1111/jbg.12496.

Cardot, V., Y. Le Roux, and S. Jurjanz. 2008. Drinking behavior of lactating dairy cows and prediction of their water intake. J. Dairy. Sci. 91:2257-2264. doi: 10.3168/jds.2007-0204.

Casas, E., S. D. Shackelford, J. W. Keele, M. Koohmaraie, T. P. L. Smith, and R. T. Stone. 2003. Detection of Quantitative Trait Loci for Growth and Carcass Composition in Cattle. J. Anim. Sci. 81:2976-2983. doi: 10.2527/2003.81122976x.

Coimbra, P. A. D., L. C. P. Machado Filho, P. A. Nunes, M. J. Hotzel, A. G. L. de Oliveira and U. Cecato. 2009. Effect of water trough type on the drinking behaviour of pasture-based beef heifers. Animal. 4(1):116-121. doi: 10.1017/S1751731109990930.

Crispim, A. C., M. J. Kelly, S. E. F. Guimaraes, F. F. e Silva, M. R. S. Fortes, R. R. Wenceslau, and S. Moore. 2015. Multi-Trait GWAS and New Candidate Genes Annotation for Growth Curve Parameters in Brahman Cattle. PLoS One. 10(10): :e0139906. doi: 10.1371/journal.pone.0139906.

Do, D., A. B. Strathe, T. Ostersen, S. D. Pant and H. N. Kadarmideen. 2014. Genome-wide association and pathway analysis of feed efficiency in pigs reveal candidate genes and pathways for residual feed intake. Frot. Genet. 5:307. doi:10.3389/fgene.2014.00307.

Doran, A. G., D. P. Berry, and C. J. Creevey. 2014. Whole genome association study identifies regions of the bovine genome and biological pathways involved in carcass trait performance in Holstein-Friesian cattle. BMC Genom. 15:837. doi: 10.1186/1471-2164-15-837.

Federation of Animal Science Societies (FASS). 2010. Guide for the Care and Use of Agricultural Animal in Research and Teaching. http://aaalac.org/about/Ag_Guide_3rd_ed.pdf

Fortes, M. R. S., A. Reverter, R. J. Hawken, S. Bolormaa, and S. A. Lehnert. 2012. Candidate genes associated with testicular development, sperm quality, and hormone levels of inhibin, luteinizing hormone, and insulin-like growth factor 1 in Brahman bulls. Biol. Reprod. 87:1-8. doi: 10.1095/biolreprod.112.101089.

Golher, D. M., B. H. M. Patel, S. H. Bhoite, M. I. Syed, G. J. Panchbhai, and P. Thirumurugan. 2021. Factors influencing water intake in dairy cows: a review. Int. J. Biometerol. 65:617-625. doi: 10.1007/s00484-020-02038-0.

Gonyou, H. W. and W. R. Stricklin. 1981. Eating behavior of beef cattle groups fed from a single stall or trough. Appl. Anim. Ethol. 7:123-133. doi: 10.1016/0304-3762(81)90090-0.

Gutierrez-Gill, B., N. Ball, D. Burton, M. Haskell, J. L. Willams, and P. Wiener. 2008. Identification of quantitative trait loci affecting cattle temperament. J. Hered. 99:629-638. doi: 10.1093/jhered/esn060.

Hanotte, O., Y. Ronin, M. Agaba, P. Nilsson, A. Gelhaus, R. Horstmann, Y. Sugimoto, S. Kemp, J. Gibson, A. Korol, M. Soller, and A. Teale. 2003. Mapping of quantitative trait loci controlling trypanotolerance in a cross oftolerant West African N'Dama and susceptible East African Boran cattle. 100:7443-7448. doi: 10.1073/pnas.1232392100.

Hicks, R. B., F. N. Owens, D. R. Gill, J. W. Oltjen, and R. P. Lake. 1990. Dry matter intake by feedlot beef steers: influence of initial weight, time on feed, and season of year received in yard. J. Anim. Sci. 68:254-265. doi: 10.1093/ansci/68.1.254.

Hoffman, M. P. and H. L. Self. 1972. Behavioral traits of feedlot steers in Iowa. J. Anim. Sci. 37:1438-1445. doi: 10.2527/jas1973.3761438x.

Holmberg, M. and L. Andersson-Eklund. 2004. Quantitative trait loci affecting health traits in Swedish dairy cattle. J. Dairy Sci. 87:2653-2659. doi: 10.3168/jds.s0022-0302(04)73391-3.

Hu, Z., C. A. Park and J. M. Reecy. 2019. Building a livestock genetic and genomic information knowledgebase through interactive developments of Animal QTLdb and CorrDB. Nucleic Acids Res. 47:D701-D710. doi: 10.1093/nar/gky1084.

Jago, J. G., J. R. Roche, E. S. Kolver, and M. W. Woolford. 2005. The drinking behaviour of dairy cows in late lactation. Proc. N. Z. Soc. Anim. Prod. 65:209-214.

Kadereit, B., P. Kumar, W. Wang, D. Miranda, E. L. Snapp, N. Severina, I. Torregroza, T. Evans, and D. L. Silver. 2007. Evolutionarily conserved gene family important for fat storage. PNAS. 105:94-99. doi: 10.1073/pnas.0708579105.

