ABSTRACT

Title of Dissertation:	ESTIMATION OF DRY MATTER INTAKE AND IDENTIFICATION OF DIETARY AND PRODUCTION PARAMETERS THAT INFLUENCE FEED EFFICIENCY OF INDIVIDUAL DAIRY COWS
	Marie Elizabeth Iwaniuk, Doctor of Philosophy, 2019
Dissertation directed by:	Professor, Dr. Richard A. Erdman Department of Animal and Avian Sciences

The objectives of this dissertation were to: 1) develop and validate equations used to estimate individual cow dry matter intake (**DMI**; kg/d) based on a nitrogen (**N**) balance approach, 2) determine the discriminatory power of several biological, production, and dietary variables on dairy feed efficiency (**FE**) as defined as energy-corrected milk (**ECM**; kg/d) per unit of DMI, 3) repeat the second objective using residual feed intake (**RFI**) to indicate FE status, and 4) determine if RFI values are dependent on the equation utilized to estimate DMI.

Results from the first experiment (Chapter 3) indicated that DMI could be successfully estimated on an individual cow basis using the following commonly measured parameters: milk yield, milk protein concentration, body weight (**BW**; kg), and dietary N concentration. These inputs are relatively simple to measure; therefore,

this equation may be used in the dairy industry as a practical method to estimate individual cow DMI when cows are fed in a group setting.

The results of the second experiment (Chapter 4) suggested that days in milk (**DIM**), milk fat yield (g/d), and BW had the most discriminatory power (89% success rate) to discriminate between cows based on their FE status when FE was defined as ECM per unit of DMI. Therefore, dairy producers can use these 3 variables to select for cows with high FE without requiring the measurement of DMI which can be costly and difficult to obtain.

Observations from the third experiment (Chapter 5) suggested that RFI is indicative of differences in metabolic efficiency between cows independent of most biological, production, and dietary variables, except DIM. These results are consistent with other studies that have suggested that RFI is indicative of true differences in metabolic efficiency between cows regardless of production parameters.

Lastly, the results of the fourth experiment (Chapter 6) suggest that RFI values generated from different DMI equations are strongly correlated such that RFI values are independent of the DMI equation utilized in the calculation. Thus, dairy producers can select the equation to estimate DMI that is most suitable for their operation without causing an "equation bias" on the RFI calculation.

ESTIMATION OF DRY MATTER INTAKE AND IDENTIFICATION OF DIETARY AND PRODUCTION PARAMETERS THAT INFLUENCE FEED EFFICIENCY OF INDIVIDUAL DAIRY COWS

by

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Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2019

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Dedication

This dissertation is dedicated to Drs. Brian Bequette and Bahram Momen.

To Dr. Brian Bequette:

"And all this science, I don't understand. It's just my job five days a week."

-Rocket Man [Elton John]

To Dr. Bahram Momen:

"You don't know what you don't know."

-Favorite motto

Acknowledgements

First and foremost, I would like to express my sincerest gratitude to **Dr. Richard Erdman** for all of his invaluable guidance and support throughout the completion of my PhD degree. Thank you for devoting your time and expertise to helping me grow as a teacher, researcher, and statistician. You are truly a wonderful advisor and I am so incredibly grateful for the opportunity to complete my M.S. and PhD degrees under your mentorship. Thank you for all of your constant support and encouragement.

I would like to thank **Dr. Rick Kohn** for serving as a committee member on both my M.S. and PhD committees. I greatly appreciate all of your invaluable expertise and guidance as well as your sharp wit and wonderful sense of humor.

I would like to thank **Dr. Erin Connor** for serving on my PhD committee and allowing me to use her USDA dataset to complete my PhD research experiments. I would not have been able to complete my PhD research without your generous "data donation" and guidance on these projects.

I would also like to thank **Dr. Kasey Moyes** and **Dr. Stephanie Lansing** for serving on my committee and devoting their time, effort, support, and guidance towards my dissertation program.

I will be forever grateful to **Dr. Brian Bequette** for his incredible mentorship throughout my undergraduate and graduate school careers. Brian encouraged me to pursue a career in science and I am truly grateful for his support, guidance, and, most importantly, friendship throughout my programs. Brian had an incredibly delightful personality and I am so lucky to have had the privilege to work with him during my programs. I will always be grateful for his lively and thought-provoking discussions.

In addition, I will forever be grateful to **Dr. Bahram Momen** for all of his constant support, mentorship, and encouragement throughout my PhD program. Dr. Momen's passion for statistics was both admirable as well as contagious and it inspired me to pursue a career as a biostatistician. Dr. Momen was one of the best professors and mentors that I have had the pleasure to work with during my graduate school career and I am so grateful for the opportunity to get to know him. I can honestly say that without him, I would not be the scientist that I am today. I am eternally grateful for all of his support and I hope that I can share his passion for teaching and statistics as I pursue my own career.

I would also like to thank my fellow "Erdman Lab" labmates, **Dr. Sarah Potts** and **Dr. Alzahraa Abdelatty**, for their assistance, support, and friendship throughout the completion of my degree. Additionally, I would like to extend my gratitude to everyone at the Central Maryland Research and Education Center (**CMREC**) for their hard work, assistance, and encouragement during my M.S. and PhD programs.

To one of my best friends and my "improv comedy wife," **Trish Rowlison**, thank you for all of your unconditional love and support throughout my PhD program. You are truly such a delightfully wonderful person and I am so incredibly lucky to have someone as hilarious and brilliant as you in my life. Whether we're on stage at an improv show in D.C. or we're frantically studying for our comprehensive exams at a Panera Bread, I always enjoy and appreciate your company. You make good days more amazing and bad days seem better. Thank you for being such a beautiful land mermaid. I like you and I love you.

I would also like to thank one of my best friends, **Leslie Juengst**, for all of her constant support and encouragement during my PhD program. Whether we were visiting a haunted penitentiary in Philly, taking a drunken 2:00 AM tour of the St. Louis Arch,

singing some sweet, sweet jams during our 16+ hour car ride to/from Cincinnati, or exploring an old pet cemetery in rural Maine, I have always truly enjoyed making memories and building an "archive" with you. You are one of my favorite people on this planet and I am so fortunate to have such a funny and caring person in my life. Thank you for all of your kindness and support. You truly are a delight.

I would also like to extend my sincerest gratitude to all of the wonderfully supportive and caring friends that I have been fortunate enough get to know during my PhD program: **Amanda Fischer**, **Cynthia Scholte**, **Ashley Franklin**, **Lindsey Vansandt**, **Halli Sigal**, **Latisha Judd**, **Kristen Brady**, and **Alex Burkard**. I am so lucky to have such a wonderful group of friends and I am forever grateful for all of your unwavering love and support. You truly enhanced my graduate school experience and I will always cherish our wonderful memories together.

I would also like to thank the Animal Sciences Graduate Student Association (**ASGSA**) for providing a space for me to meet new friends and grow both academically and personally. In addition, I would like to thank the club for its monetary support through the "Grants for Grads" program which helped finance trips to academic conferences and SAS workshops.

To my improv friends, **Sarah Spell** and **Sasha Jacob**, thank you for all of your love and support during my PhD program. You ladies are delightfully brilliant and wickedly funny and I am so grateful that I have such incredible friends in my life.

Of course, all of my success would not have been possible without the love and support of my family. I would like to thank my parents, **Linda** and **Steve Iwaniuk**, for always encouraging me to pursue my dreams. Thank you for teaching me the importance

of hard work and for always giving me the confidence needed to accept any challenge. I would also like to thank my brother, **Dan Iwaniuk**, for all of his love, guidance, and, most importantly, friendship throughout my PhD program and life. I am so incredibly grateful that I have such an amazing and supportive brother who is also one of my best friends. Thank you so much for being there for me on the good days, the bad days, and all of the days in between.

I would like to thank the Department of Animal and Avian Sciences, the Graduate School, and the University of Maryland for providing me the opportunity and financial assistance to complete my PhD degree.

I have been so incredibly lucky to have such wonderful people in my life to support, encourage, and inspire me throughout the completion of my PhD program. If I have failed to mention anyone, I would like to offer my sincerest apologies. I could not have completed my PhD without the help and support of so many wonderful individuals and I am forever grateful for all of the brilliant and kind people who have helped me along the way.

Lastly, despite all of my daydreams, I may never actually win a coveted Oscar so I am just going to officially say it here: "I'd like to thank the Academy."

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ADF	Acid detergent fiber
ADG	Average daily gain
BUN	Blood urea nitrogen
BW	Body weight
CCC	Concordance correlation coefficient
CDA	Canonical discriminant analysis
CP	Crude protein
CWT	Centum weight
DA	Discriminant analysis
DHIA	Dairy Herd Improvement Association
DietN	Dietary nitrogen
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
ECM	Energy-corrected milk
FCM	Fat-corrected milk
FCR	Feed conversion ratio
FE	Feed efficiency
IOFC	Income over feed costs
Mb	Mean bias
MBW	Metabolic body weight
MFN	Metabolic fecal nitrogen
MilkN	Milk nitrogen
MSEP	Mean square error of prediction
MUN	Milk urea nitrogen
MY	Milk yield
NDF	Neutral detergent fiber
NE_G	Net energy of gain
NE_L	Net energy of lactation
NE_M	Net energy of maintenance
NPN	Non-protein nitrogen
NRC	National Research Council
RFI	Residual feed intake
RMSE	Root mean square error
RMSEP	Root mean square error of prediction
Sb	Slope (linear) bias
SD	Standard deviation
SE	Standard error
SDA	Stepwise discriminant analysis
UN	Urinary nitrogen
WOL	Week of lactation

CHAPTER 1: INTRODUCTION

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Currently, feed costs represent the largest expense associated with milk production as they account for approximately 50% of the total production costs incurred on dairy farms (Beck and Ishler, 2016; USDA-ERS, 2018; Hardie et al., 2017). Because feed costs affect profitability, dairy producers are interested in calculating feed efficiency (**FE**) on an individual cow basis such that highly efficient cows can be selected for current and future herds through management and genetic selection (Erdman, 2011). Ultimately, selecting for high efficiency cows will reduce feed costs as well as the environmental impact of milk production while improving producer profitability and increasing milk production to meet the demands of the growing global population (Capper at al., 2009; VandeHaar et al., 2016).

There are 3 primary methods that are currently being utilized in the U.S. dairy industry to estimate dairy FE (Connor, 2015). The first method is referred to as "Income over feed costs (**IOFC**)" and IOFC values are calculated as the difference between the income related to milk production minus the cost of feed required for milk production (Beck and Ishler, 2016; Block 2010). To calculate an IOFC value, a dairy producer must have the following information: average daily milk production (kg/d/cow), current milk prices ($/cwt), average dry matter intake (**DMI**; kg/d/cow), and current feed prices (Beck and Ishler, 2016; Block 2010).

The second approach to estimating FE is to calculate the ratio of energy-corrected milk (**ECM**; kg/d; standardized for milk fat and protein concentrations) to DMI which is similar to calculating feed conversion ratios (**FCR**) used to estimate FE in poultry, swine, and beef industries (Erdman, 2011; Willems et al., 2013). To calculate a FE ratio for an individual cow, a dairy producer must have the following on-farm data: milk yield (kg/d), milk fat concentration (%), milk protein concentration (%), and DMI (DRMS, 2014; Erdman, 2011).

Lastly, the third approach to estimating dairy FE on an individual cow basis is to calculate a cow's residual feed intake (**RFI**). RFI is calculated as the difference between a cow's actual DMI and her predicted DMI based on an established DMI prediction equation (Connor, 2015; Koch et al., 1963; Macdonald et al., 2014). DMI prediction equations vary; however, most equations contain the following 3 variables: 1) a variable used to estimate milk and/or milk component yields such as energy-corrected milk production (**ECM**; kg/d), 2) a variable used to estimate body weight (**BW**; kg) such as BW itself or metabolic BW (**MBW**; $BW^{0.75}$), and 3) an estimate in change in BW (**ΔBW**) such as average daily gain (**ADG**; g/d) (Connor, 2015; Connor et al., 2013; Koch et al. in 1963). In order to calculate RFI, a dairy producer must have the following information: actual DMI, predicted DMI based on a selected prediction equation, and the data for all production variables included in the prediction equation such as milk yield, milk composition, and BW (Connor, 2015).

One of the biggest issues with calculating IOFC values or the FE ratio is that DMI is rarely measured on an individual cow basis on most dairy operations (Connor et al., 2013; Faverdin et al., 2017; Halachmi et al., 2004). Unfortunately, most dairy operations do not have the time, labor, or financial resources to measure DMI on an individual cow basis (Halachmi et al., 2004). Therefore, the vast majority of dairy cows are fed in large groups such that the DMI of an individual cow within a group is unknown (Halachmi et al., 2004). One way to overcome the lack of individual cow DMI measurements on farm is to estimate DMI using mathematical models (Halachmi et al., 2004).

Several studies have shown that there is a robust relationship between nitrogen (**N**) intake and N output in lactating dairy cows (Jonker et al., 1998; NRC, 2001, Van Horn et al., 1994). Research has shown that dairy cows secrete approximately 25-35 percent of their consumed N into milk while the majority of the remaining N is excreted in urine and feces (NRC, 2001). Van Horn et al. (1994) explored the relationships between consumed N and milk, urinary, and fecal N outputs and reported that urinary and fecal N excretions can be estimated by subtracting the milk N concentration from the concentration of N consumed (NRC, 2001). Similarly, Jonker et al. (1998) found that the N intake can be estimated using milk and urinary N (UN) concentrations in which milk N was calculated as a function of milk yield (kg/d) and the crude protein percentage of milk and UN was estimated as a function of MUN. Based on these concepts, it is possible that DMI can be estimated on an individual cow basis if the amount of excreted N in the milk, urine, and feces are known or estimated (Jonker et al., 1998).

The second biggest issue regarding FE is that various biological, production, and dietary factors have been shown to affect dairy FE ratios including: stage of lactation, parity, individual cow variation in production parameters (milk yield and milk composition), BW, calving month, dietary energy concentration, dietary neutral detergent fiber concentration, and dietary crude protein (CP) concentration (Heinrichs et al., 2016; Ishler, 2014; NRC, 2001). Although substantial research has been conducted to explore the effects of various biological, dietary, and production parameters that affect FE, the relative importance of each factor has yet to be determined. In regard to RFI, several biological, production, and/or dietary variables are included and accounted for in the model to predict DMI such that RFI values are understood to be phenotypically independent of the variables used for the DMI prediction (Connor, 2015; Potts et al., 2015; VandeHaar et al., 2016). However, it is possible that RFI may still be dependent on biological, production, and/or dietary factors that are not included in the DMI prediction equation. Research regarding this topic is limited; thus, more research is needed to explore the relationship between RFI and various biological, management, dietary, and/or behavioral factors (Connor et al., 2013; Golden et al., 2008; Nkrumah et al., 2007).

Lastly, the third major issue regarding dairy FE occurs when FE is estimated using the RFI approach. RFI is a statistical error in the regression analysis between actual and predicted DMI; thus, RFI contains both true variation in metabolic FE between cows due to genetics as well as random variation due to DMI measurement and prediction errors (VandeHaar et al., 2016). Because errors in DMI prediction are allocated to the RFI term, it may be possible that within-cow RFI values may be dependent on the equation used to predict DMI (VandeHaar et al., 2016).

The first central hypothesis of this dissertation was that an equation that estimates DMI on an individual cow basis can be developed and validated using the concept of N balance derived from common, on-farm parameters. Thus, the first objective of this dissertation was to develop and validate several equations that estimate DMI on an individual cow basis using the concept of N balance derived from common, on-farm parameters using linear and non-linear modeling techniques. The practical application of this project was to estimate DMI of an individual cow fed in groups so that dairy producers could use these novel equations on-farm to estimate DMI to allow for the calculation of IOFC and dairy FE ratios.

The second main hypothesis of this dissertation was that the relative importance of several biological, production, and dietary factors that affect dairy FE ratios and RFI can be determined and ranked. Thus, the second objective of this dissertation was to determine and rank the relative importance of several biological, production, and dietary factors that affect dairy FE ratios and RFI using a series of discriminant analyses including stepwise, canonical, and basic discriminant analyses. The practical application of the second project was to identify key factors that affect dairy FE ratios and RFI to help producers select for highly efficient animals even if FE ratios and RFI cannot be calculation from on-farm parameters.

Lastly, the third central hypothesis of this dissertation was that RFI values are dependent on the DMI equation used to predict DMI. Therefore, the third objective of this dissertation was to determine if within-cow RFI values were repeatable when different DMI equations were used to predict DMI to calculate RFI. The results of the third project may be used by dairy producers to help them select an appropriate DMI equation to predict DMI and calculate RFI on their respective dairy operations.

In summary, the combined goal of these 3 projects was to help dairy producers estimate FE on an individual cow basis so that dairy producers can select for more efficient cows within their current and future herds. Improved dairy FE will ultimately result in improved producer profitability, reduced environmental impact of milk production, and increased milk (and milk product) production to feed the growing, global population.