Kim, E.S, T. S. Sonstegard, M. V. Silva, L. C. Gasbarre, and C. P. Van Tassell. 2013. Identification of quantitative trait loci affecting gastrointestinal parasite resistance in an experimental Angus population. Anim. Genet. 45:117-121. doi: 10.1111/age.12101

Kneeland, J., C. Li, J. Basarab, W. M. Snelling, B. Benkel, B. Murdoch, C. Hansen, and S. S. Moore. 2004. Identification and fine mapping of quantitative trait loci for growth traits on bovine chromosome 2, 6, 14, 19, 21, 23 within one commercial line of Bos taurus. J. Anim. Sci. 82:3405-3414. doi: 10.2527/2004.82123405x.

Lancaster, P. A., G. E. Carstens, F. R. B. Ribeiro, L. O. Tedeschi, and D. H. Crews. 2009. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. J. Anim. Sci. 87:1528-1539. doi: 10.2527/jas.2008-1352.

Lardner, H. A., L. Braul, K. Schwartzkopf-Genswein, K. Schwean-Lardner, D. Damiran and E. Darambazar. 2013. Consumption and drinking behavior of beef cattle offered a choice of several water types. Livest. Sci. 157:577-585. doi: 10.1016/j.livsci.2013.08.016.

Leach, R. J., S. C. Craigmile, S. A. Knott, J. L. Williams, and E. J. Glass. 2010. Quantitative trait loci for variation in immune response to a Foot-and-Mouth Disease virus peptide. BMC Genom. 11:107. doi: 10.1186/1471-2156-11-107.

Lee, K., D. T. Nguyen, M. Choi, S. Cha, J. Kim, H. Dadi, H. G. Seo, K. Seo, T. Chun, and C. Park. 2013. Analysis of cattle olfactory subgenome: the first detail study on the characteristics of the complete olfactory receptor repertoire of a ruminant. BMC Genom. 14(596). doi: 10.1186/1471-2164-14-596.

Lu, D., S. Miller, M. Sargolzaei, M. Kelly, G. Vander Voort, T. Caldwell, Z. Wang, G. Plastow, and S. Moore. 2013. Genome-wide association analyses for growth and feed efficiency traits in beef cattle. 91:3612-3633. doi: 10.2527/jas.2012-5716.

Mader, T. L. and M. S. Davis. 2004. Effect of management strategies on reducing heat stress of feedlot cattle: feed and water intake. J. Anim. Sci. 82:3077-3087. doi: 10.2527/2004.82103077x.

Maltecca, C., K. A. Weigel, H. Khatib, M. Cowan, and A. Bagnato. 2008. Whole-genome scan for quantitative trait loci associated with birth weight, gestation length and passive immune transfer in a Holstein x Jersey crossbred population. Anim. Genet. 40:27-34. doi: 10.1111/j.1365-2052.2008.01793.x.

Mao, F., L. Chen, M. Vinsky, E. Okine, Z. Wang, J. Basarab, D. H. Crews Jr. C. Li. 2013. Phenotypic and genetic relationships of feed efficiency with growth performance, ultrasound, carcass merit traits in Angus and Charolais steers. J. Anim. Sci. 91:2067-2076. doi: 10.2527/jas.2012-5470.

Marete, A., M. S. Lund, D. Boichard, and Y. Ramayo-Caldas. 2018. A system-based analysis of the genetic determination of udder conformation and health phenotypes across three French dairy cattle breeds. PloS One. 13(7): e0199931. doi: 10.1371/journal.pone.0199931.

Masuda, Y. 2019. Introduction to BLUPF90 suite programs. http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=tutorial_blupf90.pdf (Accessed 5 July 2022).

McClure, M.C., N. S. Morsci, R. D. Schnabel, J. W. Kim, P. Yao, M. M. Rolf, S. D. McKay, S. J. Gregg, R. H. Chapple, S. L. Northcutt, and J. F. Taylor. 2010. A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. Anim. Genet. 41:597-607. doi: 10.1111/j.1365-2052.2010.02063.x.

Michenet, A., M. Barbat, R. Saintilan, E. Venot, and F. Phocas. 2016. Detection of quantitative trait loci for maternal traits using high-density genotypes of Blonde d'Aquitaine beef cattle. BMC Genom. 17:88. doi: 10.1186/s12863-016-0397-y.

Misztal, I., S. Tsuruta, D. Lourenco, Y. Masuda, I. Aguilar, A. Legarra, and Z. Vitezica. 2014 Manual for BLUPF90 family of programs. http://nce.ads.uga.edu/wiki/lib/exe/fetch.php?media=blupf90_all8.pdf (Accessed 5 July 2022).

Mizoshita, K. T. Wantanabe, H. Hayashi, C. Kubota, H. Yamakuchi, J. Todoroki, and Y. Sugimoto. 2004. Quantitative trait loci analysis for growth and carcass traits in a half-

sibfamily of purebred Japanese Black (Waygu) cattle. J. Anim. Sci. 82:3415-3420. doi: 10.2527/2004.82123415x.

Montanholi, Y. R., K. C. Swanson, R. Palme, F. S. Schenkel, B. W. McBride, D. Lu, and S. P. Miller. 2010. Assessing feed efficiency in beef steers through feeding behavior, infrared thermography and glucocorticoids. Animal. 5:692-701. doi: 10.1017/S1751731109991522.

Morrison, S. R. 1983. Ruminant heat stress: Effect on production and means of alleviation. J. Anim. Sci. 67:1594-1600. doi: 10.2527/jas1983.5761594x.

Mujibi, F. D. N., S. S. Moore, D. J. Nkrumah, Z. Wang, J. A. Basarab. 2010. Season of testing and its effect on feed intake and efficiency in growing beef cattle. J. Anim. Sci. 88:3789-3799. doi: 10.2527/jas.2009-2407.

Nardone, A., B. Ronchi, N. Lacetera, M.S. Ranieri, and U. Bernabucci. 2010. Effects of climate changes on animal production and sustainability of livestock systems. Livest. Sci. 130:57-69. doi: 10.1016/j.livsci.2010.02.011.

Nephawe, K. A., L. V. Cundiff, M. E. Dikeman, J. D. Crouse, L. D. Van Vleck. 2004. Genetic relationships between sex-specific traits in beef cattle: Mature weight, weight adjusted for body condition score, height and body condition score of cows, and carcass traits of their steer relatives. J. Anim. Sci. 82:647–653.

Nkrumah, J. D., D. H. Crews Jr., J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. J. Anim. Sci. 85:2382-2390. doi:10.2527/jas.2006-657.

National Research Council. Nutrient requirements of beef cattle. 2016. National Academies Press. Eighth revised edition.

Oliveira, B. R. Jr., M. N. Ribas, F.S.Machado, J. A. M. Lima, L. F. L. Cavalcanti, M. L. Chizzotti, and S. G. Coelho. 2018. Validation of a system for monitoring individual feeding and drinking behaviour and intake in young cattle. Animal. 12:634-639. doi: 10.1017/S1751731117002002.

Olivieri, B. F., M. E. Z. Mercadante, J. N. dos Santos Concalves Cyrillo, R. H. Branco, S. F. M. Bonilha, and L. G. de Albuquerque. 2016. Genomic regions associated with feed efficiency indicator traits in an experimental Nellore cattle population. PLoS ONE. 11:10. doi: 10.1371/journal.pone.0164390.

Pereira, G. M., A.A. Egito, R.C. Gomes, M.N. Ribas, R. A. A. Torres Junior, J. A. Fernandes Junior, and G. R. O. Menezes. 2021. Water requirements of beef production can be reduced by genetic selection. Animal. 15:3:100142. doi: 10.1016/j.animal.2020.100142.

Peters, S. O, K. Kizilkaya, D. J. Garrick, R. L. Fernando, J. M. Reecy, R. L. Weaber, G. A. Silver, and M. G. Thomas. 2012. Bayesian genome-wide association analysis of growth and yearling ultrasound measures of carcass traits in Brangus heifers. J. Anim. Sci. 90:3398-3409. doi: 10.2527/jas.2012-4507.

Polizel, G. H. G., L. Grigoletto, M. Carvalho, P. Rossi Junior, J. B. S. Ferraz, M. H. de Almeida Santana. 2018. Genetic correlations and heritability estimates for dry matter intake, weight gain, and feed efficiency of Nellore cattle in feedlot. Livest. Sci. 214:209-210. doi: 10.1016/j.livsci.2018.06.013.

Purwanto, B.P., M. Harada, and S. Yamamoto. 1996. Effect of drinking-water temperature on heat balance and thermoregulatory responses in dairy heifers. Aust. J. Agric. Res. 47:505-512. doi: 10.1071/AR9960505.

Puzio, N., C. Purwin, Z. Nogalski, I. Bialobrzewski, L. Tomczyk, and J. P. Michalski. 2019. The effects of age and gender (bull vs steer) on the feeding behavior of young beef cattle fed grass silage. Asian-Australas J. Anim. Sci. 32:1211-1218. doi: 10.5713/ajas.18.0698.

R Core Team. 2013. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available from http://www.r-project.org/

Ray, D. E. and C. B. Roubicek. 1971. Behavior of feedlot cattle during two seasons. J. Anim. Sci. 33:72-76. doi: 10.2527/jas1971.33172x.

Robinson, D. L. and V. H. Oddy. 2004. Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. Livest. Prod. Sci. 90:255-270.v doi: 10.1016/j.livprodsci.2004.06.011.

Rosen, B. D., D. M. Bickhart, R. D. Schnabel, S. Koren, C. G. Elsik, E. Tseng, T. N. Rowan, W. Y. Low, A. Zimin, C. Couldrey, R. Hall, W. Li, A. Rhie, J. Ghurye, S. D. McKay, F. Thibaud-Nissen, J. Hoffman, B. M. Murdoch, W. M. Snelling, T. G. McDaneld, J. A. Hammond, J. C. Schwartz, W. Nandolo, D. E. Hagen, C. Dreischer, S. J. Schultheiss, S. G. Schroder, A. M. Phillippy, J. B. Cole, C. P. Van Tasell, G.. Liu, T. P. L. Smith, J. F. Medrano. 2020. De novo assembly of the cattle reference genome with single-molecule sequencing. GigaScience. 9:giaa021.

Saatchi, M., J. E. Beever, J. E. Decker, D. B. Faulker, H. C. Freetyl, S. L. Hansen, H. Yampara-Iquise, K. A. Johnson, S. D. Kachman, M. S. Kerley, J. Kim, D. D. Loy, E. Marques, H. L. Neibergs, E. J. Pollak, R. D. Schnabel, C. M. Seabury, D. W. Shike, W. M. Snelling,

M. L. Spangler, R. L. Weaber, D. J. Garrick, and J. F. Taylor. 2014. QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. BMC Genom. 15:1004. doi: 10.1186/1471-2164-15-1004.