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CHAPTER 2: LITERATURE REVIEW

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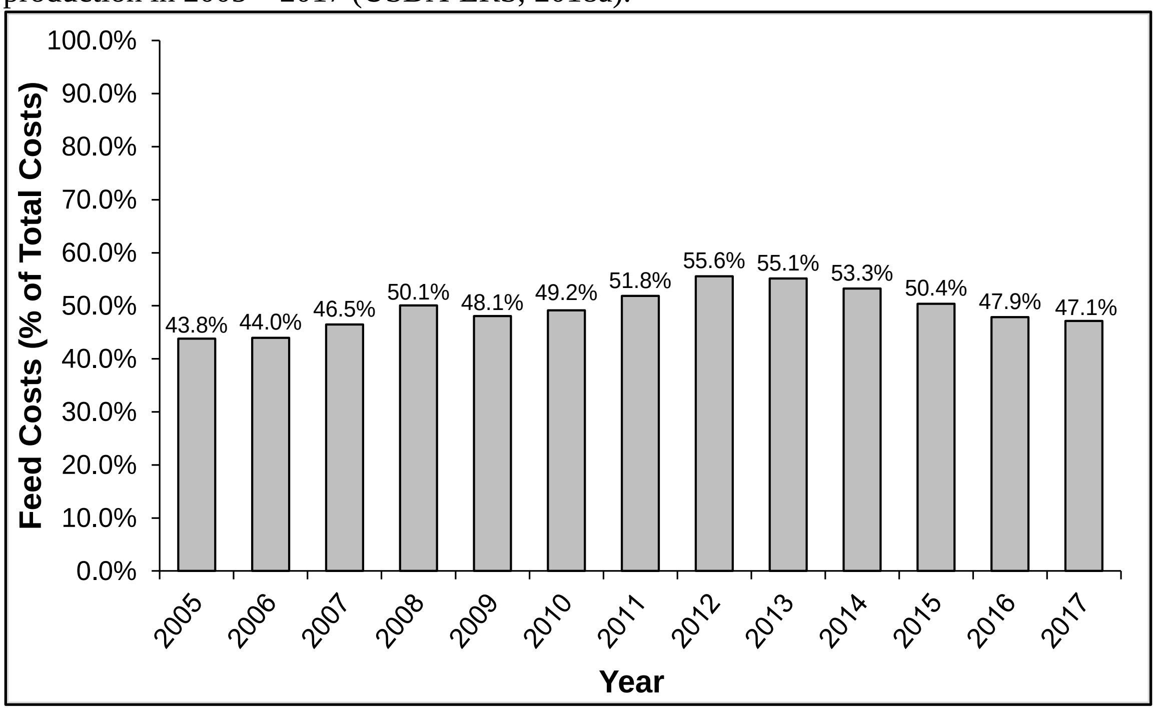
Dairy Feed Costs

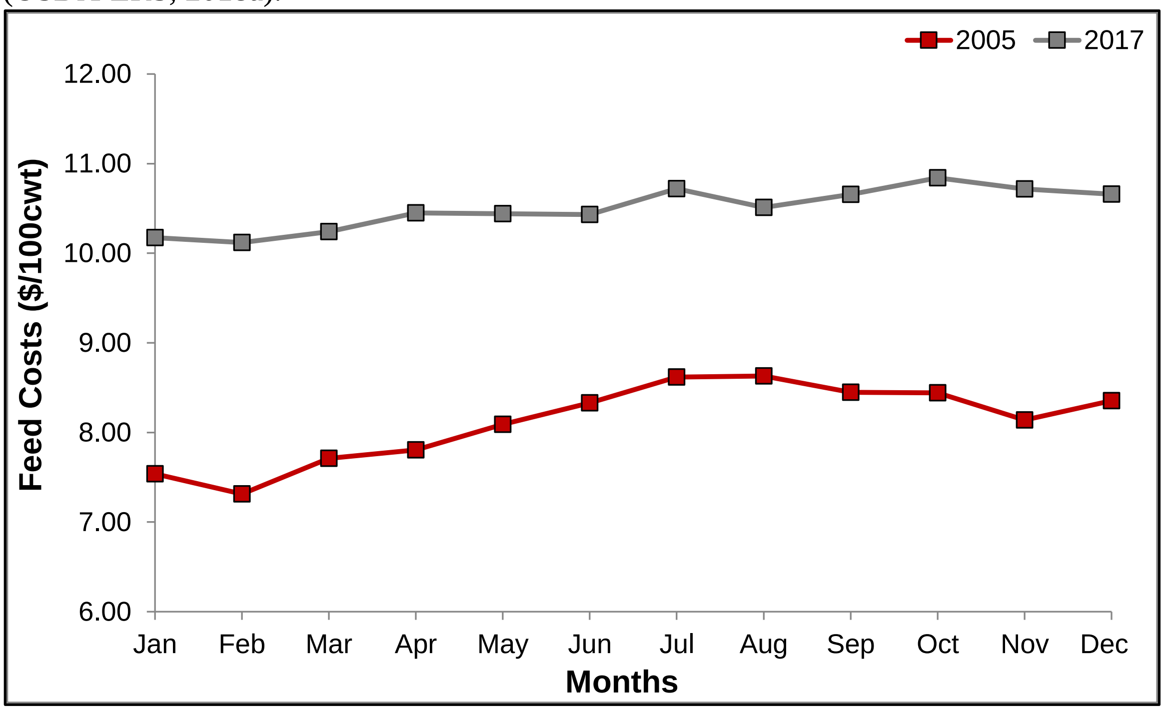
Dairy feed costs represent the single largest expense associated with milk production on dairy farms (Beck and Ishler, 2016; Hardie et al., 2017; Valvekar et al., 2010). Currently, feed costs account for approximately 50% of total production costs for milk production (Beck and Ishler, 2016; USDA-ERS, 2018a; Hardie et al., 2017). Using data published by the United States Department of Agriculture's Economic Research Service (**USDA-ERS**), Figure 2.1 illustrates the U.S. national average yearly dairy feed costs as a function of total production costs (USDA-ERS, 2018a).

Figure 2.1. Average yearly U.S. dairy feed costs as a function of the total cost of milk production in 2005 – 2017 (USDA-ERS, 2018a).



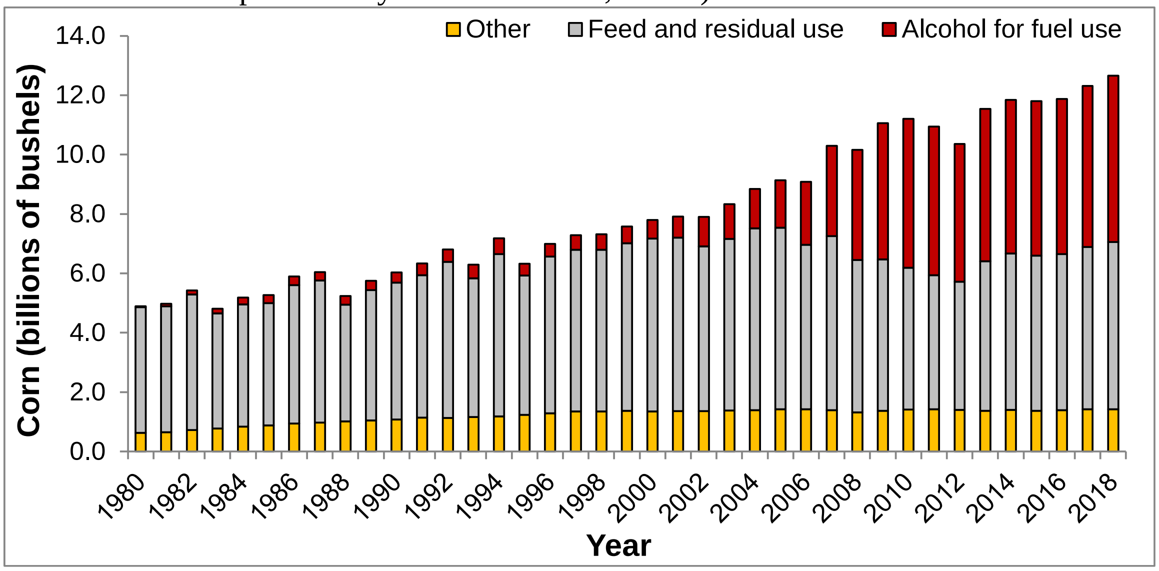
Due to several factors, feed costs have increased approximately 1.29-fold from 2005 to 2017 (USDA-ERS, 2018a). Figure 2.2 illustrates the U.S national monthly dairy feed costs per centum weight (CWT) of milk sold in the years 2005 and 2017 using data derived from the USA-ERS (2018a).

Figure 2.2. U.S. monthly dairy feed costs per centum weight of milk sold in 2005 and 2017 (USDA-ERS, 2018a).



In addition to a historic drought that caused record-high feed costs in 2012, an increased use of corn for ethanol production in the United States has caused U.S. dairy feed costs to be extremely high as corn is a staple ingredient in dairy cow rations (Buza et al., 2014; Hardie et al., 2014; USDA-ERS, 2018b). As illustrated in Figure 2.3, corn production (billions of bushels) has nearly tripled from 1980 to 2014; however, the additional corn produced is being utilized by the ethanol industry to produce fuel and is not being allocated for use as animal feed (USDA-ERS, 2018b). In fact, as the amount of corn used for ethanol production increased, there was a slight decrease in the amount of corn that was being used for animal feed (USDA-ERS, 2018b).

Figure 2.3. Comparison of the U.S. domestic corn use between 1980 and 2018 (figure is derived from data provided by the USDA-ERS, 2018b).



Feed Efficiency

Feed Conversion Ratios

High feed costs are not an issue that is unique to the dairy industry; feed costs are the largest expense of production in several animal production industries (Erdman, 2011; Willems et al., 2013). In order to assess an animal's production value, feed efficiencies (FEs) are utilized in the poultry, swine, and beef industries and benchmark FEs have been established for these industries (Erdman, 2011). Commonly, FE is assessed using feed conversion ratios (**FCR**) in which the amount of feed consumed is the numerator and the amount of body weight (**BW**) gained is the denominator (Willems et al., 2013). A standard FCR is shown in Equation 1:

$$\text{FCR} = \frac{\text{Amount of Feed Consumed}}{\text{Amount of BW Gained}} \tag{1}$$

For example, an animal that can convert 2.4 kg of feed into 2.0 kg of BW would have an FCR value of 1.2. For most animal production industries, a low FE value is highly desirable because it indicates that the animal is efficiently converting feed nutrients to a saleable product (Food and Agriculture Organization of the United Nations (**FAO**), 2010). Several factors such as gender, age, genetic composition, environmental conditions, and dietary composition may alter the FE of an individual animal (FAO, 2010). In addition, FCRs vary significantly between different animal species and average FCR values for several species have been reported in Table 2.1.

Table 2.1. Average FCR values of various species.

Animal Species	FCR
Beef Cattle[1]	6.70
Broilers (Chicken)[2]	1.60
Ducks[1]	2.59
Guinea Fowl[1]	2.98
Japanese Quail[1]	2.59
Small Ruminants[3]	7.00
Swine[4]	3.00
Turkey[1]	3.03

[1]Mean of reported FCR values extracted from Willem et al. (2013).
[2]FCR reported by Best (2011).
[3]Data derived from FAO (2010).
[4]FCR reported by Vansickle (2013).

Although FCRs are widely utilized in the poultry, swine, and beef industries, additional methods have been developed to estimate FE such as the Residual Feed Intake (**RFI**) method which will be discussed shortly (Xu et al., 2014). Therefore, there are several approaches to estimate FE in the U.S. meat industries. Similarly, the dairy industry lacks a singular equation to assess FE (Erdman, 2011). In total, there are 3 primary methods utilized by the dairy industry to estimate FE on an individual cow and/or herd basis: Income over Feed Costs (**IOFC**), FE ratios, and RFI (Beck and Ishler, 2016; Connor, 2015; Erdman, 2011).

Dairy Feed Efficiency

Income over Feed Costs

The first method used to estimate dairy FE is entitled, "Income over Feed Costs (**IOFC**)" and this calculation allows producers to estimate changes in their daily profit margins based on adjustments made to the ration formulation and/or fluctuations in the market value of select feed ingredients (Beck and Ishler, 2016; Block 2010). The broad equation used to calculate IOFC is shown in Equation 2:

$$\text{IOFC (\$/day)} = \text{Milk Income (\$/day)} - \text{Feed Costs (\$/day)} \qquad (2)$$

Although the overall IOFC equation may appear simplistic, the IOFC equation is further broken down into 2 major segments: milk income ($/day) and feed costs ($/day). The goal of the milk income calculation is to provide producers with an estimation of daily milk production profits using 3 input values: 1) average amount of milk produced per cow per day (lb/cow/d), 2) number of lactating cows on the farm, and 3) current price of a hundred-weight ($/cwt) of milk (Beck and Ishler, 2016; Block 2010). The equation used to calculate milk income is shown below in Equation 3:

$$\text{Milk Income (\$/day)} = ((\text{Milk (lb/cow/d)} \times (\text{\# of cows}))/100) \times \text{milk price (\$/cwt)} \qquad (3)$$

Once the dairy producer calculates the milk income, feed costs must be calculated using the formula provided in Equation 4:

$$\text{Feed Costs (\$/day)} = (\text{cost per lb of feed DM}) \times (\text{dry matter intake (lb/cow/d)}) \qquad (4)$$

In order to calculate the "cost per lb of feed DM" portion of the feed costs equation, the dairy producer must know the individual feed ingredient amounts per cow per day and the feed prices for each feed ingredient (Beck and Ishler, 2016; Block 2010). In addition, the producer must either record individual daily DMI or be able to estimate daily DMI on a per cow basis in order to estimate feed costs (Beck and Ishler, 2016; Block 2010). Once the producer has calculated values for both milk income and feed costs, the IOFC value can be estimated (Equation 2).

Advantages of IOFC

One major benefit of calculating IOFC is the ability to estimate changes in profitability based on projected modifications within the dietary ration (Block, 2010). An example of a hypothetical comparison that a producer may make between 2 different lactating dietary rations utilizing the IOFC technique is provided in Table 2.2.

Table 2.2. Example of a ration formulation comparison using IOFC calculations.

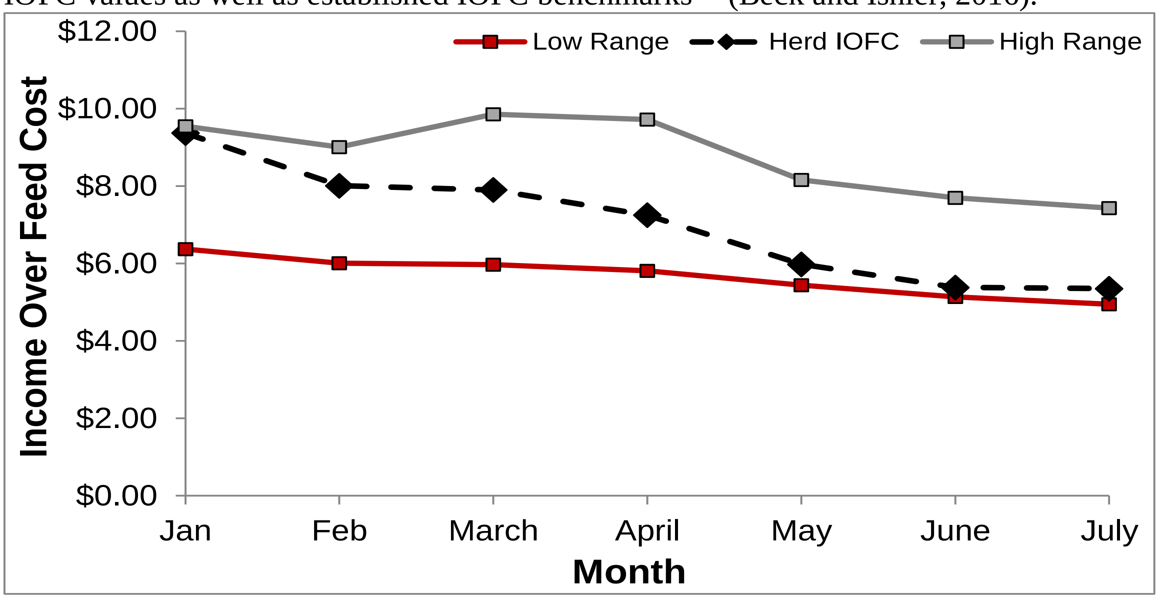
Herd Information	**Current Diet**	**Proposed Diet**
Milk Income		
Average Milk Production (lb/cow/day)	81.7	84.7
Number of Lactating Cows	327	327
Current Milk Price ($/cwt) for Class I Milk[1]	$15.98	$15.98
Total Milk Income ($/day)	$4,269.20	$4,425.96
Total Milk Income ($/cow/day)	$13.06	$13.54
Feed Costs		
Price of Feed Dry Matter ($/lb)[2]	$0.14	$0.15
Average Dry Matter Intake (lb/cow/day)	52	52
Total Feed Costs ($/day)	$2,380.56	$2,550.60
Total Feed Costs ($/cow/day)	$7.28	$7.80
IOFC		
Total IOFC ($/day)	$1,888.64	$1,875.36
Total IOFC ($/cow/day)	$5.78	$5.74
Additional IOFC ($/day)		-$13.28
Additional IOFC ($/cow/day)		-$0.04
Additional Annual IOFC ($/year)		-$4,845.81

[1]Class I milk prices ($/cwt) were derived from data published by Hoard's Dairyman (2019)
[2]Calculated based on dietary ingredients and composition as well as respective feed prices.

Based on this example, a dairy producer would lose approximately $0.04 per cow per day, $13.28 per day, and $4,845.81 per year if the dietary ration was changed from the current diet to the proposed diet. This may be surprising to the dairy producer as the proposed diet was projected to increase milk yield by 3.0 lbs per cow per day while only costing an additional $0.01/lb of feed dry matter. However, the increase in projected income did not outweigh the increase in projected feed costs; thus, this dietary manipulation would be disadvantageous for the dairy producer. Overall, the IOFC method serves as a useful tool that can provide producers with important information regarding changes in profit as a result of dietary ration alterations (Block, 2010).

In addition to helping producers conduct cost-benefit analyses, the IOFC method can also be used as a tool to help producers set personal IOFC benchmarks (High range and low range IOFC values) and assess their actual IOFC values over time (Beck and Ishler, 2016). For example, Penn State Extension developed an "Income over Feed Costs" tool in which dairy producers can enter their herd, production, and dietary ration information which the program then uses to calculate individual IOFC values as well as IOFC benchmarks (Beck and Ishler, 2016). An example output from the Penn State "Income over Feed Costs" tool is presented in Figure 2.4.

Figure 2.4. Sample output from the Penn State "IOFC" tool displaying individual herd IOFC values as well as established IOFC benchmarks[1,2] (Beck and Ishler, 2016).



[1]High range IOFC values are calculated such that milk income is 2.50x higher than feed costs.
[2]Low range IOFC values are calculated such that milk income is 1.67x higher than feed costs.

The output given by the Penn State IOFC tool can help producers determine how efficiently their ration is being utilized based on the amount (and price) of milk being produced; thus, IOFC values are indicators of overall economic dairy FE (Beck and Ishler, 2016). In the current example, the actual IOFC values for this hypothetical herd are close to the high range IOFC values for the months of January and February. Between February and April, the actual IOFC values for this hypothetical herd are in the middle between the high and low range IOFC values. Lastly, from May to June the actual IOFC values for this hypothetical herd are diminished and fall along the low range IOFC values. Therefore, if a dairy producer could implement a dietary or management change in their dairy operation during any time point between January and July, the producer would most likely choose to implement a change during the months of May, June, and/or July to attempt to increase their IOFC values. Based on this information, a dairy producer may make changes during these months in the following year to avoid a similar reduction in IOFC values. Because these months tend to be associated with hot weather, a dairy producer may attempt to improve the efficiency of their herd by implementing more effective strategies to mitigate heat stress such as water misters (evaporative cooling), fans, or dietary supplements (Polsky and von Keyserlingk, 2017). Thus, the IOFC tool published by Penn State Extension can be utilized by dairy producers to help visualize their herd's current IOFC values and the results may elicit changes in dietary or management strategies in hopes of improving economic efficiency of the operation.

The third benefit of the IOFC tool is that it helps producers plan for upcoming months (Beck and Ishler, 2016). As previously mentioned, the actual IOFC values for May, June, and July are approaching the low-range IOFC benchmark values for these respective months. If this trend continues, the current feeding program may not be suitable to maintain an appropriate IOFC value in future months. Therefore, this trend would signal to the producer that a new dietary or management strategy should be implemented in order to either decrease feed costs or increase milk revenues in order to maintain or increase profit margins for future months (Beck and Ishler, 2016). The effects of the new feeding or management strategy on overall profitability could be estimated using the IOFC calculations discussed in Table 2.2.

Disadvantages of IOFC

Although there are several advantages to using the IOFC to estimate FE in dairy cows, there are four major disadvantages to this method that prohibit it from being a reliable candidate for the sole predictor of dairy FE. First and foremost, the calculations used for each IOFC estimation require current milk and feed prices which frequently change.

Because milk and feed prices continuously fluctuate, it is impossible to create standardized IOFC benchmarks that can be used over time across different dairy operations because IOFC values are temporal indicators of profitability (Erdman, 2011). For example, the average price of milk per hundredweight in the U.S. was $13.36 during March 2018; however, the price per hundredweight of milk in the U.S. in October 2018 was $16.33 (USDA-AMS, 2019). If all other IOFC calculation input values remained the same, the aforementioned difference in milk prices would change the estimated IOFC by $730.62 per day, as shown in Table 2.3.

Table 2.3. Comparison of IOFC estimations based on 2 different milk prices.

Herd Information	Milk Price $13.36	Milk Price $16.33
Milk Income		
Average Milk Production (lb/cow/day)	82	82
Number of Lactating Cows	300	300
Current Milk Price ($/cwt) for Class I Milk[1]	$13.36	$16.33
Total Milk Income ($/day)	$3,286.56	$4,017.18
Total Milk Income ($/cow/day)	$10.05	$12.28
Feed Costs		
Price of Feed DM ($/lb)	$0.17	$0.17
Average Dry Matter Intake (lb/cow/day)	54	54
Total Feed Costs ($/day)	$2,754.00	$2,754.00
Total Feed Costs ($/cow/day)	$9.18	$9.18
IOFC		
Total IOFC ($/day)	$532.56	$1,263.18
Total IOFC ($/cow/day)	$0.87	$3.10

[1]Milk prices per hundredweight ($/cwt) are based on data derived from the USDA-AMS (2019).

Therefore, it is impossible to establish fixed IOFC benchmarks that could serve as within or across farm indicators of dairy FE because the IOFC calculations are heavily based on fluctuating market prices of milk and feed ingredients which vary depending on the region of the U.S.