Santana, M. H. A., M. C. Freua, D. N. Do, R. V. Ventura, H. N. Kadarmideen, and J. B. S. Ferraz. 2016. Systems genetics and genome-wide association approaches for analysis of feed intake, feed efficiency, and performance in beef cattle. Genet. Mol. Res. 15(4) doi: 10.4238/gmr15048930.

Schwartzkopf-Genswein, K. S., C. Huisma, and T. A. McAllsiter. 1999. Validation of radio frequency identification system for monitoring the feeding patterns of feedlot cattle. Livest. Prod. Sci. 60:27-31.

Schwartzkopf-Genswein, K. S., S. Atwood, and T. A. McAllister. 2002. Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. Appl. Anim. Behav. Sci. 76:179-188. doi:10.1016/S0168-1591(02)00009-6

Seabury C. M., D. L. Oldeschulte, M. Saatchi, J. E. Beever, J. E. Decker, Y. A. Halley, E. K. Bhattarai, M. Molaei, H. C. Freetly, S. L. Hansen, H. Yampara-Iquise, K. A. Johnson, M. S. Kerley, J. Kim, D. D. Loy, E. Marques, H. L. Neilbergs, R. D. Schnabel, D. W. Shike, M. L. Spangler, R. L. Weaber, D. J. Garrick, and J. F. Taylor. 2017. Genome-wide association study for feed efficiency and growth traits in U.S. beef cattle. BMC Genom. 18:386. doi: 10.1186/s12864-017-3754-y.

Shaffer, W. R. 2022. Modeling phenotypic plasticity as an indicator of adaptability in beef cattle. Masters Thesis. Kansas State Univ., Manhattan.

Sherman, E. L., J. D. Nkrumah, C. Li, R. Bartusaik, B. Murdoch, and S. S. Moore. 2009. Fine mapping quantitative trait loci for feed intake and feed efficiency in beef cattle. J. Anim. Sci. 87:37-45. doi: 10.2527/jas.2008-0876

Sherman, E. L., J. D. Nkrumah, and S. S. Moore. 2010. Whole genome SNP associations with feed intake and feed efficiency in beef cattle. J. Anim. Sci. 88(1):16-22. doi: 10.2527/jas.2008-1759.

Snelling, W. M., M. F. Allan, J. W. Keele, L. A. Kuehn, T. McDaneld, T. P. Smith, T. S. Sonstegard, R. M. Thallman, and G. L. Bennett. 2010. Genome-wide association study of growth in crossbred beef cattle. J. Anim. Sci. 88:837-848. doi: 10.2527/jas.2009-2257

Sowell, B. F. M. E. Branine, J. G. P. Bowman, M. E. Hubbert, H. E. Sherwood, and W. Quimby. 1999. Feeding and watering behavior of healthy and morbid steers in a commercial feedlot. J. Anim. Sci. 77:1105-1112. doi: 10.2527/1999.7751105x.

Srikanth, K., S. H. Lee, K. Y. Chung, J. E. Park, G. W. Jang, M. R. Park, N. Y. Kim, T. H. Kim, H. H. Chai, W. C. Park, and D. Lim. 2020. A Gene-Set Enrichment and Protein-Protein Interaction Network-Based GWAS with Regulatory SNPs Identifies Candidate Genes and Pathways Associated with Carcass Traits in Hanwoo Cattle. 11(3):E316. doi: 10.3390/genes11030316.

Takeda, M., Y. Uemoto, K. Inoue, A. Ogino, T. Nozaki, K. Kurogi, T. Yasumori, and M. Satoh. 2019. Genome-wide association study and genomic evaluation of feed efficiency traits in Japanese Black cattle using single-step genomic best linear unbiased prediction model. Anim. Sci. J. 91:e13316. doi: 10.1111/asj.13316.

Tetens, J., G. Thaller, and N. Krattenmacher. 2014. Genetic and genomic dissection of dry matter intake at different lactation stages in primiparous Holstein cows. J. Dairy Sci. 97:520-531. doi: 10.3168/jds.2013-7301.

The UniProt Consortium. 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 47: D506-515.

Thomson, D. U., M. E. Youngers, E. F. Schwandt, S. J. Bartle, M. Siemens, J. C. Simroth, and C. D. Reinhardt. 2017. Bulls are more efficient than steers with similar meat quality. Kansas Agricultural Experiment Station Research Reports. 3:1. doi:10.4148/2378-5977.1365.

Turner, S. D. 2018. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. J. Open Source Soft. 3:731. doi:10.21105/joss.00731.

USDA NASS, 2019. USDA National Agricultural Statistics Service, 2017 Census of Agriculture.

VanRaden, P.M. 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91:4414-4423. doi:10.3168/jds.2007-0980.

Yilmaz, A., M. E. Davis, and R. C. M. Simmen. 2004. Estimation of (co) variance compoenets for reproductive traits in Angus beef cattle divergently selected for blood serum IGF-1 concentration. J. Anim. Sci. 82:2285-2292. doi: 10.2527/2004.8282285x.

Yilmaz, A., M. E. Davis, and R. C. M. Simmen. 2005. Analysis of female reproductive traits in Angus beef cattle divergently selected for blood serum insulin-like growth factor 1 concentration. Theriogenology. 65:1180-1190. doi: 10.1016/j.theriogenology.2005.06.018.

Wang, H., I. Misztal, I. Aguilar, A.Legarra, and W. M. Muir. 2012. Genome-wide association mapping including phenotypes from relatives without genotypes. Genome Res. 94(4):73-83. doi:10.1017/S0016672312000274

Winchester, C. F., and M. J. Morris. 1956. Water intake rates of cattle. J. Anim. Sci. 15:722-740.

Wong, S.T., K. Trinh, B. Hacker, G. C. Chan, G. Lowe, A. Gaggar, Z. Xia, G. H. Gold, and D. -R. Storm. 2000. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. Neuron. 3:487-497. doi: 10.1016/s0896-6273(00)00060-x.

Zhang, F., Y. Wang, R. Mukiibi, L. Chen, M. Vinsky, G. Plastow, J. Basarab, P. Stothard, and C. Li. 2020. Genetic architecture of quantitative traits in beef cattle revealed by genome wide association studies of imputed whole genome sequence variants: I: feed efficiency and component traits. BMC Genom. 21:36. doi: 10.1186/s12864-019-6362-1.

Zhou, Y., E. E. Connor, G. R. Wiggans, Y. Lu, R. J. Tempelman, and S. G. Schroeder. 2018. Genome-wide copy number variant analysis reveals variants associated with 10 diverse production traits in Holstein cattle. BMC Genom. 19:1 doi: 10.1186/s12864-018-4699-5.

Appendix A - Additional Tables

Table A.1- Significant Single Nucleotide Polymorphism (SNP) associated with feed number of sessions with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were within the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome : Position	Gene Name	Description
ARS-BFGL-NGS-44623	rs109466582	10: 20834705	TBC1D21	TBC1 Domain Family Member 21
			LOC104973073	uncharacterized
			SDR39U1	Short Chain Dehydrogenase/Reductase Family 39U Member 1
			KHNYN	KH And NYN Domain Containing
			CBLN3	Cerebellin 3 Precursor
			NYNRIN	NYN Domain And Retroviral Integrase Containing
			LOC112448387	uncharacterized
			NFATC4	Nuclear Factor Of Activated T Cells 4
			LOC101905010	uncharacterized
			RIPK3	Receptor Interacting Serine/Threonine Kinase 3
			ADCY4	Adenylate Cyclase 4
			LTB4R	Leukotriene B4 Receptor
			LTB4R2	Leukotriene B4 Receptor 2
			CIDEB	Cell Death Inducing DFFA Like Effector B
			NOP9	NOP9 Nucleolar Protein
			DHRS1	Dehydrogenase/Reductase 1
			RABGGTA	Rab Geranylgeranyltransferase Subunit Alpha
			TGM1	Transglutaminase 1
			TINF2	TERF1 Interacting Nuclear Factor 2
			GMPR2	Guanosine Monophosphate Reductase 2
			NEDD8	NEDD8 Ubiquitin Like Modifier

			MDP1	Magnesium Dependent Phosphatase 1
			CHMP4A	Charged Multivesicular Body Protein 4A
			TSSK4	Testis Specific Serine Kinase 4
			LOC112448388	uncharacterized
			TM9SF1	Transmembrane 9 Superfamily Member 1
			IPO4	Importin 4
			REC8	REC8 Meiotic Recombination Protein
			IRF9	Interferon Regulatory Factor 9
			RNF31	Ring Finger Protein 31
			PSME2	Proteasome Activator Subunit 2
			EMC9	ER Membrane Protein Complex Subunit 9
			PSME1	Proteasome Activator Subunit 1
			FITM1	Fat Storage Inducing Transmembrane Protein 1
			DCAF11	DDB1 And CUL4 Associated Factor 11
			PCK2	Phosphoenolpyruvate Carboxykinase 2, Mitochondrial
			NRL	Neural Retina Leucine Zipper
			CPNE6	Copine 6
			CARMIL3	Capping Protein Regulator And Myosin 1 Linker 3
BovineHD1000016131	rs134486905	10: 54343151	TEX9	Testis Expressed 9
			RFX7	Regulatory Factor X7
			TRNASTOP-UCA	transfer RNA opal suppressor
			NEDD4	NEDD4 E3 Ubiquitin Protein Ligase
ARS-BFGL-NGS-119388	rs110698940	19: 30952848	MAP2K4	Mitogen-Activated Protein Kinase Kinase 4
			MYOCD	Myocardin
			LOC112442638	uncharacterized
			LOC104975039	small integral membrane protein 20-like
			ARHGAP44	Rho GTPase Activating Protein 44

Table A.2-Significant Single Nucleotide Polymorphism (SNP) associated with Feed Intake Rate with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome: Position	Gene Name	Description
BovineHD1700013389	rs42480618	17:46716862	RIMBP2	RIMS binding protein 2
			PIWIL1	Piwi Like RNA-Mediated Gene Silencing 1
			TRNAG-CCC	transfer RNA glycine (anticodon CCC)
			FZD10	Frizzled Class Receptor 10
ARS-USDA-AGIL-chr10-37393241-000186	No rsID	10:37276549	MGA	MAX Gene-Associated Protein
			MAPKBP1	Mitogen-Activated Protein Kinase Binding Protein 1
			JMJD7	Jumonji Domain Containing 7
			PLA2G4B	Phospholipase A2 Group IVB
			SPTBN5	Spectrin Beta, Non-Erythrocytic 5
			EHD4	EH Domain Containing 4
			PLA2G4E	Phospholipase A2 Group IVE
			LOC112448545	uncharacterized
			PLA2G4D	Phospholipase A2 Group IVD
BovineHD2800011375	rs42148856	28:40568738	GRID1	Glutamate Ionotropic Receptor Delta Type Subunit 1
			TRNAC-GCA	TRNA-Cys (Anticodon GCA) 7-1
BovineHD1500024034	rs135671861	15:81138619	YPEL4	Yippee Like 4
			CLP1	Cleavage Factor Polyribonucleotide Kinase Subunit 1
			LOC112441555	basic proline-rich protein-like