The second major disadvantage of the IOFC calculation is that it does not take milk composition into consideration. Milk is comprised of several components such as fat, protein, lactose, ash, and water and the concentration of these components can vary based on several factors such as breed, parity (age), stage of lactation, season, and diet composition (Field and Taylor, 2012; Harding, 1999; Looper, 2012). The lack of consideration for milk composition in the IOFC calculation is problematic because calculated IOFC gross profits are solely based on fluid milk yield; however, a majority of dairy producers are paid for milk component yield, specifically milk fat and protein (Geuss, 2015). Therefore, gross income projections may be inaccurate depending on in which milk payment system the dairy producer is enrolled (Geuss, 2015). In addition, milk composition should be considered when determining the FE of an individual cow as different milk components require different amounts of energy to produce which can affect overall milk yield (Harding, 1999; Gaines and Davidson, 1923). This concept is further discussed in the next section of this dissertation. In summary, it is imperative to consider milk composition when calculating gross profits and/or FE of dairy cows; thus, the IOFC calculation is flawed as it does not take milk composition into consideration.

The third disadvantage of the IOFC calculation is that it essentially serves to estimate economic efficiency, not efficiency of nutrient utilization. Although the tool effectively provides dairy producers with an estimate of gross profits after feed costs are removed, the IOFC value is primarily based on monetary outputs and inputs and those values are calculated based on projected, average values of milk yield (kg/d) and dry matter intake (**DMI**; kg/d), respectively (Beck and Ishler, 2016). Thus, IOFC does not actually provide a dairy producer with information regarding the efficiency of nutrient utilization for milk production which is the basis of dairy FE.

Finally, the fourth major limitation of the IOFC method is that the IOFC calculations are typically based on average herd production values, not on an individual cow basis. With feed costs consisting of approximately 50% of total production costs, dairy producers are interested in selecting high efficiency cows that can effectively utilize their dietary ration for milk production for their herd (Connor et al., 2013). In order to select high producing cows, dairy producers must be able to calculate IOFC on an individual cow basis (Beck and Ishler, 2016). Although it is possible to calculate the IOFC of an individual cow, IOFC requires both milk production and DMI to be measured on an individual cow basis; however, individual cow DMI is rarely measured on most dairy operations as this measurement tends to be costly and labor intensive (Connor et al., 2013; Faverdin et al., 2017; Halachmi et al., 2004). Therefore, IOFC is a great tool that can be used by producers to estimate the efficiency of their herd; however, it is not commonly used to estimate FE on an individual cow basis due to the frequent lack of DMI measurements on an individual cow basis.

In conclusion, the IOFC method does have several advantages for dairy producers to estimate their profitability with respect to potential ration formulation changes, alterations in production responses, or implementation of new on-farm strategies (Block, 2010). However, the IOFC cannot be utilized as a universal tool to indicate dairy FE because the IOFC calculations depend on current market prices for milk and feedstuffs which results in regional and time-dependent IOFC values (USDA-NASS, 2018). Most importantly, IOFC is not designed to be used to calculate individual cow FE for genetic and/or management selection to improve FE (Beck and Ishler, 2016). Therefore, an alternate calculation should be used to estimate dairy FE. For the remaining portion of this dissertation, dairy FE will be estimated using FE ratios and RFI.

Dairy FE Ratios

Although IOFC and RFI methods are becoming more popular in the dairy industry, dairy FE is most commonly estimated as a ratio that compares the amount milk produced (kg/cow/d) to the DMI (kg/cow/d) needed for the milk production (Connor, 2015).

Although all milk to feed ratios use DMI as the denominator, the numerator of the ratio may vary based on study. There are five numerator variables that are commonly used in dairy FE and each FE equation will be discussed in further detail.

Dairy FE Numerators

Milk Yield

The first and most basic dairy FE equation utilizes overall milk yield as the numerator of the ratio and it compares milk produced to DMI, as shown in Equation 5.

$$\text{Dairy FE} = \text{Milk Yield (kg)} / \text{DMI (kg)} \tag{5}$$

Although this ratio is the most simplistic and easiest method of calculating a dairy FE ratio, dairy FE is incorrectly predicted using this formula because overall milk yield does not account for changes in the composition of the milk produced by individual cows or herds. As shown in Table 2.4, milk is comprised of several components including: water, lactose, fat, protein, and ash (vitamins and minerals) (Field and Taylor, 2012).

Table 2.4. Average milk composition and heat of combustion values for Holstein milk.

Milk Component	**Percentage in Milk[1] (%)**	**Heat of Combustion[2] (Mcal/kg)**
Water	88.08	0.00
Lactose	4.61	3.95
Fat	3.56	9.29
Protein	3.02	5.71
Ash	0.73	0.00
Component Total	100.00	---

[1]Values derived from Harding (1999).
[2]Values derived from NRC (2001).

Although all milk contains similar nutrients, the relative abundance of each nutrient varies based on breed (Harding, 1999). For example, the relative abundance (%) of each milk nutrient for several dairy breeds is shown in Table 2.5.

Table 2.5. Relative abundance (%) of milk nutrients based on cattle breeds[1].

Breed	**Water**	**Lactose**	**Fat**	**Protein**	**Ash**
Holstein	88.08	4.61	3.56	3.02	0.73
Brown Swiss	87.50	4.80	3.80	3.18	0.72
Ayrshire	87.40	4.63	3.97	3.28	0.72
Guernsey	86.40	4.78	4.58	3.49	0.75
Jersey	85.91	4.70	4.97	3.65	0.77

[1]Data derived from Harding (1999).

In addition to animal species and breed, milk composition may also vary on an individual animal basis due to genetics (Harding, 1999). As shown in Table 2.4, each milk component has a different heat of combustion value; therefore, each nutrient requires a different amount of dietary energy to be produced. Thus, the amount of energy required to produce a specific amount of milk depends on the milk composition. The simplistic FE equation that utilizes overall milk yield as the numerator of the ratio does not account for energy differences in milk production; therefore, this ratio should not be used to calculate dairy FE. Instead, milk yields should be standardized based on the nutrient composition of the milk.

4.0% Fat-Corrected Milk

In 1923, Gaines and Davidson (1923) developed the first formula to standardize milk yield based on its composition. As shown in Tables 2.4 and 2.5, milk fat is the most energy dense nutrient in milk and it varies from cow to cow (Harding, 1999; Gaines and

Davison, 1923). In order to account for the energy differences of milk yield due to individual cow milk fat variation, Gaines and Davidson (1923) developed the 4.0% fat-corrected milk (4.0% FCM) formula, which standardizes milk yield to the energy output of a cow producing milk with 4.0% fat. To develop the 4.0% FCM formula, Gaines and Davidson (1923) used the heats of combustion for milk fat (9.28 kcal/g) and milk solids-non-fat (SNF; 4.09 kcal/g) to create coefficients for milk yield (kg/d) and milk fat yield (kg/d). The final 4.0% formula is presented in Equation 6.

$$4.0\%\ \text{FCM} = (0.40 \times \text{kg milk}) + (15.00 \times \text{kg milk fat}) \tag{6}$$

There is one major flaw associated with this equation. By using only one coefficient for milk SNF, Gaines and Davidson (1923) assumed that milk lactose, protein, and ash are always present in the same ratio in milk. However, as shown in Table 2.5, the percentages of milk lactose, protein, and ash vary by cow breed and can even vary by individual cow (Harding, 1999). This flaw may lead to over or under-predictions in 4.0% FCM if the actual ratio of milk lactose, protein, and ash deviates from the ratio proposed by Gaines and Davidson (1923). Although this flaw is present in the 4.0% FCM equation, this formula is still used in the dairy industry to predict FE because differences in milk energy output are typically related to differences in milk fat content, which are appropriately accounted for in this equation (Erdman, 2011).

3.5% Fat-Corrected Milk

One of the most commonly used numerators in the dairy FE ratio is the 3.5% fat-corrected milk (3.5% FCM) formula which was derived from the 4.0% FCM equation (Erdman, 2011). Gaines and Davidson (1923) developed a FCM equation standardized to 4.0% milk fat because this value fell between the milk fat content of Holstein (3.4%) and Jersey (5.4%) breeds. However, average dairy cows in the United States today do not produce 4.0% milk fat. Instead, U.S. dairy cows tend to produce approximately 3.25 to 3.80% (average 3.5%) milk fat due to 2 main reasons. First, a 3.5% milk fat value is more closely related than 4.0% to the average milk fat percentage for the Holstein breed and, currently, 85-90% of the cows in the United States are Holsteins (Capper et al., 2009). Second, many genetic advancements have been made within the last century that have enabled cows to produce more milk over time; however, the caveat to this improvement is that dietary energy is being allocated for milk volume and milk fat concentrations decrease (Blayney, 2002). Because of these reasons, the 3.5% FCM formula was adapted from the original 4.0% FCM formula to provide a standardized milk yield that better reflected the current U.S. dairy industry (Erdman, 2011). Similar to the 4.0% FCM formula, heat of combustion values (kcal/g) for milk fat and SNF were used to develop coefficients for milk yield (kg/d) and milk fat yield (kg/d), respectively. The formula for 3.5% FCM is provided in Equation 7.

$$3.5\%\ \text{FCM} = (0.4318 \times \text{kg milk}) + (16.23 \times \text{kg milk fat}) \quad (7)$$

Because it was derived from the 4.0% FCM formula, the 3.5% FCM intrinsically possesses the same flaw as the 4.0% FCM formula discussed previously; the 3.0% FCM formula assumes a constant ratio of milk lactose, protein, and ash in the SNF content of milk (Erdman, 2011). However, it is important to note that milk fat content is the factor that has the greatest effect on milk energy output; therefore, the 3.5% FCM formula is still a reasonably accurate indicator of milk energy output.

Solids-Corrected Milk

In 1965, Tyrell and Reid developed a new standardized milk formula that aimed to address the inherent flaw associated with the 4.0% FCM formula by appropriately accounting for all components of milk. To develop the new equation, Tyrell and Reid (1965) analyzed milk samples from 42 cows that varied in composition in order to establish heats of combustion for each milk component using an oxygen-bomb, adiabatic calorimeter and determine the relationship between milk composition and overall milk yield. Tyrell and Reid (1965) concluded that milk energy output is dependent on the content of lactose, fat, and protein in the milk; however, milk ash content did not affect milk energy output as ash has no heat of combustion. Using the heats of combustion for each milk component determined in their study, Tyrell and Reid (1965) developed a new equation to predict milk energy output using coefficients for milk fat content, milk SNF content (lactose and protein), and overall milk yield. The equation developed by Tyrell and Reid (1965) is known as the solids-corrected milk (**SCM**) formula and it is shown in Equation 8.

$$\text{SCM} = (12.3 \text{ x lbs milk fat}) + (6.56 \text{ lbs SNF}) - (0.0752 \text{ x lbs milk}) \tag{8}$$

In regard to predicting milk energy outputs, the SCM formula has been shown to be a better predication equation compared to the 4.0% FCM formula, especially at more extreme levels of milk fat content (Erdman, 2011). However, the SCM formula still contains an inherent error regarding the assumed milk lactose, protein, and ash ratio in the SNF content of milk (Erdman, 2011). Regardless of its improvement compared to the 4.0% FCM formula, the SCM formula is still not utilized as frequently as the 3.5% FCM equation to standardize milk yield.

Energy-Corrected Milk

The last equation that has been developed to standardize milk yield based on milk composition is the energy-corrected milk (**ECM**) formula (Erdman, 2011). Based on the regression equations developed by Tyrell and Reid (1965), the ECM formula was created by the Dairy Herd Improvement Association (**DHIA**) to standardize milk yields for lactation records based on 3.5% milk fat and 3.2% milk protein (DRMS, 2011; Erdman, 2011). The ECM formula is presented in Equation 9.

$$\text{ECM} = (12.95 \times \text{lbs milk fat}) + (7.65 \times \text{lbs milk protein}) + (0.327 \times \text{lbs milk}) \qquad (9)$$

Although the ECM individually accounts for milk fat and protein, the coefficient for overall milk yield still contains the inherent error regarding the assumption of a constant milk lactose to ash ratio in the SNF content of milk (Erdman, 2011). However, lactose and ash concentrations in milk are fairly constant; therefore, the inherent error in the ECM equation is relatively small compared to the errors present in the 4.0% FCM, 3.5% FCM, and SCM equations (Erdman, 2011). Because the ECM formula provides adequate milk energy output predictions, it is one of the most widely utilized milk standardization equations in the U.S. dairy industry (Erdman, 2011).

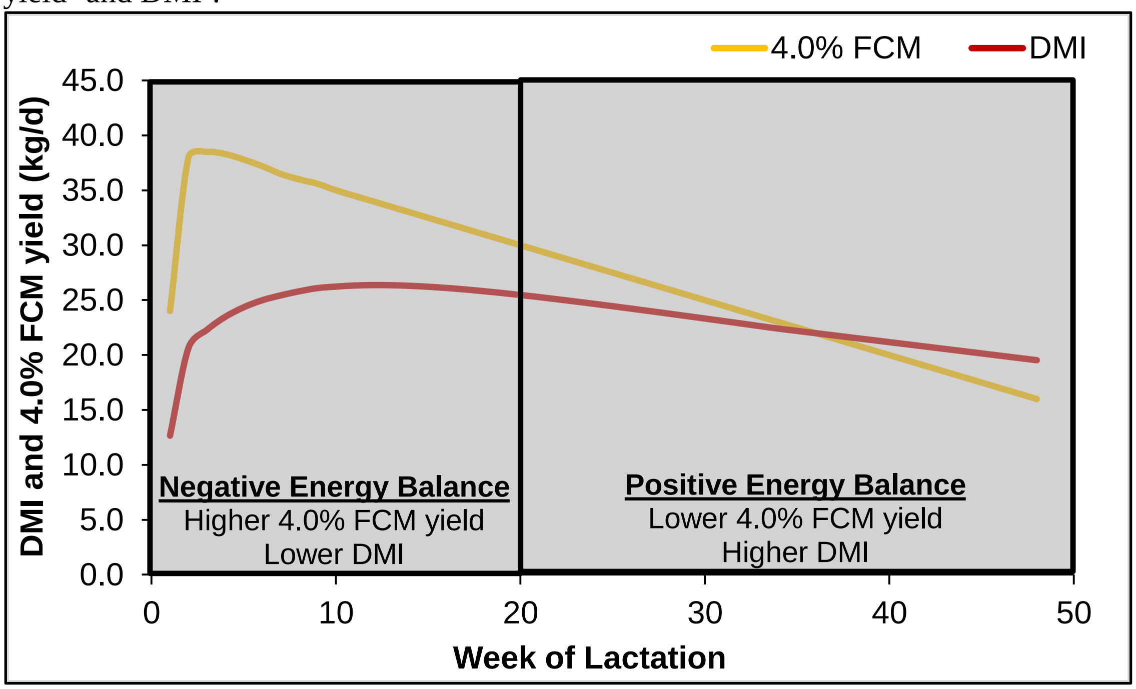
Dairy FE Ratio Denominator

Dry Matter Intake

Although there may be some discrepancies regarding the numerator of the dairy FE equation, the universal denominator of the dairy FE equation is DMI (Erdman, 2011). DMI was selected as the denominator of the FE equation because, in lactating cows, DMI represents the food "cost" of producing any given quantity and composition of milk.

In all animals, feed is digested into utilizable nutrients which are partitioned to various body tissues depending on the animal's physiological status (Bauman and Currie, 1980). First and foremost, the body utilizes nutrients for maintenance functions such as turning-over body tissue and replenishing body stores (Field and Taylor, 2012). If additional nutrients are supplied in the diet, the animal can utilize these nutrients for functions such as growth, pregnancy (fetal development), and/or lactation (Field and Taylor, 2012). For first-lactation cows, dietary energy is first allocated to fulfill maintenance requirements; however, remaining dietary energy is partitioned to both milk production and growth, because these animals have only reached approximately 85% of mature body weight (Field and Taylor, 2012; NRC, 2001). For second-lactation and beyond cows, dietary energy is used to fulfill maintenance requirements, additional growth requirements (second and third parity cows exhibit minimal growth) as well as the energy demands of lactation (Field and Taylor, 2012). In both cases, it is extremely difficult for early-lactation cows to consume enough energy from the diet; therefore, these cows are considered to be in a negative energy balance (**NEB**) in which they utilize their body reserves to support lactation (Field and Taylor, 2012; NRC, 2001). After early lactation, milk yield slowly decreases until the dry-off period while DMI remains fairly constant (NRC, 2001). During this period, cows are considered to be in a positive energy balance (**PEB**) in which dietary energy is apportioned to replenish body stores as well to support the subsequent pregnancy (Field and Taylor, 2012). The transition between NEB and PEB throughout lactation is depicted below in Figure 2.5 (NRC, 2001).

Figure 2.5. Transition between NEB and PEB throughout lactation based on 4.0% FCM yield[1] and DMI[2].

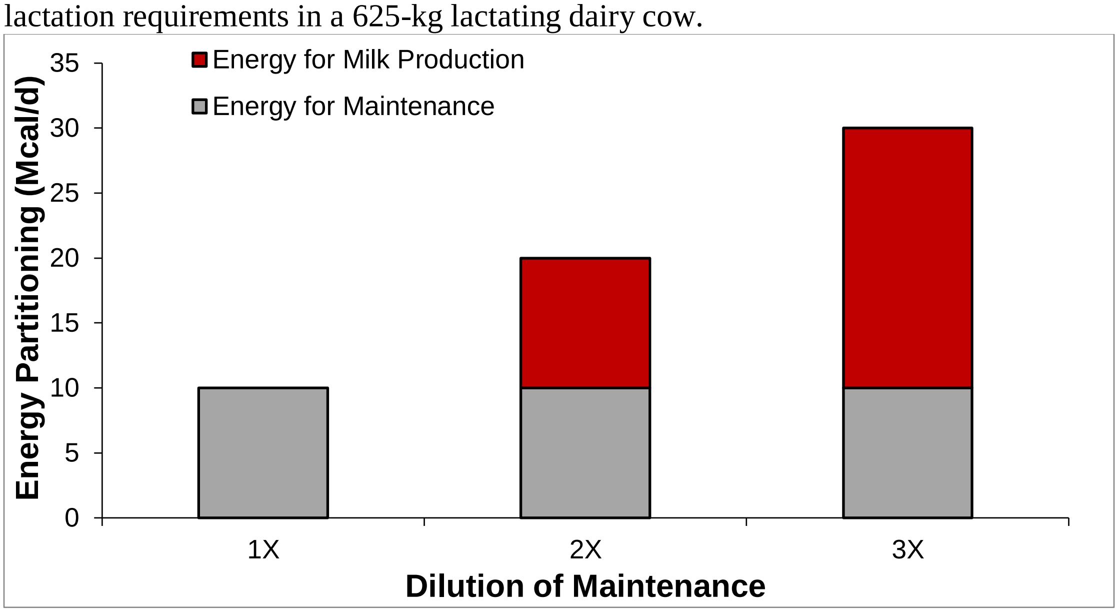


[1]Data for 4.0% FCM was adapted from the NRC (2001).

[2]DMI was generated based on the following equation: DMI (kg/d) = ((0.372 x 4.0% FCM + 0.0968 x $BW^{0.75}$)*($1-^{(-0.192 \text{ x } (\text{Week of Lactation} + 3.67))}$) (NRC, 2001).

Although dietary nutrients are needed to support several different functions in dairy cows, dairy FE is dependent on the allocation of nutrients between maintenance and lactation energy demands (VandeHaar and St-Pierre, 2006). Regardless of feed intake, the maintenance requirement of a dairy cow remains constant; however, as the cow consumes more feed, more energy is allocated to milk production (VandeHaar and St-Pierre, 2006). Essentially, as a cow consumes more feed, a smaller portion of the feed energy is partitioned to maintenance requirements and a larger portion of the feed energy is partitioned to milk production, as shown in Figure 2.6 (VandeHaar and St-Pierre, 2006). This dilution of maintenance effect is important for improving dairy FE because a small increase in DMI (denominator) can cause a significant increase in 3.5% FCM (numerator) which, in combination, results in improved dairy FE.