			ZDHHC5	Zinc Finger DHHC-Type Palmitoyltransferase 5
			MED19	Mediator Complex Subunit 19
			TMX2	Thioredoxin Related Transmembrane Protein 2
			SELENOH	Selenoprotein H
			LOC101905538	uncharacterized
			CTNND1	Catenin Delta 1
			LOC101905743	olfactory receptor family 6 subfamily Q member 1
			LOC107131403	uncharacterized
			LOC112441662	uncharacterized
			LOC524304	olfactory receptor family 9 subfamily I member 17
			LOC615808	olfactory receptor family 9 subfamily I member 2
			OR9Q2	Olfactory Receptor Family 9 Subfamily Q Member 2
			LOC107133203	olfactory receptor family 1 subfamily S member 8
Hapmap34036-BES10_Contig636_1251	rs41732798	14:27952572	NKAIN3	Sodium/Potassium Transporting ATPase Interacting 3
			GGH	Gamma-Glutamyl Hydrolase
			TTPA	Alpha Tocopherol Transfer Protein
			YTHDF3	YTH N6-Methyladenosine RNA Binding Protein 3
BTB-00876819	rs42035549	24:2953070	LOC112444173	uncharacterized
			LOC101904059	uncharacterized

Table A.3- Significant Single Nucleotide Polymorphism (SNP) associated with Feed Time per session with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome: Position	Gene Name	Description
BovineHD1000016131	rs134486905	10: 54343151	TEX9	Testis-Expressed Protein 9
			RFX7	Regulatory Factor X7
			TRNASTOP-UCA	transfer RNA opal suppressor (anticodon UCA)
			NEDD4	NEDD4 E3 Ubiquitin Protein Ligase
BovineHD1500023539	rs135936657	15: 79666048	LOC522775	olfactory receptor family 8 subfamily J member 2
			LOC100298605	olfactory receptor family 5 subfamily T member 23
			LOC104969845	olfactory receptor family 5 subfamily T member 2
			LOC510433	olfactory receptor family 8 subfamily H member 14
			LOC506891	olfactory receptor family 9 subfamily G member 4
			LOC509594	olfactory receptor family 9 subfamily G member 10C
			LOC785896	olfactory receptor family 8 subfamily K member 63
			LOC785914	olfactory receptor family 8 subfamily K member 60
			LOC790274	olfactory receptor family 8 subfamily K member 5
			LOC786201	olfactory receptor family 8 subfamily K member 1B
			LOC100336901	olfactory receptor family 8 subfamily J member 17

			LOC100336916	olfactory receptor family 8 subfamily J member 15
			LOC100300446	olfactory receptor family 8 subfamily J member 3
			LOC100300488	olfactory receptor family 8 subfamily U member 1
			LOC100299725	olfactory receptor family 8 subfamily U member 9
			LOC100300575	olfactory receptor family 5 subfamily AL member 2
			LOC100299764	olfactory receptor family 5 subfamily AL member 1
			LOC100299808	olfactory receptor family 8 subfamily U member 3
			OR5M3	olfactory receptor family 5 subfamily M member 3
			LOC788130	olfactory receptor 5M3-like
			LOC618091	olfactory receptor family 5 subfamily M member 13D
			LOC782555	olfactory receptor family 5 subfamily M member 10
			LOC781287	olfactory receptor family 5 subfamily AP member 2
			OR5AR1	olfactory receptor family 5 subfamily AR member 1
			LOC615810	olfactory receptor family 2 subfamily AH member 1
			OR9G9	olfactory receptor family 9 subfamily G member 9
			LOC509124	olfactory receptor family 9 subfamily G member 4D
Hapmap58334-rs29012728	rs29012728	9:87701436	IYD	olfactory receptor family 9 subfamily G member 4D

			LOC100848331	retinoic acid early transcript 1E-like
			PLEKHG1	pleckstrin homology and RhoGEF domain containing G1
			LOC112448078	uncharacterized

Table A.4- Significant Single Nucleotide Polymorphism (SNP) associated with Feed Session Size with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome: Position	Gene Name	Description
Hapmap53387-rs29010859	rs29010859	3:13768233	LOC101906408	SLAM family member 5-like
			TRNAS-GGA	transfer RNA serine (anticodon GGA)
			ETV3	ETS Variant Transcription Factor 3
			ETV3L	ETS Variant Transcription Factor 3 Like
			ARHGEF11	Rho Guanine Nucleotide Exchange Factor 11
			LRRC71	Leucine Rich Repeat Containing 71
			LOC104971464	uncharacterized
			PEAR1	Platelet Endothelial Aggregation Receptor 1
			NTRK1	Neurotrophic Receptor Tyrosine Kinase 1
			INSRR	Insulin Receptor Related Receptor
ARS-BFGL-NGS-19199	rs110052485	10:57415611	LOC101904374	uncharacterized
			ONECUT1	One Cut Homeobox 1
			TRNAC-GCA	transfer RNA cysteine (anticodon GCA)
BovineHD1600022863	rs133983237	16:76632577	CRB1	Crumbs Cell Polarity Complex Component 1
			MIR2284N	microRNA mir-2284n
			LOC112441872	uncharacterized
			DENND1B	DENN Domain Containing 1B
			C16H1orf53	chromosome 16 open reading frame, C1orf53
			LHX9	LIM Homeobox 9
BovineHD0100027341	rs109939302	1:95417319	FNDC3B	Fibronectin Type III Domain Containing 3B
			LOC112447727	uncharacterized
			LOC101904810	mpv17-like protein 2
			LOC112447728	uncharacterized

			TMEM212	Transmembrane Protein 212
BovineHD0800025093	rs42867118	8:82934733	ERCC6L2	ERCC Excision Repair 6 Like 2
			LOC112447893	uncharacterized
			LOC112447829	uncharacterized
			HSD17B3	Hydroxysteroid 17-Beta Dehydrogenase 3
			LOC112447897	U6 spliceosomal RNA
			SLC35D2	Solute Carrier Family 35 Member D2
			ZNF367	Zinc Finger Protein 367
			LOC100140121	uncharacterized
			HABP4	Hyaluronan Binding Protein 4

Table A.5- Significant Single Nucleotide Polymorphism (SNP) associated with Feed Session Interval with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rs ID	Chromosome: Position	Gene Name	Description
ARS-BFGL-NGS-24783	rs42032214	12:65289522	LOC112449137	uncharacterized
			LOC101907906	uncharacterized
ARS-BFGL-NGS-54655	rs109190582	10:21841817	PABPN1	poly(A) binding protein nuclear 1
			BCL2L2	Bcl-2-like protein 2
			PPP1R3E	Protein phosphatase 1 regulatory subunit 3E
			HOMEZ	Homeobox and leucine zipper encoding
			RNF212B	Ring finger protein 212B
			SLC7A8	Solute carrier family 7 member 8
			LOC112448584	U6 spliceosomal RNA
			LOC112448665	U6 spliceosomal RNA
			CEBPE	CCAAT/enhancer-binding protein
			LMLN2	ciliated left-right organizer metallopeptidase
			C10H14orf119	Chromosome 10 C14orf119 homolog
			ACIN1	Apoptotic chromatin condensation inducer 1
			LOC112448395	uncharacterized
			CDH24	Cadherin 24
			PSMB11	Proteasome subunit beta
			PSMB5	Proteasome subunit beta type-5
			C10H14orf93	Chromosome 10 C14orf93 homolog
			AJUBA	LIM domain-containing protein ajuba
			HAUS4	HAUS augmin like complex subunit 4
			TRNAR-ACG-5	uncharacterized
			PRMT5	Protein arginine N-methyltransferase 5
			RBM23	RNA binding motif protein 23

			REM2	RRAD and GEM like GTPase 2
			LRP10	LDL receptor related protein 10
			MMP14	39S ribosomal protein L52, mitochondrial
			MRPL52	Uncharacterized
			LOC112448396	Solute carrier family 7 member 7
			SLC7A7	Mitochondrial inner membrane protein OXA1L
			OXA1L	Mitochondrial inner membrane protein OXA1L
			LOC615014	olfactory receptor family 6 subfamily E member 1D

Table A.6- Significant Single Nucleotide Polymorphism (SNP) associated with Dry Matter Intake with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rs ID	Chromosome: Position	Gene Name	Description
BovineHD0700008050	rs43076526	7:27252564	MARCH3	membrane associated ring-CH-type finger 3
			LMNB1	Lamin B1
			LOC112447582	U6 spliceosomal RNA
			TEX43	Testis expressed 43
			ALDH7A1	Aldehyde dehydrogenase 7 family member A1
			GRAMD2B	GRAM domian containing 2B
			LOC112447385	uncharacterized
			MIR2458	microRNA mir2458
			LOC112447386	uncharacterized
			PHAX	phosphorylated adaptor for RNA export
BovineHD0800017083	rs109372479	8:56452230	LOC112447807	nuclear pore-associated protein 1-like
BovineHD0900017249	rs42644402	9:61913789	TRNAD-GUC	transfer RNA aspartic acid (anticodon GUC)
			CNR1	cannabinoid receptor 1
			SPACA1	sperm acrosome associated 1
			TRNAE-UUC-46	transfer RNA glutamic acid (anticodon UUC)
BovineHD2000020297	rs42865604	20:69425225	IRX1	Iroquois homeobox 1

Table A.7- Significant Single Nucleotide Polymorphism (SNP) associated with Water Number of Sessions with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rs ID	Chromosome: Position	Gene Name	Description
BovineHD2400002130	rs136946884	24:7336349	RTTN	Rotatin
			CD226	CD226 molecule
			DOK6	Docking protein 6
			LOC101907606	Uncharacterized
BovineHD0700014659	rs136215737	7: 48783256	SPOCK1	SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1

Table A.8- Significant Single Nucleotide Polymorphism (SNP) associated with Water Intake Rate with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome: Position	Gene Name	Description
BovineHD2000011769	rs136922488	20:40926479	NPR3	Natriuretic Peptide Receptor 3
			LOC104975283	uncharacterized
			SUB1	SUB1 Regulator Of Transcription
			TRNAG-CCC	transfer RNA glycine (anticodon CCC)
BovineHD0200025079	rs135885260	2:87929185	SATB2	SATB Homeobox 2
			LOC112442950	uncharacterized
			LOC112443725	U6 spliceosomal RNA
ARS-BFGL-NGS-115197	rs110017100	25:39767015	SDK1	Sidekick Cell Adhesion Molecule 1
			LOC112444323	uncharacterized
BovineHD2500011433	rs132772434	25:40056270	SDK1	Sidekick Cell Adhesion Molecule 1
			LOC112444323	uncharacterized
			MIR2390	microRNA mir-2390
BovineHD4100008521	No rsID	11:6760989	MAP4K4	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4
			LOC112448746	uncharacterized
			IL1R2	Interleukin 1 Receptor Type 2
			IL1R1	Interleukin 1 Receptor Type 1
			LOC112448931	small nucleolar RNA SNORD86