Figure 2.6. Dilution of maintenance effect on energy partitioned to maintenance and lactation requirements in a 625-kg lactating dairy cow.



[1]Data and figure were adapted from VandeHaar and St-Pierre (2006).

Although increasing feed intake increases milk production, there is a caveat to the dilution of maintenance effect; increased feed intake results in decreased feed digestibility (VandeHaar and St-Pierre, 2006). Based on a review by VandeHaar and St-Pierre (2006), decreases in digestibility can be predicted using Equation 10.

$$\text{Digestibility Decrease} = 4.0\% \times (\text{Multiple of Maintenance} - 1)^{0.80} \tag{10}$$

Based on this equation, diet digestibility decreases by 4.0, 7.0, and 9.6% for 2X, 3X, and 4X maintenance (X), respectively (VandeHaar and St-Pierre, 2006). For example, if the energy digestibility of a diet at maintenance feeding is 67.0%, the energy digestibility of the same diet fed at 4X maintenance would be 57.4%. Essentially, as more feed is being ingested and passed through the digestive tract of the cow, fewer nutrients are being broken down and absorbed by the animal (VandeHaar and St-Pierre, 2006). Although Equation 10 provides an adequate prediction of decreases in diet digestibility, the rate of decline in diet digestibility is dependent on the source(s) of dietary energy (VandeHaar and St-Pierre, 2006). Thus, decreases in diet digestibility may fluctuate based on ration ingredients such as grains and forages (Erdman, 2011; VandeHaar and St-Pierre, 2006). Based on the concept of dilution of maintenance and the resulting decrease in diet digestibility, it is more important to optimize, not maximize, DMI in order to ultimately improve dairy FE (Heinrichs et al., 2016; VandeHaar and St-Pierre, 2006).

In conclusion, DMI serves as the denominator of the dairy FE ratio because it represents the metabolic "cost" of milk production. By calculating the ratio of ECM yield (kg/d) to DMI (kg/d), one can estimate the efficiency at which feed nutrients are being utilized for milk production purposes (Connor, 2015).

Calculating and Utilizing Dairy FE Values

After calculating ECM yield (kg/d) and DMI (kg/d) from on-farm production measurements, FE ratios can be calculated by dividing ECM by DMI (Ishler, 2014). High FE values are desired as the ratio is calculated as "products" over "cost" unlike aforementioned FCRs which are calculated as "cost" over "products" so a smaller value is preferred (FAO, 2010; Ishler, 2014). Typically, dairy FE ranges between 1.30 and 1.80 for lactating cows on U.S. dairies (Ishler, 2014).

Once a producer has calculated FE for an individual cow or cohort of cows, the producer can assess the efficiency of the cow or group of cows by comparing the calculated FE to established FE benchmarks shown in Table 2.6 (Hutjens, 2007; Ishler, 2014). Calculated FE values that fall below the established benchmarks for a specified group of cows may encourage a producer to elicit changes in the herd and/or operation in regard to management, dietary, or genetic strategies in order to improve their herd efficiency (Heinrichs et al., 2016).

Table 2.6. Dairy FE benchmarks established by Hutjens (2007).

Group	Days in Milk	FE[1]
One group, all cows	150 to 225	1.4 to 1.6
Primiparous cows[2]	< 90	1.5 to 1.7
Primiparous cows[2]	> 200	1.2 to 1.4
Multiparous cows[3]	< 90	1.6 to 1.8
Multiparous cows[3]	> 200	1.3 to 1.5
Fresh cows	< 21	1.3 to 1.6
Problem herds/groups	150 to 200	< 1.3

[1]FE = ECM (kg/d) divided by DMI (kg/d).

[2]Primiparous cows are cows in their first lactation.
[3]Multiparous cows are cows in their second or beyond lactation.

Additionally, producers may use calculated FE values to select for more efficient cows within their herd to improve the FE of the current and/or future herds (Heinrichs et al., 2016). Ultimately, FE can serve as a diagnostic tool for dairy producers to use to select efficient cows within a herd and/or implement management, dietary, or genetic strategies to improve the FE and, subsequently, profitability of their dairy operation (Heinrichs et al., 2016).

Advantages of the FE Ratio

There are several advantages to utilizing the FE ratio to estimate FE of dairy cows. First, FE ratios are the simplest method used to estimate FE and, because of their simplicity, they are widely utilized in the U.S. dairy industry (Arndt et al., 2015; Connor, 2015; Heinrichs et al., 2016). In the poultry, swine, and beef industries, FCRs are the predominant method used to calculate FE and the most similar approach utilized by the dairy industry is the FE ratio (Linn, 2006). Although the dairy FE ratio is more complex as it includes 3 product parameters (milk yield, milk fat yield, and milk protein yield) compared to the one product parameter (body weight) utilized by FCR calculations, it is still relatively simple compared to other methods that have been established to estimate dairy FE such as IOFC or RFI (Connor, 2015; Linn, 2006). Unlike IOFC calculations that require current feed and milk costs and RFI that requires predictive modeling, FE ratios are simply calculated as the ratio of standardized milk to feed intake based on the following on-farm parameters: milk yield, milk fat percentage, milk protein percentage, and DMI (Beck and Ishler, 2016; Connor, 2015; Erdman, 2011). Once all of the necessary on-farm data have been collected and recorded, FE ratios are easy to calculate and interpret which attributes to their popularity among dairy producers (Ishler, 2014; Linn, 2006).

The second major advantage of dairy FE ratios is that general benchmarks have been established such that a dairy producer can utilize FE ratios on their operation as both diagnostic and selection tools (Heinrichs et al., 2016). As shown in Table 2.6, dairy FE benchmarks have been created so that producers can compare individual cows or cohorts of cows within their herd to suggested FE guidelines based on the age and stage of lactation of the cow(s) (Heinrichs et al., 2016). These benchmarks allow producers to utilize FE ratios as a diagnostic tool to select efficient cows within a herd and/or implement management, dietary, or genetic strategies to improve the FE of their herd (Heinrichs et al., 2016). For example, a dairy producer calculates the FE of the fresh cows within their herd and finds that the average FE of fresh cows is 1.17 (Ishler, 2014). When compared to the benchmarks established by Heinrichs et al. (2016) in Table 2.6, fresh cows should have a FE that ranges between 1.30 and 1.60. Therefore, the producer may view the discrepancy in actual versus suggested FE values as an opportunity to improve the management and/or dietary strategies of the fresh cows to improve FE of their operation (Heinrichs et al., 2016; Ishler, 2014). Thus, established benchmarks allow for FE values to serve as on-farm diagnostic tools of individual cow or cohort efficiency.

The third major advantage of using milk-to-feed ratios to estimate dairy FE is that the traits involved in the calculation have shown to be highly heritable for genetic selection (Cassell, 2009; Holstein Association USA, 2018). Heritability is calculated as the ratio of genotypic variance (σ^2_G) to phenotypic variance (σ^2_P) and its values range between 0.0 and 1.0 (Kempthorne, 1957). In terms of genetic selection, heritability (h^2) is a measure of how likely a trait is to be passed down from parents to offspring or a measure of the strength of relationship between genotype and phenotype (Cassell, 2009). Traits with high heritability are often used in genetic selection to influence various production characteristics and traits with a heritability above 0.10 are considered to be advantageous in the genetic selection of dairy cows (Cassell, 2009; Holstein Association USA, 2018). The 4 traits used to calculate FE are milk yield, milk fat yield, milk protein yield, and DMI and these traits have heritabilities of 0.30, 0.58, 0.51, and 0.30, respectively, in Holstein dairy cows (Cassell, 2009; Holstein Association USA, 2018). Compared to other traits that are currently being used for genetic selection, such as body condition score ($h^2 = 0.25$) or days to first breeding ($h^2 = 0.04$), the traits associated with FE are considerable highly heritable which means that FE may be used to make genetic progress in improved efficiency (Cassell, 2009; Holstein Association USA, 2018). Although the underlying traits associated with FE are heritable, it is important to note that the sum of the traits (FE) may not be heritable. Thus, the heritability of FE itself must be further explored. Ultimately, using the milk-to-feed ratio approach to estimate efficiency allows producers to improve the FE of their current herd by selecting for high efficiency cows while simultaneously improving the FE of their future herd because the traits associated with high FE are considerable moderately-to-highly ($h^2 = 0.30 – 0.58$) heritable (Cassell, 2009; Holstein Association USA, 2018).

The last major advantage of using the ratio approach to estimate dairy FE is that increasing the FE ratio results in more milk produced per unit of feed which has been shown to reduce feed costs and improve profitability for dairy producers (Casper, 2008; Heinrichs et al., 2016; Tuck, 2010). For example, Erdman et al. (2011) increased the Dietary Cation-Anion Difference (**DCAD**; mEq/g; Na + K – Cl) concentration of lactating cow diets from

251 to 336 mEq/kg using potassium carbonate which resulted in a FE (3.5% FCM per DMI) increase from 1.78 to 2.00 and translated into a $0.38 reduction of feed cost per cow per day. As shown in Table 2.7, this $0.38 per cow per day reduction in feed cost would translate into an annual savings of $13,870 for a 100-cow herd over the span of 365 d (Erdman et al., 2011).

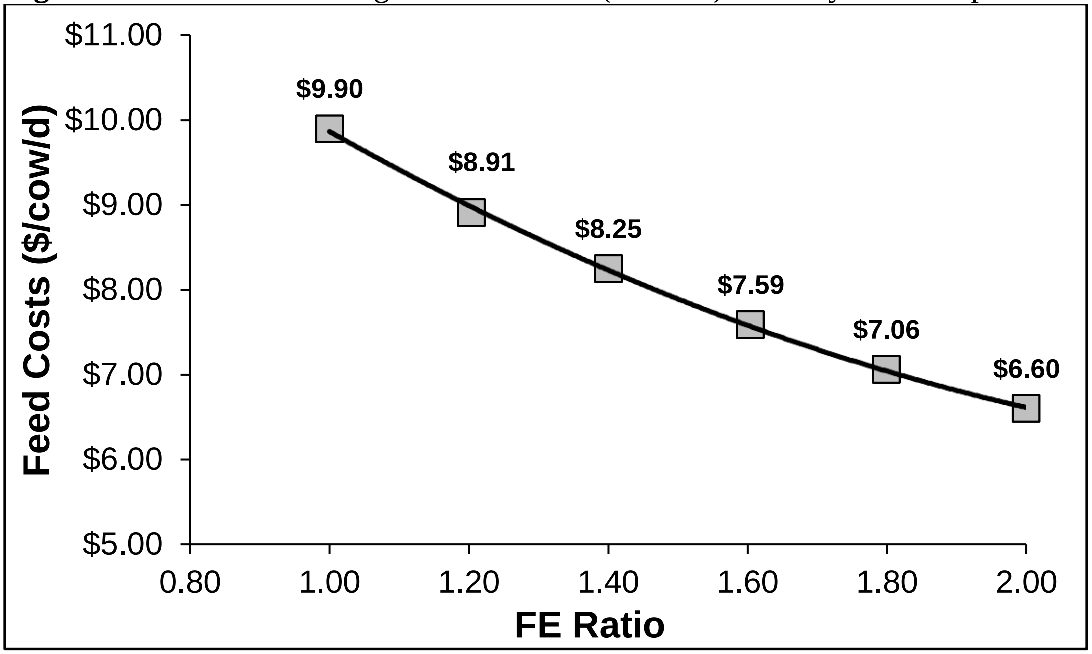
Table 2.7. Reduction in feed costs due to increased dairy FE[1] in a 100-cow dairy herd.

	Dietary Treatment	
Item	**CS**	**CS-DCAD**
DCAD[2]	251	336
DMI, kg	22.7	20.7
3.5% FCM, kg	40.4	41.4
FE[3]	1.78	2.00
Feed Cost, $/1000kg[4]	$265.60	$272.69
Feed Cost, $/kg[5]	$0.27	$0.27
Feed Cost, $/cow/d[6]	$6.03	$5.64
Feed Cost Reduction, $/cow/d[7]	.	-$0.38
Annual Feed Cost Reduction[8]	.	-$13,870.00

[1]Data adapted from Erdman et al. (2011).
[2]DCAD (mEq/kg) = Na + K – Cl.
[3]FE = 3.5% FCM (kg) per unit of DMI (kg).
[4]Feed costs ($/1000kg) are based on the May 2011 Northeast cost for the selected dietary components used in this specific study (Erdman et al., 2011).
[5]Feed costs ($/kg) = feed costs ($/1000kg) divided by 1000.
[6]Feed costs ($/cow/d) = feed costs (kg) multiplied by average DMI (kg/d).
[7]Feed cost reduction ($/cow/d) = feed costs of CS-DCAD diet minus the feed costs of CS diet.
[8]Annual feed cost reduction was predicted assuming a reduction of $0.38/cow/d for a 100-cow herd over 365 d.

Using simulated data, theoretical changes in feed costs have been regressed on dairy FE values as shown in Figure 2.7 (Casper, 2008).

Figure 2.7. Theoretical changes[1] in feed costs ($/cow/d)[2] as dairy FE[3] is improved.



[1]Concept derived from Casper (2008).
[2]Feed costs = $0.33/kg feed DM.
[3]Simulated milk yield and DMI ranged from 30 to 40 kg/d and 20 to 30 kg/d, respectively.

Based on Figure 2.7, improving FE from 1.80 to 2.00 results in a $0.46/cow/day reduction in feed costs which is consistent with the aforementioned Erdman et al. (2011) projection of a $0.38/cow/day reduction in feed costs as FE increased from 1.78 to 2.00 (Casper, 2008). Increasing the FE ratio values does result in economic improvements for dairy producers; thus, estimating FE using this approach can be advantageous for dairy producers.

In summary, utilizing the FE ratio to estimate dairy FE is advantageous for dairy producers as the ratio method 1) is simple to calculate and easy to interpret, 2) has established benchmarks so FE can be used as a diagnostic or selection tool for an individual cow or a cohort of cows, 3) contains 4 production parameters that are moderately-to-highly heritable which promotes future genetic improvements in FE, and 4) has a practical application that can be utilized to help dairy producers improve their profitability.

Disadvantages of the FE Ratio

First and foremost, the biggest disadvantage of using the FE ratio is that DMI is used as the denominator of the equation and DMI on individual cows is rarely measured on most dairy operations (Connor et al., 2013; Faverdin et al., 2017; Halachmi et al., 2004). Unfortunately, most dairy operations do not have the time, labor, or financial resources to measure DMI on an individual cow basis (Halachmi et al., 2004). Therefore, the vast majority of dairy cows are fed in large groups such that the DMI of an individual cow within a group is unknown (Halachmi et al., 2004).

One way to overcome the lack of individual cow DMI measurements on farm is to estimate DMI using mathematical models (Halachmi et al., 2004). Several published DMI equations exist and these equations were developed using one of 2 common approaches: 1) DMI can be estimated by accounting for energy sinks such as milk and milk component production because cows consume feed to meet their energy requirements or 2) DMI can be estimated using regression analysis with dietary and production parameters included in the estimation model (Krizsan et al., 2014; NRC, 2001). Regardless of the method used to estimate DMI, the following parameters are commonly used in DMI equations: milk yield (kg/d), milk fat yield (g/d), milk protein yield (g/d), BW or metabolic $BW^{0.75}$ (kg), and week of lactation (**WOL**) (Krizsan et al., 2014; NRC, 2001l Roseler et al., 1997). For example, the DMI estimation equation published by the NRC (2001) is one of the most commonly utilized and studied energy-based DMI equations and it is shown below in Equation 11.

$$\text{DMI (kg/d)} = (0.372 \text{ x FCM} + 0.0968 \text{ x } BW^{0.75}) \text{ x } (1 - e^{(-0.192x(WOL+3.67))}) \quad (11)$$

Although the 2001 NRC DMI equation is widely utilized, recent studies have evaluated the 2001 NRC DMI equation and have found that it displays mean prediction biases (Huhtanen et al., 2011; Jensen et al., 2013; Zom et al., 2012). For example, Krizsan et al. (2014) found that the 2001 NRC DMI equation over-predicted DMI when compared to actual DMI. Conversely, Rim et al. (2008) evaluated the 2001 NRC DMI equation using data from commercial farms as well as controlled experiments and found that, in both cases, the equation under-estimated DMI. In heifers, Hoffman et al. (2008) found that the 2001 NRC DMI equation over-predicted DMI in heavy Holstein and crossbred heifers, but under-predicted DMI in light Holstein and crossbred heifers. In summary, the 2001 NRC DMI equation has been shown to result in biased estimations of DMI; thus, new DMI estimation equations have since been developed in hopes of correcting for prediction biases.

In addition to estimating DMI based on energy outputs, it may be possible to estimate DMI based on nitrogen (**N**) outputs (Van Horn et al., 1994). To understand the method in which DMI could be estimated from N outputs, a brief review of ruminant protein metabolism is provided.

There are 3 types of protein (or N) sources in the diets of dairy cows: rumen undegradable protein (RUP), rumen degradable protein (RDP), and non-protein nitrogen (NPN) sources (Van Soest, 1982). As its name suggest, RUP bypasses the rumen and is subsequently broken down to amino acids (**AA**) and peptides which are absorbed in the small intestine and can be utilized for multiple metabolic processes, including milk production (Kohn, 2007). If not required for milk synthesis, excess AA and peptides are shuttled to the liver where they are deaminated and the amine groups (N**:**) are converted to urea which becomes part of the animal's blood urea pool (Kohn, 2007). In the rumen, RDP is degraded to AA which are used for ammonia ($NH4^+$) production by rumen bacteria (Kohn, 2007). The ammonia diffuses across the rumen wall and is rapidly converted to urea in the liver as ammonia is toxic to the cow (Kohn, 2007). This urea is added to the cow's blood urea pool. Lastly, NPN can be converted to ammonia by bacteria within the rumen as well and this ammonia is also diffused across the rumen wall and converted to urea by the liver (Kohn, 2007). Thus, NPN also increase the cow's blood urea pool.

Once in the blood urea pool, urea can be recycled via saliva to the rumen or it can diffuse across the rumen wall directly into the rumen to be utilized by bacteria to synthesize rumen microbial protein (**MCP**) which is degraded and absorbed in the small intestine of the cow (Kohn, 2007; Van Soest, 1982). In addition, urea can be filtered out of the blood via the kidneys and it is excreted via urine production (Kohn, 2007). Lastly, urea can be secreted into milk which occurs because urea is constantly diffusing in and out of the mammary gland (Kohn, 2007). The concentration of urea in the blood dictates the amount of urea that diffuses into the mammary gland as well as the amount of urea that is excreted via urine (Kohn, 2007). Thus, MUN is proportional to blood urea nitrogen (**BUN**) and MUN has been shown to be linearly related to total urinary N excretion (Broderick and Clayton, 1997; Ciszuk and Gebregziabher, 1994; Jonker et al., 1998; Kohn, 2007; Roseler et al., 1993).

Several studies have shown that there is a robust relationship between N intake and N output in lactating dairy cows (Jonker et al., 1998; NRC, 2001, Van Horn et al., 1994). Research has shown that dairy cows secrete approximately 25-35 percent of their consumed N into milk while the majority of the remaining N is excreted in urine and feces (NRC, 2001). Van Horn et al. (1994) explored the relationships between consumed N and milk, urinary, and fecal N outputs and reported that urinary and fecal N excretions can be estimated by subtracting the milk N concentration from the concentration of N consumed (NRC, 2001). Similarly, Jonker et al. (1998) found that the N intake can be estimated using milk and urinary N (UN) concentrations in which milk N was calculated as a function of milk yield (kg/d) and the crude protein percentage of milk and UN was estimated as a function of MUN. Based on these concepts, it is possible that DMI can be estimated on an individual cow basis if the amount of excreted N in the milk, urine, and feces are known or estimated (Jonker et al., 1998). The estimation of DMI on an individual cow basis based on N excretion is the focus of the experiment featured in Chapter 3 of this dissertation.