Table A.9- Significant Single Nucleotide Polymorphism (SNP) associated with Water Session Size with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome: Position	Gene Name	Description
Hapmap50662-BTA-33967	rs41634083	13: 13132195	CELF2	CUGBP Elav-Like Family Member 2
			MIR7861	microRNA mir-7861
BovineHD1400003099	rs109807965	14: 9876129	ADCY8	Adenylate Cyclase 8
BovineHD2400003540	rs133469167	24: 12240445	SERPINB8	Serpin Family B Member 8
			TRNAK-UUU	transfer RNA lysine (anticodon UUU)
			LOC112444149	uncharacterized
			LOC281376	serpin family B member 2-like

Table A.10-Significant Single Nucleotide Polymorphism (SNP) associated with Water Session Interval with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rs ID	Chromosome: Position	Gene Name	Description
BovineHD1400012108	rs41729388	14: 40398857	PEX2	Peroxin-2
			LOC101904449	39S ribosomal protein L33, mitochondrial
BTA-87355-no-rs	rs41660319	26:27000142	LOC107131335	uncharacterized

Table A.11- Significant Single Nucleotide Polymorphism (SNP) associated with Water Time per session with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rsID	Chromosome: Position	Gene Name	Description
ARS-BFGL-NGS-104120	rs110942558	29: 37411101	LOC112444852	uncharacterized
			CCDC86	Coiled-Coil Domain Containing 86
			PTGDR2	Prostaglandin D2 Receptor 2
			TRNAF-GAA	transfer RNA phenylalanine (anticodon GAA)
			PRPF19	Pre-MRNA Processing Factor 19
			LOC100847182	uncharacterized
			TMEM109	Transmembrane Protein 109
			TMEM132A	Transmembrane Protein 132A
			SLC15A3	Solute Carrier Family 15 Member 3
			CD6	CD6 Molecule
			LOC112444913	uncharacterized
			LOC112444914	uncharacterized
			CD5	CD5 molecule
			VPS37C	VPS37C Subunit Of ESCRT-I
			LOC613739	pregnancy-associated glycoprotein 2
			LOC100847667	uncharacterized
			PAG10	pregnancy-associated glycoprotein 10
			PAG2	pregnancy-associated glycoprotein 2
			PAG12	pregnancy-associated glycoprotein 12
ARS-BFGL-NGS-28913	rs42155131	28:42608083	PTPN20	protein tyrosine phosphatase non-receptor type 20
			FRMPD2	FERM and PDZ domain containing 2

			MAPK8	mitogen-activated protein kinase 8
			ARHGAP22	Rho GTPase Activating Protein 22

Table A.12- Significant Single Nucleotide Polymorphism (SNP) associated with Daily Water Intake with genomic location and the gene candidates in the linkage disequilibrium range (± 250 kb) associated with each SNP. If no genes were with the linkage disequilibrium range, then the SNP was not listed in the table.

SNP ID	rs ID	Chromosome: Position	Gene Name	Description
BovineHD1000007082	rs109310532	10:22062093	CDH24	Cadherin 24
			PSMB11	Proteasome subunit beta 11
			PSMB5	Proteasome subunit beta 5
			C10H14orf93	Chromosome 10 C114orf93 homolog
			AJUBA	Ajuba LIM protein
			HAUS4	HAUS augmin like complex subunit 4
			TRNAR-ACG-5	Transfer RNA arginine (anticodon ACG)
			PRMT5	Protein arginie methyltransferase 5
			RBM23	RNA binding motif protein 23
			REM2	RRAD and GEM GTPase 2
			LRP10	LDL receptor related protein 10
			MMP14	matrix matellopeptidase 14
			MRPL52	mitochondrial ribosomal protein L 52
			LOC112448396	uncharacterized
			SLC7A7	Solute carrier family 7 member 7
			OXA1L	OXA1L mitrochondrial innter membrane protein
			LOC615014	olfactory receptor family 6 subfamily E member 1D
			LOC619067	olfactory receptor family 6 subfamily E member 1B

			LOC104973083	olfactory receptor family 6 subfamily E member 1C
			LOC104973084	olfactory receptor family 6 subfamily E member 1
			LOC615040	olfactory receptor family 6 subfamily E member 1E
			ABHD4	Abhydrolase domain containing 4, N-acyl phospholipase B
			DAD1	Defender against cell death 1
			LOC100336282	uncharacterized
BTB-01841682	rs42951507	26:12274011	LOC784522	zinc finger protein 332
			HTR7	5-hydroxytryptamine receptor 7
			RPP30	ribonuclease P/MRP subunit p30
Hapmap50507-BTA-94221	rs41595591	24:54187542	LOC100137989	uncharacterized
			DYNAP	Dynactin associated protein
			RAB27B	RAB27B, member RAS oncogene family
			CCDC68	coiled-coil domain containing 68