The second major disadvantage of using the FE ratio to estimate FE of individual dairy cows is that several factors have been shown to affect the FE. The effect of the following factors on FE will be discussed in detail below: stage of lactation (days in milk; **DIM**), parity, individual cow variation in production parameters (milk yield and milk composition), BW, calving month, dietary energy concentration (net energy of lactation **NE_L**; Mcal/kg), dietary neutral detergent fiber (**NDF**) concentration (%), and dietary crude protein (**CP**) concentration (%).

Stage of Lactation

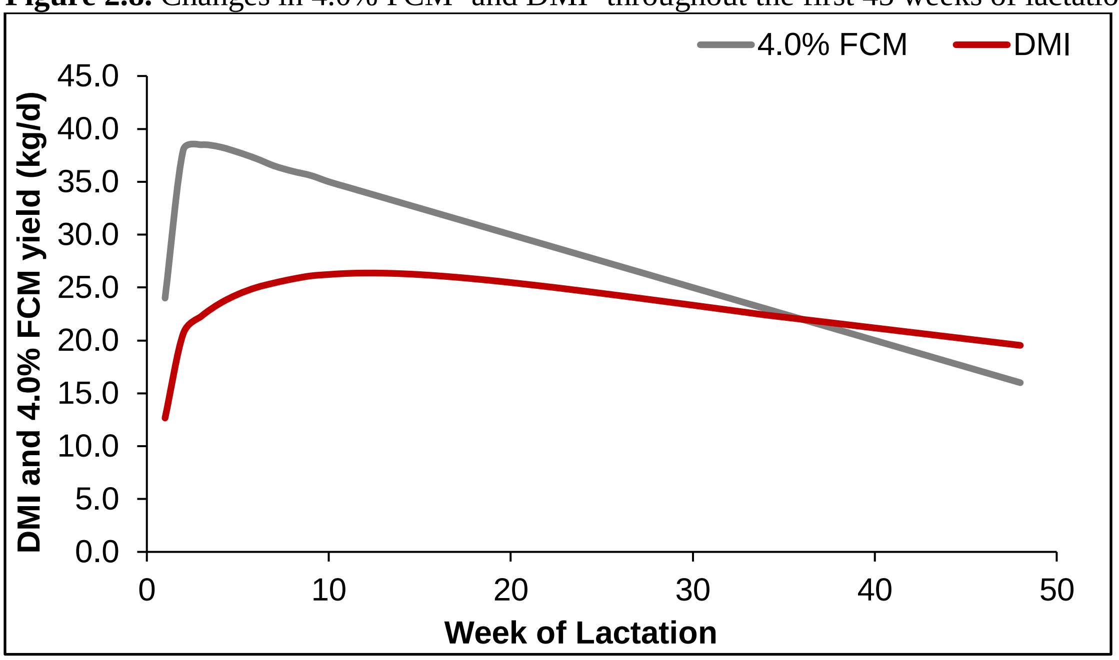
One of the most important biological factors that has been shown to affect dairy FE is the stage of lactation of the dairy cow (St-Pierre, 2012). In 1967, Wood defined the curve of an average lactation by developing an equation (Eq. 12) that predicted average daily milk production based on week of lactation (n) and 3 coefficients (A, b, and c).

$$\text{Average Daily Milk Yield Prediction } (y_n) = An^{b}e^{cn} \tag{12}$$

Using the Wood equation as a basis, Kellogg et al. (1977) developed gamma curve equations to investigate the effect of parity on lactation curve coefficients and found that parity significantly affects 2 lactation curve coefficients: A and c. Several other articles have been published that suggest that lactation curves are affected by parity (Jingar et al., 2014; Nasri et al., 2008; Wood, 1970, Wood, 1980). Based on these results, lactation curves tend to be discussed in relation to the parity of the dairy cow.

In regard to dairy FE, stage of lactation has a huge impact on FE values because daily milk yield and DMI change inversely over time, as shown below in Figure 2.8.

Figure 2.8. Changes in 4.0% FCM[1] and DMI[2] throughout the first 45 weeks of lactation.



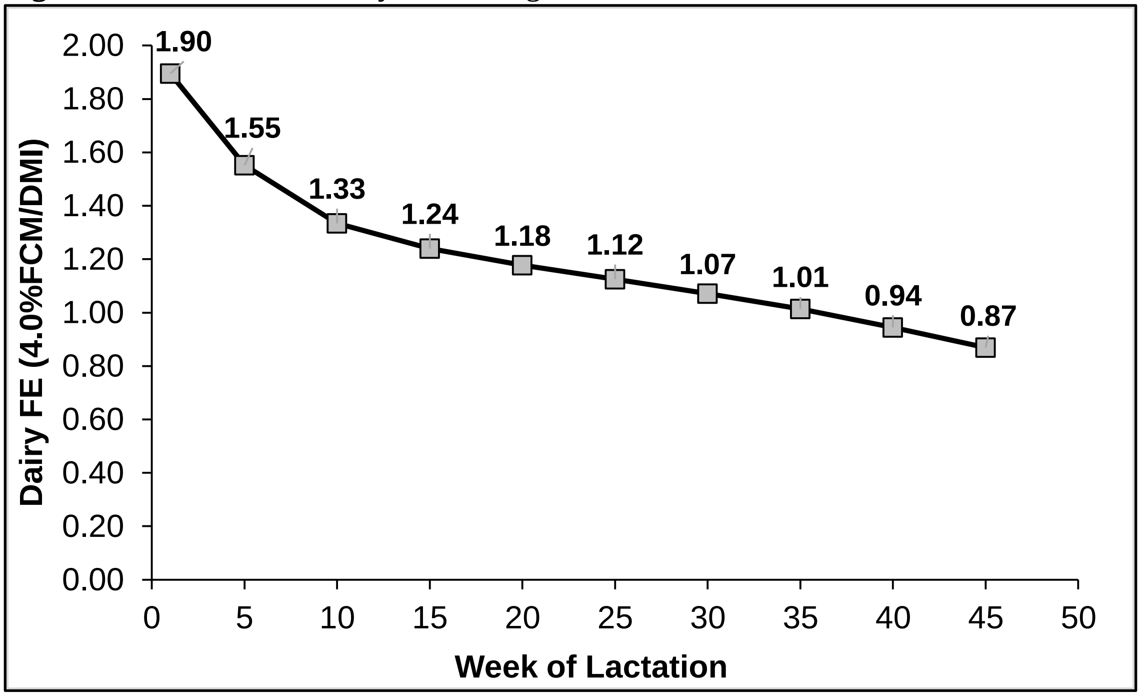
[1]Data for 4.0% FCM was adapted from the NRC (2001).

[2]DMI was generated based on the following equation: DMI (kg/d) = ((0.372 x 4.0% FCM + 0.0968 x $BW^{0.75}$)*($1-^{(-0.192 \text{ x (Week of Lactation} + 3.67))}$) (NRC, 2001).

As presented in Figure 2.8, milk production peaks at approximately 4 weeks into the lactation. During this time, cows are mobilizing their body tissue stores in order to meet the high energy demands of milk production as the nutrient intake from feed is insufficient (Erdman, 2011; NRC, 2001). After peak milk yield, milk production steadily decreases for the remaining portion of the lactation. At approximately 9 to 12 weeks, DMI peaks and it will eventually reach a plateau. During this time, cows are consuming more DMI than previously in order to replenish the body stores that were lost during peak milk production and to continue to support the remaining lactation energy demands (NRC, 2001).

Because milk production peaks at the beginning of lactation and then steadily decreases while DMI peaks later in lactation, dairy FE is highest at the beginning of lactation and decreases over time (St-Pierre, 2012). Figure 2.9 shows the expected FE at various stages of lactation. Dairy FE is 1.90, 1.12, and 0.87 at weeks 1, 25, and 45 of lactation, respectively. Based on this figure, it is clearly evident that the stage of lactation has a great impact on dairy FE.

Figure 2.9. Decreases in dairy FE[1] throughout the first 45 weeks of lactation.

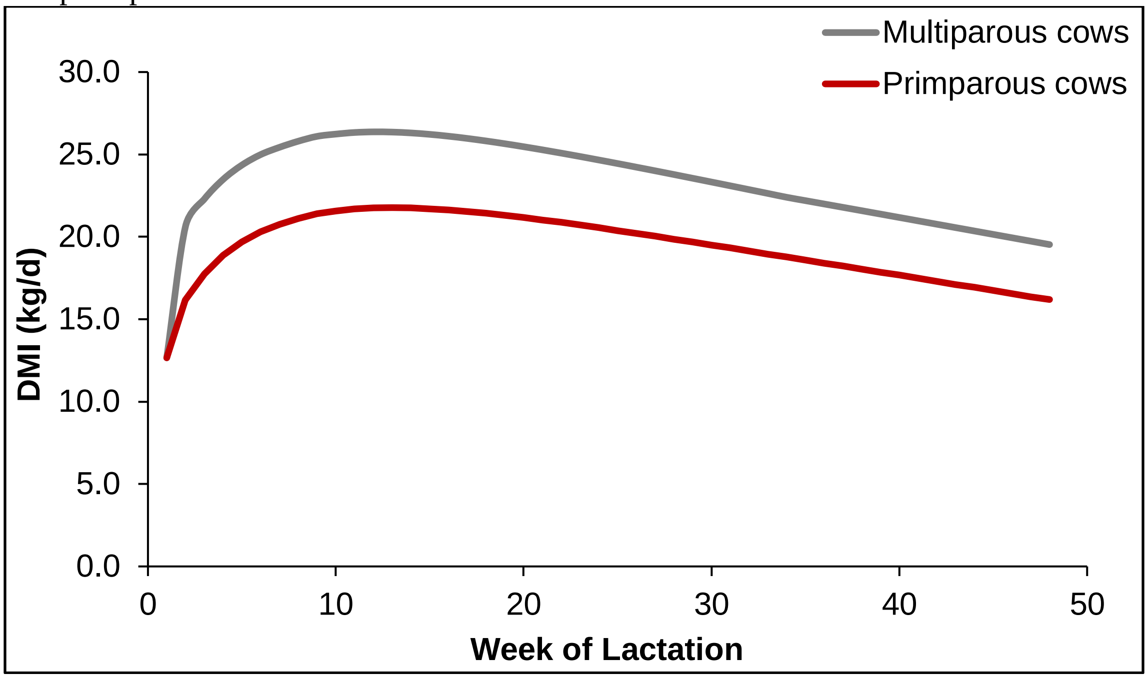


[1]Dairy FE values were calculated using the 4.0% FCM and DMI data (NRC, 2001) presented in Figure 2.8. Here, dairy FE = 4.0% FCM (kg/d)/DMI (kg/d).

Parity

Similar to stage of lactation, the parity of the dairy cow also affects milk production (Field and Taylor, 2012). Lee and Kim (2006) found that there was a significant linear increase in the average 305-day milk production from first (8,431 kg) to fourth-lactation (10,812 kg) Holstein cows. The differences in milk production between primiparous (first lactation) and multiparous (second lactation or beyond) dairy cows can be attributed to the fact that primiparous cows are still growing; thus, a portion of their energy intake is partitioned to growth instead of milk production (NRC, 2001). In addition to sanctioning nutrients towards growth, primiparous cows are also typically smaller in stature and BW compared to multiparous cows, which results in reduced DMI as shown in Figure 2.10 (NRC, 2001).

Figure 2.10. Changes in DMI throughout the first 45 weeks of lactation for multiparous[1] and primiparous[2] cows.



[1]DMI was generated based on the equation: DMI (kg/d) = ((0.372 x 4.0% FCM + 0.0968 x $BW^{0.75}$)*(1-$^{(-0.192 \text{ x } (\text{Week of Lactation} + 3.67))}$) (NRC, 2001) where BW = 650 kg.
[2]DMI was generated based on the equation: DMI (kg/d) = ((0.372 x 4.0% FCM + 0.0968 x $BW^{0.75}$)*(1-$^{(-0.192 \text{ x } (\text{Week of Lactation} + 3.67))}$) (NRC, 2001) where BW = 500 kg.

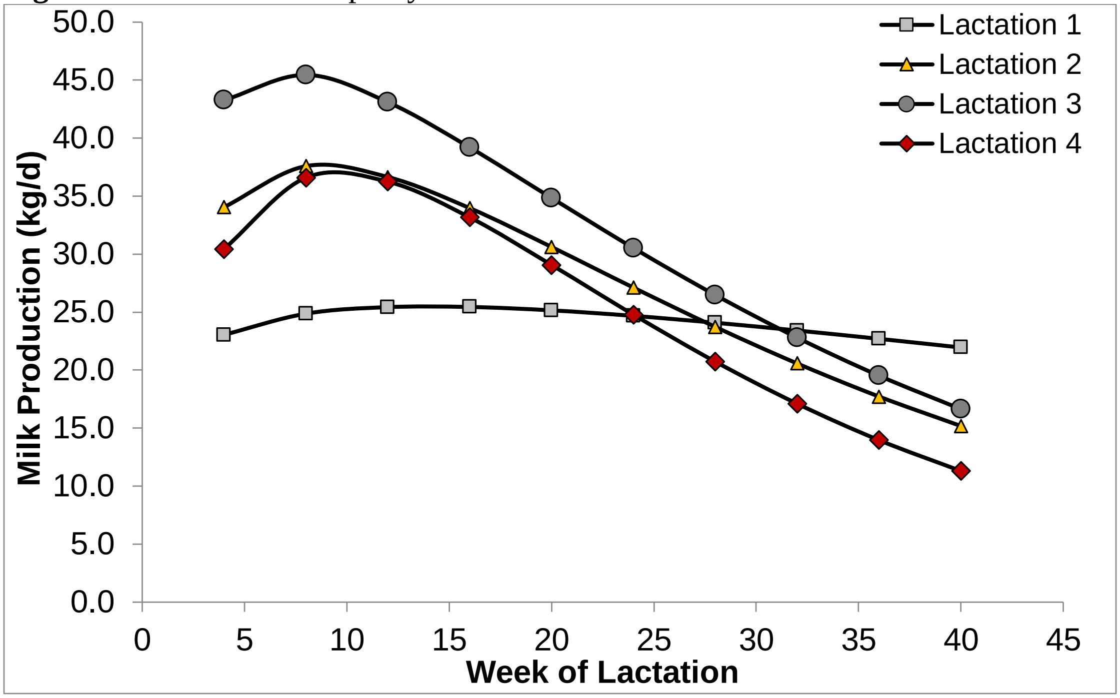
Because primiparous cows are using a portion of their nutrient intakes towards growth in combination with the fact that they also consume less feed than multiparous cows, it is no surprise that FE is higher in multiparous cows compared to primiparous cows (Heinrichs et al., 2016; Maulfair et al., 2011; NRC, 2001).

Parity also affects the lactation curves of the dairy cows. As shown in Figure 2.12, the lactation curve of a primiparous cow is lower and relatively flatter compared to that of a multiparous cow (Kellogg et al., 1977). Differences in the lactation curves between primiparous and multiparous cows are, in large part, due to differences in the synthetic capability of the mammary gland (Miller et al., 2006). Essentially, milk production is a function of the quantity and activity of secretory cells in the mammary gland (Capuco et al., 2001; Miller et al., 2006). Miller et al. (2006) reported that primiparous cows had a significantly lower density of mammary secretory cells as compared to multiparous cows which may explain the lowered milk production observed in primiparous dairy cows. In addition, Miller et al. (2006) reported that the expression of specific genes related to mammary metabolic activity was decreased in early lactation in primiparous cows compared to multiparous cows, suggesting that the mammary gland of primiparous cows is less metabolically active compared to multiparous cows. This observation further explains the decreased milk production in early lactation in primiparous cows as compared to multiparous cows (Miller et al., 2006).

Throughout lactation, mammary secretory cells undergo apoptosis, or programmed cell death, which reduces the number of viable secretory cells and consequently reduces milk production (Capuco et al., 2001; Miller et al., 2006). Miller et al. (2006) reported that the secretory cells in primiparous cows had a greater capacity for cell renewal throughout lactation as compared to multiparous, resulting in a higher persistency in milk production during mid-to-late lactation for primiparous cows as compared to multiparous cows. This result may explain the relatively flat shape of the primiparous lactation curve compared to the more dynamic lactation curve exhibited by multiparous cows.

Thus, differences in mammary secretory cell number and activity may contribute to the differences in lactation curves observed between primiparous and multiparous dairy cows (Capuco et al., 2001; Miller et al., 2006).

Figure 2.11. The effect of parity on the lactation curves of a Holstein cow[1].



[1]Milk yield was calculated based on estimated lactation curves reported by Kellogg et al. (1977) for cow 627 ($\bar{y} = A*t^{b}e^{-ct}$; A, b, and c = coefficients; t = week of lactation).

In this example, milk yield peaked at 24.9 kg/d during the first lactation and 45.5 kg/d for the third lactation (Kellogg et al., 1977). Using parity-adjusted DMI prediction equations from the 2001 NRC, the predicted DMIs during the second month of lactation were 16.46 kg/d and 19.48 kg/d for the first and third lactation cows, respectively. Using these DMI predictions, the ratio of milk yield to DMI would be 1.51 and 2.33 for the first and third lactation cows, respectively. Although the ratio of unadjusted milk yield to DMI is not the most appropriate calculation for dairy FE, this data does suggest that parity affects overall milk production, which is a large component of the ECM equation. Therefore, it is highly likely that parity would affect dairy FE as both DMI and milk production are affected along the shape of the lactation curve.

Individual Cow Variation in Production Parameters

Due to advancements in the genetic selection of dairy cows, individual cows are now able to produce more than 20,000 kg of milk per lactation and the amount of milk produced per cow per lactation has more than doubled during the last 45 years (Oltenacu and Broom, 2010). Although these genetic improvements have impacted the U.S. Holstein population as a whole, large individual cow variation still exists for several production parameters such as milk production and DMI due to genetics (Connor, 2015; Shonka and Spurlock, 2013; St-Pierre and Weiss, 2009). In particular, Moyes et al. (2009) reported that the large individual cow variation can be attributed to factors such as breed/genetics, parity, stage of lactation, and season.

In regard to milk production, 2 distinct production classes exist: high producing and low producing dairy cows. Compared to low producing dairy cows, high producing dairy cows have higher FE because high producing cows have a larger dilution of feed used for maintenance. Therefore, high producing cows allocate a smaller proportion of their energy intake towards meeting maintenance requirements, but partition a larger proportion of energy intake to support milk production (Linn, 2006). In a dairy FE review, Erdman (2011) estimated the effects of production level on dairy FE using average 305-day production records from the 2009 herd summary from the USDA, ARS, Animal Improvement Program Laboratory (**AIPL**; Beltsville, MD). Erdman (2011) used the herd summary data to group herds into 30th, 50th, 70th, and 90th percentile groups, in which a higher percentile indicates higher milk production. Erdman (2011) estimated that the 150-day dairy FE values were 1.43, 1.49, 1.55, and 1.63 for 30th, 50th, 70th and 90th percentile dairy herds, respectively. Thus, milk production levels affect ECM yield and dairy FE such that dairy FE is highest in high producing dairy herds (Erdman, 2011).

In addition to overall milk yield, differences among cows in regard to milk composition also affect dairy FE. The numerator of the dairy FE is ECM which is calculated based on milk yield, milk fat yield, and milk protein yield (Connor, 2015; Heinrichs et al., 2016; Ishler, 2014). Thus, differences in milk fat and protein concentrations impact overall dairy FE. For example, Rico et al. (2014) conducted an experiment which examined the effects of fat source (palmitic or stearic acid supplement) on production parameters in lactating dairy cows and found that increasing dietary fat with palmitic acid significantly increased milk fat percentage (P = 0.01) from 3.55 to 3.66% which subsequently increased ECM yield from 46.1 to 47.7 kg/d (P < 0.01). Because overall milk yield (kg/d) was not significantly affected by dietary treatment (P = 0.22), it can be concluded that the significant increase in milk fat percentage resulted in a significant increase in ECM (Rico et al., 2014). In addition, DMI was not affected by dietary treatment (P = 0.39). Because the numerator of the FE equation (ECM) was significantly affected by fat supplementation while the denominator remained similar between treatments, it can be hypothesized that dairy FE would have been affected by treatment in this study (Rico et al., 2014). Thus, it is evident that altering milk fat concentration and/or milk fat yield can significantly affect dairy FE (Rico et al., 2014).

Similarly, altering milk protein concentrations may also affect FE as milk protein concentration is used to calculate ECM, the numerator of the FE ratio (Heinrichs et al., 2016; Ishler, 2014). One strategy that has recently been implemented to increase overall milk protein concentration and yield is to formulate dietary rations to meet metabolizable protein requirements instead of overall CP concentrations (Overton, 2016). In addition, supplementation of rumen-protected forms of methionine and lysine has also been shown to increased milk protein yield (Overton, 2016; Vyas and Erdman, 2009). For example, Vyas and Erdman (2009) reported that increasing methionine intake from 30 to 70 g/d and lysine intake from 85 to 200 g/d resulted in an approximately 400 g/d and 550 g/d increases in milk protein yield, respectively. Regardless of the approach utilized, increases in milk protein concentration and yield theoretically result in increased ECM yields, which may significantly improve overall dairy FE (Heinrichs et al., 2016; Ishler, 2014).

Although it may not directly affect dairy FE, dairy producers can utilize milk urea nitrogen (**MUN**; mg/dL) concentrations in milk to estimate the overall status of protein metabolism in dairy cows (Isler, 2016). As previously discussed, MUN is a measure of the urea (CH_4N_2O) concentration in milk and MUN is strongly correlated to the concentration of urea present in the cow's blood (Ishler, 2016). For example, Roseler et al. (1997) observed an increase in MUN concentrations when dietary protein concentration was fed in excess and a decreased in MUN concentrations when dietary protein concentration was limited. Thus, MUN can be utilized by dairy producers as a tool to estimate protein status in the animal (Ishler, 2016; Kohn, 2007; Roseler et al., 1997). Because MUN is indicative of a cow's protein status and her protein status affects her FE, it is possible that MUN concentrations could be highly correlated to FE. Therefore, MUN's relationship to FE will be further explored in this dissertation.

Body Weight (BW)

In addition to individual cow variation and production parameters, BW has also been shown to affect dairy FE. Research has shown that although larger cows may be able to produce more milk compared to smaller cows, FE tends to be inversely related to BW (Linn, 2006; VandeHaar et al., 2016). For example, Linn (2006) compared the FE (3.5% FCM per unit of DMI) of smaller cows to larger cows producing the same milk quantity (34.0 kg/d with 3.6% milk fat) and found that FE decreased from 1.52 to 1.30 as BW increased from 544 to 816 kg. The decreased FE is a result of increased DMI as the larger cows require more nutrients to meet maintenance requirements compared to smaller cows (Linn, 2006; NRC, 2001). Thus, increasing BW increases maintenance requirements which can result in increased feed intake and reduced FE, depending on the cow's milk production (Heinrichs et al., 2016).

Calving Month

The month in which a cow calves and enters milk production can have a significant impact on dairy FE due to environmental effects that influence production parameters (Torshizi, 2016). For example, heat stress has been shown to lower milk production by 25 to 40% due to a reduction in DMI (Tao et al., 2018; Torshizi, 2016). Thus, cows that calve during hot, summer months tend to have decreased milk yield and milk composition which can result in decreased FE. Torshizi (2016) examined the effects of season of calving on genetic and phenotypic production parameters and found that cows that calved in autumn and winter had higher levels of milk production compared to cows that calved in spring and summer ($P < 0.05$). Although FE was not reported, increased milk production increases ECM in the dairy FE ratio which may result in increased FE. Utrera et al. (2013) evaluated the effects of calving season on milk yield and efficiency (calculated as a function of standardized milk yield and BW) and found that cows that calved during months with cooler temperatures had significantly higher milk production (kg/d) and efficiency compared to cows that calved during warmer months. Based on these experiments, it is apparent that calving month affects production parameters and, subsequently, dairy FE.

In addition to heat stress, photoperiod has also been shown to affect FE in dairy cows (Dahl et al., 2000). Photoperiod is the period of time per day in which a cow is exposed to natural or artificial light (Dahl et al., 2000). Natural photoperiod length varies depending on the time of year such that short-day photoperiods occur between September and April and long-day photoperiods occur between May and August in the U.S. (Dahl et al., 2000). Research has shown that cows exposed to long-day photoperiods (16 to 18 h of light/d) produced an average of 2.5 kg/cow/d more milk compared to cows exposed to short-day photoperiods (≤ 12 h of light/d) due to changes in endocrine mechanisms that regulate lactation (Dahl et al., 2000). Because calving month dictates the month in which a cow enters lactation, it is possible that cows that calve during months associated with long-day photoperiods may have increased milk production, and subsequently FE, compared to cows that calve during months associated with short-day photoperiods (Dahl et al., 2000). Thus, calving month may indirectly affect dairy FE as it is confounded with photoperiod effects on lactation.

Dietary Energy Concentration (NEL)

Typically, a cow's energy requirements for both maintenance and lactation are expressed together in net energy of lactation (**NE_L**) units (NRC, 2001). One approach to increasing the energy density of a lactating dairy cow ration is to increase the dietary fat concentration. Because dietary fat (9.3 kcal/g) is more energy dense than either carbohydrates (4.1 kcal/g) or protein (5.65 kcal/g), increasing the fat concentration of the diet of a lactating cow (by reducing the carbohydrate concentration) would provide more energy per unit of feed that can be utilized for milk production purposes (NE_L) (Onetti et al., 2001; Weiss and Pinos-Rodriguez, 2009; Zou et al., 2007).

Studies have shown that increasing the fat concentration in the diet has resulted in increased milk fat percentage, milk production, and dairy FE (Karimian et al., 2015; Lock et al., 2013; Rabiee et al., 2012). Using a meta-analysis and meta-regression approach, Rabiee et al. (2012) reported that milk and milk fat production increased while DMI decreased in response to dietary fat supplementation; therefore, fat supplementation increased dietary energy density which resulted in improved FE. Similarly, Lock et al. (2013) reported that fat supplementation resulted in decreased DMI (kg/d), increased fat percentage and yield (kg/d), and subsequently, increased dairy FE. Lastly, Karimian et al. (2015) reported that the addition of a dietary fat supplement resulted in decreased DMI which translated into increased milk efficiency (4.0% FCM/DMI). Based on this evidence, it can be concluded that increasing dietary energy concentration through dietary fat supplementation results in improved dairy FE through changes in milk yield, milk composition, and/or DMI.

Dietary Neutral Detergent Fiber (NDF) Concentration

For lactating dairy cows, the most common measure of dietary fiber is neutral detergent fiber (**NDF**) and NDF is comprised of 3 major structural components of plant cell walls: hemicellulose, cellulose, and lignin (NRC, 2001). Adequate dietary NDF (at least 25-33% of diet DM) is required for maintaining proper rumen health and buffering capacity of the cow (NRC, 2001; Oba and Allen, 2009). Although cows require sufficient dietary NDF to maintain proper rumen function and maximize production, excess dietary NDF has been shown to decrease DMI because of the physical limitation of rumen fill (Kendall et al., 2009; Oba and Allen, 2009). As a result of decreased DMI, milk production and milk fat yield also decrease as dietary NDF concentration increases (Kendall et al., 2009; Oba and Allen, 2009; Ruiz et al., 1995). For example, Kendall et al. (2009) reported that increasing NDF concentration from 28 to 32% resulted in significant reductions in DMI, milk production, and milk fat percentage ($P < 0.05$). Similarly, Zhao et al. (2015) reported that increasing the dietary NDF-to-starch ratio from 0.86 to 2.34 resulted in a 4.90, 4.90, and 4.00 kg/d decrease in DMI, milk yield, and ECM, respectively ($P < 0.01$). Because DMI and ECM are components of the FE equation, it can be postulated that dietary NDF concentration may significantly affect dairy FE.

In addition to dietary NDF concentration, NDF digestibility also affects production parameters such that increased NDF digestibility increases DMI, milk yield, and milk composition (Kendall et al., 2009; Oba and Allen, 2009). For example, Oba and Allen (2009) reported that a one-unit increase in NDF digestibility resulted in a 0.17 kg and 0.25 kg increase in DMI and 4.0% FCM yield, respectively. Similarly, Kendall et al. (2009) reported that increasing NDF digestibility resulted in increased milk and 4.0% FCM production. Therefore, it can be concluded that increasing NDF digestibility can increase DMI, milk yield, and milk component yields which may increase dairy FE. In summary, both dietary NDF concentration and digestibility affect dairy FE.

Dietary Protein Concentration

Similar to NDF concentration, dietary crude protein (**CP**) concentration has also been shown to affect production responses associated with dairy FE such as DMI, milk yield, and milk fat yield (Cabrita et al., 2011; Kalscheur et al., 1999; Reid et al., 2015). Dietary CP (in the form of RDP) is required by dairy cows to meet the protein needs of the rumen microbes for microbial fermentation and to meet the animal's metabolizable protein (**MP**) requirement (Kalscheur et al., 1999). Because dietary CP concentrations affect the rumen environment, production responses can be altered by manipulating the CP concentration of the diet. For example, Kalscheur et al. (1999) reported that increasing CP from 13.4 (mean CP% of low CP diets) to 15.3% resulted in increased milk yield, milk fat yield, and 4.0% FCM yield (Experiment 1). Although not reported in the study, dairy FE increased from 1.66 (average of low CP diets) to 1.79 when CP% increased from 13.4 to 15.3% (Kalscheur et al., 1999). In addition, a regression analysis that was conducted to investigate the effects of CP concentration on milk production revealed that increasing CP concentration resulted in a quadratic milk yield (kg/d) response with maximum milk yield occurring when CP was 23% of diet DM (NRC, 2001). Similarly, Broderick et al. (2015) reported that increasing dietary CP from 15 to 17% resulted in increased milk fat yield and a trend for increased milk yield. Lastly, Cabrita et al. (2011) reported that increasing dietary CP from 14 to 16% resulted in increased DMI (kg/d) and milk production (kg/d).

In summary, CP concentration has been shown to affect both milk yield and milk fat yield and changes in these 2 parameters would most likely result in changes in ECM yield and, subsequently, dairy FE.

Relative Importance of Factors Affecting FE Ratios

Although substantial research has been conducted to explore the effects of various biological, dietary, and production parameters on dairy FE, the relative importance of each factor has yet to be determined. Using a discriminant analysis approach, the relative importance of several factors that affect dairy FE will be determined and these experiments are the focus of Chapter 4 of this dissertation.

Residual Feed Intake

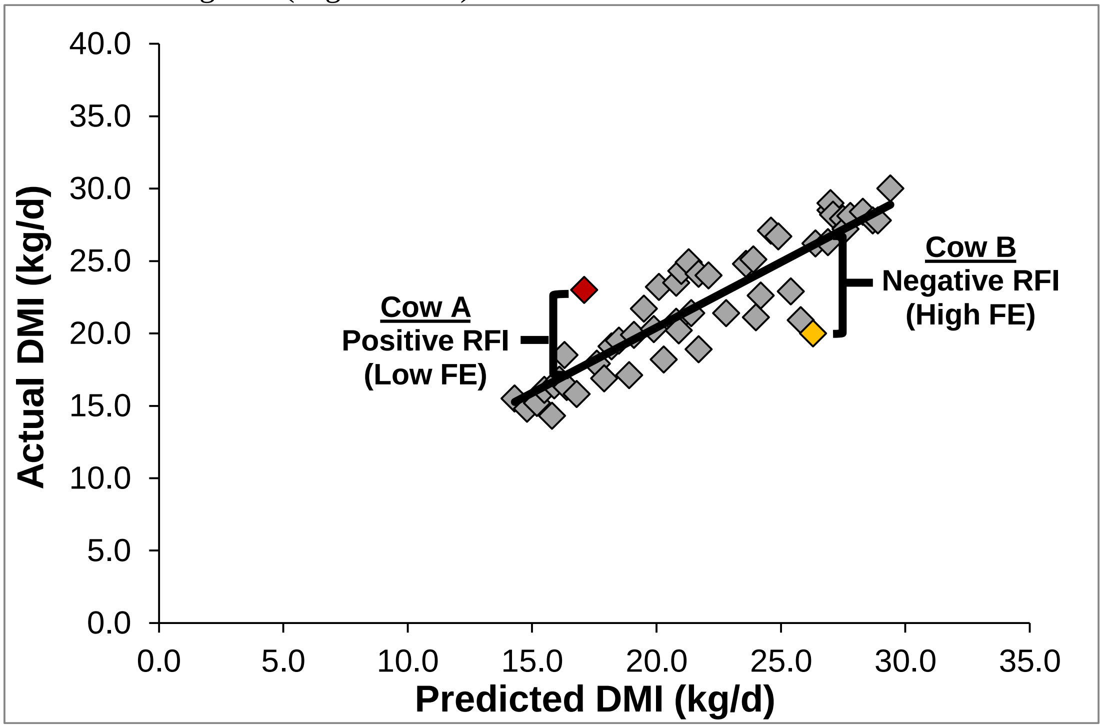
Since its development by Koch et al. in 1963, residual feed intake (**RFI**) has been used in the poultry, swine, beef, dairy industries as a tool to estimate FE (Berry and Crowley, 2013; Potts et al., 2015). In order to calculate RFI on individual cows, measurements of individual cow DMI, BW, milk production, and milk composition must be recorded during an established period of time (Macdonald et al., 2014). Once all measurements have been made, the collected data are used to estimate DMI using a least squares multiple regression analysis in which RFI is calculated as the difference between actual and predicted DMI (Connor, 2015; Macdonald et al., 2014; Potts et al., 2015). Several different RFI DMI prediction equations have been published for dairy cows; however, there are 3 major components that are found in most dairy DMI prediction equations: 1) a term that accounts for BW, 2) a term that accounts for changes in BW, and 3) a term that accounts for milk energy, as shown in Equation 13 (Berry and Crowley, 2013; Connor, 2015; Potts et al., 2015).

$$\text{Predicted DMI} = \beta 0 + \beta_1 BW^{0.75} + \beta_2 \Delta BW + \beta_3 \text{MilkEnergy} + \varepsilon \qquad (13)$$

Additional terms that account for variance such as age, parity, stage of lactation, body condition score (**BCS**), diet, or feeding frequency may also be added to the dairy RFI equation (Berry and Crowley, 2013; Connor, 2015; Potts et al., 2015).

Once the DMI prediction equation has been established and DMI has been predicted for each cow, the RFI of an individual cow is estimated by subtracting the cow's predicted DMI from its observed DMI (Berry and Crowley, 2013; Connor, 2015; Potts et al., 2015). A negative RFI indicates that a cow consumes less DMI than expected to produce a given quantity of milk while a positive RFI indicates that a cow consumes more DMI than expected to produce a given quantity of milk (Potts et al., 2015). In terms of feed efficiency, a cow that has a negative RFI is considered to be more efficient relative to a cow with a positive RFI as shown in Figure 2.12 (Potts et al., 2015).

Figure 2.12. Example of an RFI analysis in which Cow A has a low FE (positive RFI) and Cow B has a high FE (negative RFI).



Based on the data provided in this example, Cow A consumes more food than predicted in order to produce a specific amount of milk; therefore, this cow has low feed efficiency. Conversely, Cow B consumes less feed than predicted in order to produce the same amount of milk; therefore, this cow has high feed efficiency (Connor, 2015; Potts et al., 2015).

Advantages of RFI

There are several advantages to using RFI to estimate FE of lactating dairy cows. First and foremost, RFI is a calculated value that is indicative of an individual cow's metabolic efficiency after variation associated with biological and production factors have been removed (Connor, 2015; Crews, 2005; Tempelman et al., 2014). As previously mentioned, DMI prediction equations are used to determine the predicted feed intake of individual cows using model parameters such as age, parity, and stage of lactation (Berry and Crowley, 2013; Connor, 2015; Potts et al., 2015). By accounting for variables that affect DMI in the prediction model, RFI should only theoretically reflect differences in metabolic efficiency of nutrient utilization, not differences due to biological, production, and/or dietary factors (VandeHaar, 2016). Factors such as stage of lactation and parity can greatly affect other measures of dairy FE such as FE ratios, as discussed above; however, accounting for these factors in the DMI prediction model ensures that their effects are removed from the RFI value itself (Kellogg et al., 1977; NRC, 2001; VandeHaar, 2016). For example, RFI values can be compared between 2 cows even if the cows are not in the same stage of lactation or parity or consuming the same dietary ration, assuming that those factors are included in the proposed DMI prediction equation (Connor et al., 2013; Potts et al., 2015; VandeHaar, 2016). Thus, RFI values for an individual cow are robust across various factors and RFI reflects metabolic efficiency after various biological, production, and/or dietary factors have been accounted for in the DMI prediction model (Crews, 2005; Connor et al., 2013).

The second major advantage of the RFI approach is that RFI has been shown to be repeatable for individual cows within and across lactations (Connor, 2015; Tempelman et al., 2014). In order to determine within-lactation repeatability for RFI, several RFI values for each individual cow are calculated at various points throughout lactation and the within-cow correlation between RFI values are calculated (Connor, 2015; Tempelman et al., 2014). Connor et al. (2013) measured RFI in 292 individual Holstein dairy cows for the first ~90 days in lactation and found that within-cow repeatability of RFI throughout lactation had a correlation coefficient (r) of 0.47 ($R^2 = 0.22$). In addition, Tempelman et al. (2015) conducted a similar study using 4,893 individual cows from 3 research stations (UK, US, and the Netherlands) and found that the average repeatability for RFI across the 3 research stations was r = 0.77 (R^2 = 0.59) . Although the correlation between RFI values within-lactation is only low-to-moderate, the results of these studies suggest that RFI may be measured at any stage of lactation and still reflect a fairly accurate prediction of metabolic efficiency for an individual cow (Connor, 2015; Tempelman et al., 2014; VandeHaar et al., 2016).

Similarly, RFI has also been shown to be repeatable across lactations for an individual cow (Connor, 2015; Tempelman et al., 2014). In the same study discussed above, Connor et al. (2013) compared the 90-day average of all weekly RFI values per cow across various parities and found the correlation to be moderately high (r = 0.56). Tempelman et al. (2014) found the average repeatability of RFI within cow across lactations to be approximately 0.27 (R^2 = 0.07). Although a correlation of r = 0.27 may seem low, it is similar to repeatability correlation values for common production parameters such as milk yield, fat yield, and protein yield which are 0.34, 0.35, and 0.29, respectively (Roman et al., 2000). Therefore, RFI is repeatable across lactations for an individual cow and a rate similar to other production parameters. As a practical application, a dairy producer could theoretically measure the RFI of an individual cow during her first lactation and be able to predict her metabolic efficiency for subsequent lactations without requiring any additional measurements. Thus, selection for efficient cows is possible using the RFI approach.

The third major advantage of RFI is that RFI is relatively heritable compared to other production traits (Connor, 2015; Tempelman et al., 2014). Connor et al. (2013) and

Tempelman et al. (2014) each calculated the average heritability of RFI and found that $h^2 = 0.36$ and $h^2 = 0.17$, respectively. As discussed previously, heritability values above 0.10 are considered to be advantageous in the genetic selection of dairy cows (Cassell, 2009; Holstein Association USA, 2018). In fact, RFI heritability is moderately heritable compared to the following production traits which are currently being used for genetic selection: DMI ($h^2 = 0.30$), milk yield ($h^2 = 0.30$), age at first calving ($h^2 = 0.14$), lifetime net income (merit; $h^2 = 0.20$), body condition score ($h^2 = 0.25$), and days to first breeding ($h^2 = 0.04$) (Cassell, 2009; Holstein Association USA, 2018). Assuming there is sufficient variation in RFI between cows within the target population, RFI can be used as a trait to genetically select for metabolically efficient dairy cows to improve FE.

The last major advantage of utilizing RFI to estimate FE of dairy cows is that RFI can be assessed on heifers (Groen and Vos, 1995; Nieuwhof et al., 1992). Nieuwhof et al. (1992) measured FE (energy intake per unit of weight gain) in heifers from 44 to 60 weeks of age and then subsequently measured the heifers RFI values during the first 105 days of lactation and observed a strong, positive correlation between growing heifer and lactating cow feed intake and RFI values ($r = 0.58$). Other studies have also reported strong correlations between RFI values measure during growth in heifers and RFI values measured during subsequent lactations (Arthur et al., 2001; Davis et al., 2014; Durunna et al., 2012). Thus, these results suggest the FE of heifers is indicative of metabolic FE during subsequent lactations (Macdonald et al., 2014; Nieuwhof et al., 1992). Therefore, a dairy producer could measure the FE of a growing heifer and predict differences in RFI for future lactations. This concept could have an enormous impact on the dairy industry as other measures of FE, such as the FE ratio, cannot determine FE of a dairy cow until she enters lactation. A heifer must be housed, fed, and managed on a dairy farm with a substantial cost until she is bred, calves, and produces milk to determine efficiency. In the case of feed inefficient cows, it is extremely costly to house, feed, manage, and breed a cow only to discover that she is incredibly inefficient (USDA-ERS. 2018a). Thus, RFI measured in growing heifers could allow dairy producers to make informed management decisions for animal selection earlier which would save producers time, money, and labor and improve the overall profitability of their dairy operation.

Disadvantages of RFI

Although utilizing RFI values to evaluate dairy FE may be a useful tool for some dairy producers, there are several issues with this method. First and foremost, the biggest disadvantage of using RFI is the same issue as utilizing FE ratios to estimate FE; DMI of individual cows is rarely measured on farm (Connor et al., 2013; Faverdin et al., 2017; Halachmi et al., 2004). As previously discussed, measurements of DMI on individual animals would be incredibly costly and labor-intensive for a commercial dairy operation that is not is not equipped to feed cows individually (Halachmi et al., 2004). Therefore, a vast majority of dairy cows are fed in large groups such that the DMI of an individual cow within a group is unknown (Halachmi et al., 2004). Thus, the lack of DMI estimate is a major disadvantage to using RFI to estimate FE on an individual cow basis.

Secondly, a unified standard equation to predict DMI for the RFI calculation does not exist; therefore, RFI values may be dependent on the equation used for DMI prediction. As it was previously mentioned, there are several factors that can affect DMI such as stage of lactation, parity, BW, calving season, and diet composition (Berry and Crowley, 2013;

Connor et al., 2013; Lin et al., 2013; Macdonald et al., 2014). Because numerous factors can affect DMI, various prediction equations have been developed to predict DMI for RFI calculations and these equations vary regarding their inclusion of parameters in the prediction model (Connor et al., 2013; Connor, 2015; Potts et al., 2015l Vallimont et al., 2011). As shown above in Figure 2.12, RFI is a statistical residual which is calculated by subtracting the predicted DMI from the actual DMI (Berry and Crowley, 2013; Potts et al., 2015; VandeHaar et al., 2016). Due to the nature of residuals, RFI contains true variation in metabolic efficiency between cows due to epigenetics (genetics, environmental conditions, and their interactions) as well as random variation due to errors in DMI measurements and predictions (VandeHaar et al., 2016). Therefore, any modeling errors that arise during the prediction of DMI may inflate the measured RFI values as the variation due to these random errors falls into the residual term (VandeHaar, et al., 2016). Because different DMI equations account for different amounts of variation associated with DMI, it is possible that RFI values are dependent on the equation used to predict DMI. This hypothesis is explored and discussed in Chapter 6 of this dissertation.

On a similar note, the third disadvantage of using RFI to estimate FE is that RFI inherently contains error and statistical bias. In regard to error, residuals fundamentally contain random variation (noise) associated with the regression analysis so RFI values are intrinsically flawed. As for statistical biases, RFI values are calculated for individual animals based on the predicted DMI line of best fit for a cohort of dairy cows; therefore, RFI values also assume that all cows within the cohort in the analysis share the same DMI prediction slope which is highly unlikely. Thus, inherent error exists when a statistical residual is used as an indicator of metabolic efficiency.

The last critical issue associated with using RFI to predict dairy FE is that the calculations are not very practical for dairy producers (Connor, 2015; VandeHaar et al., 2016). First, the DMI predication equations needed for the RFI calculation require producers to have access to RFI literature so that producers can identify and select a proper DMI prediction equation based on the parameters collected on their dairy operation (Connor, 2015). Secondly, as compared to IOFC and FE ratios, RFI values are much more labor intensive to calculate because the RFI calculations require dairy producers to perform statistical modeling in order to obtain predicted DMI and a regression analysis to calculate RFI (Connor, 2015). Lastly, RFI values are not intuitive; negative RFIs indicate better FE than positive RFIs which can be confusing to interpret and discuss. In summary, RFI is currently not a practical tool for dairy producers to make management or nutrition decisions on farm. However, it could be utilized by nutritionists and geneticists for the genetic selection of metabolically efficient dairy cows (Connor, 2015; VandeHaar et al., 2016).

Factors That Affect RFI Values

As discussed previously, several factors have been shown to affect dairy FE ratios and/or the production parameters associated with the ratio (Erdman, 2011; Field and Taylor, 2012; St-Pierre, 2012). However, factors that affect RFI values between lactating dairy cows are not well understood and more research is needed to characterize important factors that may influence RFI such as biological, management, dietary, and/or behavioral factors (Connor et al., 2013; Golden et al., 2008; Kkrumah et al., 2007). Using a discriminant analysis approach, the relative effect and importance of several factors such as stage of lactation, parity, production parameters, BW, and dietary composition on RFI will be determined and these experiments are the focus of Chapter 5 of this dissertation.

Summary

Feed costs in the dairy industry account for approximately 50% of total cost of producing milk (Beck and Ishler, 2016; USDA-ERS, 2018a; Hardie et al., 2017). Because feed costs are high, dairy producers are interested in approaches that can estimate, and ultimately improve FE, in lactating dairy cows. The 3 main methods used in the U.S. dairy industry to estimate FE are IOFC, FE ratios, and RFI (Connor, 2015). Because IOFC does not estimate FE on an individual cow basis, it will not be utilized in the research experiments in this dissertation.

The DMI estimates on an individual cow basis are a critical component to calculate FE ratios as well as RFI. Therefore, the experiment discussed in Chapter 3 of this dissertation aims to develop and validate novel equations that estimate DMI on an individual cow basis. In addition, several factors have been shown to affect dairy FE; however, the relative importance of these factors have yet to be determined (Erdman, 2011; Field and Taylor, 2012; St-Pierre, 2012). Thus, the first series of experiments discussed in Chapter 4 of this dissertation aims to explore the relative importance of several well-known factors that affect FE ratios. Similarly, RFI can also be affected by biological, production, and dietary factors; however, the relationships between RFI and these factors are not well understood (Connor et al., 2013). Therefore, the second series of experiments discussed in Chapter 5 of this dissertation aims to explore the effect and relative importance of several factors on RFI. In addition, it is possible that RFI values are dependent on the equation used to predict DMI as statistical residuals inherently contain errors associated with model prediction. Therefore, the objective of the experiment in Chapter 6 of this dissertation is to determine the relationship between RFI values calculated within-cows using different equations to predict DMI. Lastly, results from all experimental chapters will be summarized and reported in Chapter 7 of this dissertation.

Hypotheses and Objectives

Based on the previous review of the literature, 4 hypotheses were investigated:

1. An equation that estimates DMI on an individual cow basis can be developed and validated using the concept of N balance derived from common, on-farm parameters.

2. The relative importance of several biological, production, and dietary factors that affect dairy FE ratios can be determined and ranked

3. The relative importance of several biological, production, and dietary factors that affect RFI can be determined and ranked

4. Residual feed intake values may be dependent on the equation used to predict DMI as statistical residuals inherently contain errors associated with predictions and these errors may vary depending on the DMI equation model used

To test these hypotheses, 4 study objectives were completed:

1. Equations that estimate DMI on an individual cow basis were developed using the concept of N balance derived from common, on-farm parameters using linear and non-linear modeling techniques

2. The relative importance of several biological, production, and dietary factors that affect dairy FE ratios were determined and ranked using a series of discriminant analyses including stepwise, canonical, and basic discriminant analyses

3. The relative importance of several biological, production, and dietary factors that affect RFI were determined and ranked using a series of discriminant analyses including stepwise, canonical, and basic discriminant analyses

4. Dependency of RFI values on the equation used to predict DMI was assessed using correlation analyses between RFI values generated from four DMI equations

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CHAPTER 3: EXPERIMENT 1

Estimation of dry matter intake of individual cows fed in a group setting using common on-farm measurements[1]

[1]Iwaniuk, M. E., E. E. Connor, and R. A. Erdman. Estimation of dry matter intake of individual cows fed in a group setting using common on-farm measurements. In preparation for submission to the Journal of Dairy Science.

INTERPRETIVE SUMMARY

Estimation of dry matter intake of individual cows fed in a group setting using common on-farm measurements. *Iwaniuk et al., page 000.* Using a dataset provided by the USDA, eight novel DMI estimation equations were developed using the concept that N intake can be estimated if the N outputs in milk, urine, feces, and body tissue are known (Jonker et al., 1998). To be included in the dataset, each individual daily cow record required the following parameters: body weight (BW), milk yield, milk protein percentage, and milk urea N (MUN). If values were missing, the parameter was estimated using a generalized linear modeling technique. The DMI equations were developed using non-linear modeling techniques and evaluated using regression analyses. The 3 most successful equations were further evaluated for mean and linear biases and were validated using 4 independent validation datasets. The results of this study indicate that DMI can be successfully estimated in individual cows using common, on-farm measurements such as milk yield, milk protein percentage, MUN, BW, and dietary N (CP) concentration.

Estimation of dry matter intake of individual cows fed in a group setting using common on-farm measurements

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ABSTRACT

Due to high feed costs, increased efforts to reduce the environmental impact of animal production and elevated concerns for feeding the growing global human population, improving feed efficiency (**FE**) has become a major focus of research in the field of dairy science. In order to calculate FE on an individual cow, her dry matter intake (**DMI**) must be known. However, most cows are fed in a group setting such that DMI on an individual cow basis is not known. The objective of this study was to develop and validate several equations that estimate DMI of individual cows using dietary and production measurements that are already commonly recorded on dairy farms. The DMI estimation equations were developed using a dataset provided by the United States Department of Agriculture (Beltsville Agricultural Research Center, Beltsville, MD) containing 8,081 weekly production records averaged by cow for 524 cows in an experiment that spanned 342 wk. Eight preliminary equations were developed using an approach similar to the one developed by Jonker et al. (1998) in which DMI was estimated based on estimated N outputs in milk, tissue, urine, and feces using the following dietary and production parameters: dietary crude protein (**CP**, %), milk yield (kg/d), milk protein (%), body weight (**BW**; kg), and milk urea N (**MUN**; mg/dL). To ensure that each cow had a daily record containing all 5 key parameters prior to model development, missing values were replaced with estimated values generated by estimation equations for BW (kg), milk yield (kg/milking), milk protein (% per milking), and MUN (mg/dL per milking). The 3 best DMI estimation equations (Equations 2, 3, and 6) were selected based on the results of the regression analyses between actual versus estimated DMI (R^2, root-mean-square error (**RMSE**), and *P*-values). Further evaluation of these 3 selected equations showed that all of the equations lacked mean biases, but linear biases were detected though minimal as they were less than the SE of DMI measurements reported in the literature. The 3 selected equations then validated using 4 independent validation datasets: Validation Dataset 1 (Iwaniuk et al., 2015; n = 80; Exp. =2), Validation Dataset 2 (Iwaniuk et al., 2015; n = 80; Exp. = 3), Validation Dataset 3 (Weidman et al., 2018; unpublished; n = 52), and Validation Dataset 4 (Moallem et al., 2014; unpublished; n = 407). Overall, Equation 6 was selected as the best equation developed to estimate DMI. On average, Equation 6 had minimal mean bias, root of the mean square error (**RMSEP**; kg/d), and mean and linear biases as a percentage of mean square error of prediction (**MSEP**) as well as the robust accuracy and precision as indicated by R^2 and concordance correlation coefficient (**CCC**) values. In conclusion, we demonstrated that DMI could be successfully estimated on an individual cow basis using commonly measured on-farm parameters. Dairy producers can use the results of this study to estimate DMI, and subsequently FE, on an individual cow basis to select for the most efficient cows in current and future herds.

Key Words: Dry matter intake, estimation, nitrogen balance, feed efficiency

INTRODUCTION

Improving feed efficiency (**FE**) has become a paramount topic of research in the dairy industry within the last decade due to 3 primary factors. First, feed costs represent approximately 50% of the total operating costs associated with milk production (USDA-ERS, 2018); therefore, dairy producers are interested in improving FE to reduce feed costs and subsequently increase profitability. Second, improving FE has been shown to reduce the negative impacts of production on the environment. Capper et al. (2009) reported that greenhouse gas (**GHG**) emissions from the U.S. dairy industry have decreased by approximately 60% within the last 60 yr due to improvements in FE (VandeHaar et al., 2016). In addition, Capper et al. (2009) reported that manure production by dairy cows associated with producing an equivalent volume of milk decreased by 24% from 1944 to 2007 due to improvements in FE. Less manure production subsequently results in a reduction of environmental pollution due to decreased nutrient excretion of nitrogen (**N**) and phosphorus (**P**) which have been shown to have detrimental impacts on the environment through the process of eutrophication (Hristrov et al., 2006; Klop et al., 2013; Ledgard et al., 1999). Therefore, improving FE reduces the negative impact of production on the environment (Place and Mitloehner, 2010). Lastly, the third benefit of improved dairy FE is the reduction in the utilization of resources such as land, feed, water, animals, and fuel by dairy farms to produce milk (Capper et al., 2009; Neumeier and Mitloehner, 2013; Place and Mitloehner, 2010). Due to improvements in dairy FE, Capper et al. (2009) reported that U.S. dairy farms in 2007 were able to produce the same amount of milk (1 billion kg) as dairy farms in 1944 using 10% less land, 23% less feed, 35% less water, and 21% fewer animals. Therefore, improving dairy FE results in the reduction of resources used for milk production and these valuable resources can be allocated for other purposes required to support the rapidly growing world population (Place and Mitloehner, 2010).

There are several established methods to estimate the FE of dairy cattle such as milk-to-feed ratios, residual feed intake, and income over feed costs (Block, 2010; VandeHaar, 2016). Regardless of the method used to calculate FE, a measure of dry matter intake (**DMI**; kg/d) is required to estimate FE on an individual cow basis. Unfortunately, most dairy operations do not have the time, labor, or financial resources to measure DMI in individual cows (Halachmi et al., 2004). The vast majority of dairy cows are fed in large groups such that the DMI of a group of cows is known, but the DMI of individual cows within a group is unknown (Halachmi et al., 2004). One way to overcome the lack of individual cow DMI measurements on farm is to estimate DMI using mathematical models.

Published equations that estimate DMI do exist; however, many of these equations were developed based on "average cow" measurements so they do not estimate individual cow intakes (NRC, 2001). In addition, other equations developed to estimate DMI are based on developmental phases and these estimates are not suitable as daily estimations of DMI (NRC, 2001). Lastly, some of the published equations that estimate DMI have yet to be statistically evaluated and/or validated (NRC, 2001).

Previous research indicated that excess N has a detrimental impact on the environment via contamination in water and ammonia pollution in air (NRC, 2001). Due to its environmental implications, N utilization has become an important focus of research in the dairy industry (NRC, 2001). Research has shown that dairy cows secrete approximately 25-35% of their consumed N into milk while the majority of the remaining N is excreted in urine and feces (NRC, 2001). Van Horn et al. (1994) explored the relationships between consumed N (N intake; g/d) and milk, urinary, and fecal N outputs and reported that urinary and fecal N excretions can be estimated by subtracting the milk N concentration from the concentration of N consumed (NRC, 2001). Similarly, Jonker et al. (1998) found that the N intake could be estimated using milk and urinary N (**UN**) concentrations in which milk N was calculated as a function of milk yield (kg/d) and the crude protein percentage and UN was estimated as a function of milk urea nitrogen (**MUN**; mg/dL).

Because N intake is directly related to DMI and the crude protein (**CP**) percentage of the diet, we hypothesized that it may possible to estimate DMI on an individual using the following individual parameters for each cow: body weight (**BW**), milk yield (**MY**), milk protein percentage, MUN, and dietary N. Therefore, the 3 objectives of this study were as follows: 1) to develop several equations that estimate DMI on an individual cow basis, 2) to select the 3 best models that estimate DMI on an individual cow basis, and 3) to evaluate the 3 best DMI estimation models using independent datasets. The results of this study may be used to estimate DMI on an individual cow such that FE can be calculated and dairy producers can select for more efficient cows within their current and future herds.

MATERIALS AND METHODS

Initial Database

The data used for this modeling project were obtained from the laboratory of Dr. Erin Connor at the United States Department of Agriculture (**USDA;** Beltsville Agriculture Research Center, Beltsville, MD). All data collection involving animals was approved by the Northeast Area Animal Care and Use Committee. The initial dataset contained production records for 529 lactating Holstein cows, which resulted in 95,633 daily production observations. To remove natural variation associated with production parameters for cows in the transition period as well as late lactation, individual cow observations with days in milk (**DIM**) less than or equal to 21 DIM or greater than or equal to 150 DIM were removed from the dataset. Removing individual cow observations based on DIM resulted in an initial dataset that contained production records for 529 lactating Holstein cows and 70,672 daily production observations.

Estimation Equations and Outlier Removal for Key Production Variables

To be included in the final dataset, each daily individual cow production record was required to have the following parameters: DMI (kg/d), BW (kg/d), MY (kg/d), milk protein (%), MUN (mg/dL), and dietary CP concentration. If a daily production record was missing DMI, the entire record was removed from the dataset. If a daily production record was missing BW, MY, milk protein (%), or MUN, the parameters were individually estimated by cow and lactation number using PROC GLM (SAS 9.4; SAS Institute, Cary, NC) using the estimation equations shown in Table 3.1. Milk yield, milk protein percentage, and MUN were estimated per milking (2X/d; AM vs. PM). To determine the success of the estimation equation, measured parameter values were regressed on estimated parameter values using PROC REG (SAS 9.4, Cary, NC) and estimations were evaluated based on the following criteria: coefficient of determination (**R^2**), root-mean-square error (**RMSE**), and *P*-value as shown in Figures 3.1 – 3.4. During the regression analysis, outliers for each parameter were removed if the R-Studentized residual was less than -3 or greater than +3. If a parameter had a missing value (either inherently missing or removed during outlier detection), these values were replaced with the estimated values generated using PROC GLM (SAS 9.4). The use of estimated values in this dataset was particularly critical for the BW, milk protein (%), and MUN variables as the milk parameters were only measured weekly during alternate morning and evening milkings every week and BW was obtained every 2 wk immediately after the morning milking. DMI and MY (AM and PM) were measured and recorded daily. After the estimation equations and outliers were removed for the key production variables, the dataset contained 70,175 observations which contained a daily measured DMI and either measured or estimated values for BW, MY, milk protein (%), and MUN for each cow.

Data Management and Weekly Cow Means

New variables were created in the dataset to be used as terms within the DMI estimation equations. As shown in Equations 1 and 2 below, milk N was calculated from milk protein yield (g/d) and dietary N (**Diet N**) was calculated from the dietary crude protein (**CP**) percentage, respectively:

$$\text{Milk N (g/d)} = (\text{Milk protein yield (g/d)})/(6.25)/(0.93) \qquad (1)$$

$$\text{Diet N (g/d)} = (\text{Dietary CP (\%)})/(6.25)*10 \qquad (2)$$

In these equations, the conversion of milk protein to milk N is calculated using 6.25 as milk protein contains approximately 16% N (100/16 = 6.25) and the concentration of milk N derived from protein is 93%, as 7% of milk N is derived from NPN sources (NRC, 2001).

After the new variables were added to the dataset, individual cow production records were averaged by cow by week. Individual weekly cow means were removed from the dataset if an individual cow had less than 5 out of 7 daily production records for a week. This data removal reduces variation within the dataset and ensures that weekly means have relatively similar weighting. After weekly production means were calculated for each cow and data were removed, the dataset contained 10,089 weekly mean observations.

Final Outlier Removal for Key Variables Used in the DMI Equations

A final procedure was performed to remove any outliers that may have been generated from the estimations of BW, MY, milk protein (%), or MUN as well as any outliers that may have been present in the newly calculated variables (Milk N or Diet N). Outlier removal was performed using PROC UNIVARIATE (SAS 9.4) such that any values greater than the 99% quantile or less than the 1% quantile for each variable were removed. After these outliers were removed from the dataset, the dataset contained 8,971 weekly cow mean observations.

Grouping the Data into Two-week Intervals for Model Development

The last data management step that was conducted prior to model development and evaluation involved grouping the individual cow weekly means data into 2-wk intervals. If a 2-wk interval had fewer than 30 weekly cow means observations, then that 2-wk interval was removed from the dataset. This data removal was performed to reduce variation within the dataset and allow for more robust estimations of the individual DMI equation parameter coefficients. The final dataset contained 8,081 weekly cow mean observations split into 171 2-wk intervals. The descriptive statistics for the final dataset are presented in Table 3.2.

Model Development

Several studies have shown that there is a robust relationship between N intake and N output in lactating dairy cows (Jonker et al., 1998; NRC, 2001; Van Horn et al., 1994). Van Horn et al. (1994) demonstrated that urinary and fecal N excretions could be estimated by subtracting milk N concentration from the total amount of N consumed (NRC, 2001). Similarly, Jonker et al. (1998) reported that milk and UN can be used to calculate N intake when milk N was calculated as a function of milk yield (kg/d) and milk protein concentration (%) and UN was calculated as a function of MUN (mg/dL). The Jonker et al. (1998) equation is presented below:

$$\text{DMI (kg/d)} = ((\text{MilkN} + (\text{MUN} \times 12.54) + 97)/(0.83))/(\text{CP}/10) \qquad (3)$$

In this equation, DMI (kg/d) is equal to the sum of 3 N outputs (milk N, UN, and endogenous N) divided by the concentration of available dietary N (Jonker et al., 1998). Milk N (**MilkN**; g/d) was estimated using Equation 1, which was previously described. Jonker et al. (1998) reported that MUN and UN had a strong, linear relationship such that UN can be estimated from MUN using the slope of the regression line (12.54) as a coefficient. Thus, the second term in the numerator accounts for UN (g/d) excretion. In addition, Jonker et al. (1998) regressed N utilization (g/d) on N intake (**NI**; g/d) and determined endogenous N (97 g/d; intercept of the regression line) and the true digestibility of N (0.83; slope of the regression line) using a Lucas test. Therefore, these constants also appear in this equation to account for endogenous N outputs (97 g/d) and the digestibility of dietary N (0.83). Lastly, dietary N is a function of dietary crude protein (**CP**) such that the true digestibility of N (0.83) is multiplied by CP divided by 10 in the denominator of the equation to determine the concentration of available dietary N (g/d) (Jonker et al., 1998).

Therefore, N intake can be estimated if the following N outputs are known/estimated: milk, urinary, fecal, and endogenous N. Once N intake is known, it is possible to estimate DMI using N intake and the CP (%) of the dietary ration. Based on this concept of N balance, 8 novel equations were developed to estimate DMI on an individual cow basis using common on-farm measurements as shown below and in Table 3.3.

Equation 1: DMI (kg/d) = (MilkN + (A × BW × MUN))/(0.83 × DietN - 3) (4)

Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3) (5)

Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) (6)

Equation 4: DMI (kg/d) = (MilkN + D + (C × MUN))/(0.83 × DietN + 5 – E) (7)

Equation 5: DMI (kg/d) = (MilkN + (C × MUN) + (F × Milk × MUN)/(DietN - (I × DietN) – MFN) (8)

Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN) (9)

Equation 7: DMI (kg/d) = (MilkN + (C × MUN))/(I × DietN - MFN) (10)

Equation 8: DMI (kg/d) = (D × (MilkN + MUN))/(0.83 × DietN - 3) (11)

In addition, the original DMI equation developed by Jonker et al. (1998; Table 3.3: Eq. 9; described above) and a modified version of this equation (Table 3.3; Eq. 10; Kauffman and St-Pierre, 2001) were also analyzed in this study to compare the new DMI estimation equations to the original and modified Jonker equations from which the new equations were derived.

Equation 10: $\text{DMI (kg/d)} = ((\text{MilkN} + (\text{MUN} \times \text{BW} \times 0.026) + 97)/(0.83))/(\text{CP}/10)$ (12)

After the 8 DMI estimation equations were developed, parameter estimates were generated using PROC NLIN (SAS 9.4). For each equation, DMI estimations were generated by 2-wk intervals of weekly mean cow observations. The coefficient estimates and their respective standard errors (SE) are reported in Table 3.3. The general concept behind the development of each estimation equation was that DMI is equal to N outputs (milk, urinary, fecal, and/or endogenous N) divided by the digestible portion of dietary N.

In all 10 DMI estimation equations, milk N is estimated based on milk protein yield (g/d) as shown above in Equation 1. Essentially, the milk protein concentration is converted to milk N concentration by dividing the milk protein concentration by a known factor of 6.25. Lastly, the milk N concentration is divided by 0.93 as the digestibility of N in milk is 93%.

To estimate UN, MUN (mg/dL) is used as a term in several DMI estimation equations (Equations 1, 2, 4, 5, 7, 8, 9, and 10) as MUN is relatively easy to measure on-farm through milk composition analysis and it has been shown to have a positive linear correlation with UN (Jonker et al., 1998; Kauffman and St-Pierre, 2001).

To account for fecal N excretion, metabolic fecal N (**MFN**) was subtracted from the denominator portion of each DMI estimation equation except for the Jonker and modified-Jonker equations (Eqs. 9 and 10). In DMI estimation Equations 5, 6, and 7, MFN was estimated using PROC NLIN (SAS 9.4). In DMI estimation Equations 1, 2, 3, and 8, MFN was included in the model at a constant value of 3 whereas MFN was held at a constant value of 5.0 in DMI estimation Equation 4. Swanson (1977) reported a mean estimate of 4.70 g/kg DM of MFN based on a subtraction of 10% of feed N from fecal N (NRC, 2001). Using 4.70 g/kg DM as a starting value for MFN, several MFN values (MFN: 1 – 10 g/kg DM) were tested within each DMI estimation equation. The final MFN value used in each DMI estimation was selected based on the DMI equation with the lowest value of the Akaike information criterion (**AIC**).

BW was used as term in several DMI estimation equations (Eq. 1, 2, 3, and 6) to estimate N outputs related to endogenous N.

Lastly, each DMI estimation equation contains a denominator that accounts for the digestible portion of dietary N concentrations. In several DMI estimation equations (Eq. 1, 2, 3, 4, 8, 9, and 10), diet N is multiplied by a factor of 0.83 which represents the true digestibility of diet N determined by Jonker et al. (1998). In the remaining DMI estimation equations (Eq. 5, 6, and 7), the indigestible (**I**) portion of diet N was estimated using PROC NLIN (SAS 9.4).

It is important to note that 4 DMI estimation equations (Eq. 4, 5, 9, and 10) have unique parameters included within the equation. Equation 4 contains an additional parameter (**E**) in the denominator which was used to adjust for variations associated with both diet N availability and MFN. The DMI estimation Equation 5 contains a parameter (**milk**) that is used to account for variation associated with milk yield. The DMI estimation Equation 9 is the original equation developed by Jonker et al. (1998) from which Equations 1-8 were derived. In this equation, DMI (kg/d) is equal to milk N (g/d), UN (g/d; MUN (mg/dL)*12.54), and endogenous N (97 g/d) divided by available dietary N (0.83*CP (%)/10). Finally, DMI estimation Equation 10 is a modified version of the Jonker equation (Eq. 9) in which MUN is multiplied by 0.0259 and BW to estimate UN (Kauffman and St-Pierre, 2001).

Regression analyses between measured DMI and estimated DMI values were completed using PROC REG (SAS 9.4) and the results of this analysis are reported in Table 3.4. As shown in Figure 3.5, the 3 best DMI estimation equations (Equations 2, 3, and 6) were selected based on the following regression analysis statistics: R^2, RMSE, and *P*-value. The 3 best DMI estimation equations were then evaluated using 4 independent validation datasets as described below. Statistical significance was declared at P-value ≤ 0.05 and a trend towards significance was declared if $0.05 < P \leq 0.10$.

Determination of Mean and Linear Biases in the 3 Selected DMI Equations

As shown in Figure 3.6, the presence of mean and linear biases in the 3 selected DMI estimation equations were determined using the methods described by Nennich et al. (2006). Essentially, regression analyses were performed by regressing residuals (actual – estimated DMI values) on centered estimated DMI values which were calculated by subtracting the mean of all estimated values from each DMI estimate (Nennich et al., 2006). Linear (slope) biases were determined using the slopes of the regression equations and mean biases were determined using the intercepts of the regression equation (Nennich et al., 2006). If linear biases were detected, the magnitude of the linear biases were determined by calculating the biases at the minimum and maximum estimated DMI values were determined for each equation as described by St-Pierre (2003).

Model Evaluation

To validate the 3 selected DMI estimation equations, 4 independent, external experimental datasets were used: Validation Dataset 1 (Iwaniuk et al., 2015; n = 80; Exp. = 2), Validation Dataset 2 (Iwaniuk et al., 2015; n = 80; Exp. = 3), Validation Dataset 3 (Weidman et al., 2018; unpublished; n = 52), and Validation Dataset 4 (Moallem et al., 2014; unpublished; n = 407). The descriptive statistics for these 4 validation datasets are presented in Table 3.5.

These datasets were selected to explore the robustness of each of the three selected DMI equations when cows are fed diets with differing compositions. The goal of the validation analyses was not to explore the interaction between dietary treatments and DMI equations, rather the goal was to determine if the three selected DMI equations were successful even when cows were fed dietary rations with different nutrient compositions. Therefore, these analyses provide information regarding the scope of inference in which these DMI estimation equations can be successfully utilized within the dairy industry.

In the first Validation Dataset, 20 Holstein dairy cows (8 primiparous; 12 multiparous) averaging 39.8 ± 1.9 kg/d milk yield and 95 ± 75 DIM at the start of the experiment were used in a 4 x 4 Latin Square design experiment to determine the effects of dietary cation-anion difference (DCAD) concentration on production parameters (Iwaniuk et al., 2015). Treatments consisted of a basal diet containing approximately 64%

corn silage, 6% alfalfa hay, and 30% concentrates (ground corn, soybean meal 48%, and a vitamin/trace mineral premix) plus potassium carbonate (K_2CO_3; DCAD Plus, Church & Dwight Co. Inc., Piscataway, NJ) supplementation that resulted in the 4 dietary treatments: 1) 250 mEq/kg DCAD (DM basis), 2) 375 mEq/kg DCAD, 3) 500 mEq/kg DCAD, and 4) 625 mEq/kg DCAD. Dietary and production parameters such as DMI, milk yield, and milk composition were collected as described by Iwaniuk et al. (2015).

Similar to Validation Dataset 1, the second validation dataset was conducted using 20 Holstein dairy cows (8 primiparous; 12 multiparous) averaging 41.4 ± 1.4 kg/d milk yield and 95 ± 25 DIM at the start of the experiment were used in a 4 x 4 Latin Square design experiment to determine the effects of cation source (sodium (**Na**) versus potassium (**K**)) used to increase DCAD concentration (mEq/kg, DM basis) on production parameters (Iwaniuk et al., 2015). Treatments consisted of a basal diet containing 65% corn silage and 35% concentrates (ground corn, soybean meal 48%, and a vitamin/trace mineral premix) with a DCAD concentration of 250 mEq/kg (DM basis) plus 150 mEq/kg DCAD increased by either potassium carbonate (K source; K_2CO_3; DCAD Plus, Church & Dwight Co. Inc.) or sodium sesquicarbonate (Na source; SQ-810,Church & Dwight Inc.) supplementation that resulted in 4 dietary treatments: 1) 100:0, 2) 67:33, 3) 33:67, and 4) 0:100% (K:Na). Information regarding the collection of dietary and production parameters is presented in Iwaniuk et al. (2015).

In the third validation dataset, 18 Holstein dairy cows (6 primiparous; 12 multiparous) averaging 38 kg/d milk yield and 75 ± 38 DIM at the start of the experiment were used in a 3 x 3 Latin Square design experiment to investigate the effects of DCAD concentration, monensin supplementation, and the interactive effects of DCAD concentration and monensin supplementation on production parameters (Weidman et al., unpublished). The basal diet contained 58% corn silage, 8% alfalfa hay, and 34% concentrates (ground corn, soybean meal 48%, and a vitamin/trace mineral premix) and had a DCAD concentration of 250 mEq/kg. Monensin was supplemented at either 0 or 13.2 mg/kg DM and the DCAD concentration of the treatments were either 250 mEq/kg, 450 mEq/kg DCAD with K supplementation, or 450 mEq/kg DCAD with Na supplementation. Treatments were arranged in a 2 x 3 factorial treatment design to produce the following 6 treatments: 1) 0 mg/kg monensin + 0 mEq/kg DCAD (Control diet), 2) Control diet + 200 mEq/kg DCAD supplementation with K, 3) Control diet + 200 mEq/kg DCAD supplementation with Na, 4) 13.2 mg/kg monensin + 0 mEq/kg DCAD (monensin diet), 5) monensin diet + 200 mEq/kg DCAD supplementation with K, and 6) monensin diet + 200 mEq/kg DCAD supplementation with Na. Dietary and production measurements were collected using the same protocol as described for validation Experiments 1 and 2 (Iwaniuk et al., 2015).

In the fourth validation dataset, 44 Holstein dairy cows (all multiparous) averaging 50 kg/d milk yield and 132 DIM at the start of the experiment were used in a completely randomized design experiment to investigate the effects of yeast supplementation (*Saccharomyces cerevisiae)* on production responses in lactating cows. The basal diet contained 19% wheat silage, 11% hay, and 60% concentrates (ground corn, rolled barley, rolled wheat, soybean meal 48%, canola meal, cottonseed, wheat bran, corn gluten feed, dried distillers grains, and a vitamin/trace mineral premix). Two dietary treatments were investigated: 1) control diet and 2) control diet plus yeast supplementation using a *Saccharomyces cerevisiae* fermentation product. Milk production and DMI were measured and recorded weekly throughout the experiment. Total milk urea concentrations (g/100 mL) were measured during this study and converted to MUN concentrations (mg/dL) using Equation 4 (shown below) in which the 0.467 coefficient represents the molecular weight (MW) contribution of N in urea (MW = 14*2 = 28 g/mol) divided by the MW of urea (60 g/mol):

$$\text{MUN (mg/dL)} = \text{Urea (g/100 mL)}*1000*0.467 \tag{13}$$

Within each validation dataset, DMI was estimated using the 3 selected DMI estimation equations (Equations 2, 3, and 6) using PROC NLIN (SAS 9.4). The experiments included in Validation Datasets 1, 2, and 3 were conducted as Latin square experiments in which experimental periods and individual cow effects served as blocks. Because the experiment conducted in Validation Dataset 4 did not account for individual cow effects, DMI estimations were estimated by cow to reduce random variation in the analysis as large individual cow variation exists for several production parameters such as milk production and DMI (Connor, 2015; Shonka and Spurlock, 2013; St-Pierre and Weiss, 2009). The coefficients estimated using the validation datasets as well as their respective standard errors (SE) are reported in Table 3.6. Once DMI was estimated for each of the 3 selected DMI equations within each validation dataset, regression analyses were performed between measured DMI and estimated DMI values and the results are reported in Table 3.7. Equations were evaluated based on the results of the regression analyses including the following statistics: R^2, RMSE, and *P*-values.

In addition to generating new DMI estimates by analyzing each validation dataset using PROC NLIN (SAS 9.4), DMI estimates were also calculated using the coefficients generated from the modeling dataset (Table 3.3) and the production data from each of the validation datasets. The relationship between measured DMI and estimated DMI values was analyzed using PROC REG (SAS 9.4) and the results of the regression analyses are presented in Table 3.8. Similarly, the estimation equations were evaluated using the R^2, RMSE, and *P*-value statistics.

Using the Model Evaluation System (**MES**, College Station, TX; http://nutritionmodels.com/mes.html) described by Tedeschi (2006), model evaluations of the 3 selected DMI estimation equations were performed. To assess the accuracy of the models, the following model evaluation statistics were calculated: mean bias (**MB**), mean square error of prediction (**MSEP**), and the square root of the mean square error of prediction (**RMSEP**). The MB is the mean difference between the measured DMI and the estimated DMI values and it is one of the most widely-used statistics to determine model accuracy (Tedeschi, 2006). The MSEP is the expected squared difference between the model-estimated DMI values and the measured DMI values and it is one of the most reliable measurements of model accuracy (Dórea et al., 2017; Tedeschi, 2006). The MSEP can be decomposed into 3 sources of variation: MB, slope (linear) bias, and random error (Tedeschi, 2006). The MB represents errors in central tendency (mean shift), slope bias represents errors associated with regression, and random errors represent natural (unaccounted for) variation between estimated DMI and measure DMI values (Tedeschi, 2006). The RMSEP was also calculated to assess the accuracy of the DMI estimation equations. In addition to accuracy, the precision of the 3 selected DMI estimation equations was tested using the coefficient of determination (R^2) between measured DMI and estimated DMI values. Lastly, both accuracy and precision were tested simultaneously using the concordance correlation coefficient (**CCC**). The CCC is calculated by multiplying the bias correction factor (**C_b**) by the correlation coefficient estimate (**r**) between the observed and estimated values (Tedeschi, 2006). The Cb is a measure of accuracy as it indicates how far the regression line deviates from the slope of unity (45°) while r is a measure of precision as it indicates how closely the estimated values are to each other along the regression line (Tedeschi, 2006). These evaluation analyses were completed for the following 2 validation approaches: 1) DMI is estimated using the validation datasets and PROC NLIN (SAS 9.4) and 2) DMI is estimated using the parameter coefficients estimated with the modeling dataset (Table 3.3) and the production data from the validation datasets. The results of these analyses are presented in Tables 3.9 and 3.10, respectively.

RESULTS AND DISCUSSION

Estimations of 4 Key Production Parameters

Prior to the development and evaluation of the DMI estimation equations, several key production parameters were estimated on a daily, individual cow basis to ensure that each daily cow record contained the specific production parameters that would be used in the equations to estimate DMI. The equations used to estimate BW (kg/d), milk yield (kg/milking), milk protein (% per milking), and MUN (mg/dL per milking) are presented in Table 3.1 and the results of the regression analyses between measured production parameters and their estimated values are presented in Figures 3.1 – 3.4. Individual cow BW (kg/d) was estimated using DIM and DIM^2 as the equation parameters and these parameters accounted for approximately 98.5% of the total variation in BW measurements

(R^2 = 0.9852; RMSE = 8.2386; P < 0.0001; Figure 3.1). Similar to the BW estimation equation, the estimation equation for milk yield (kg/milking) also contained DIM and DIM^2 as equation parameters as well as time (AM vs. PM) as the milk yield variable was expressed as kilograms per milking and cows were milked 2X daily. The milk yield estimation equation accounted for approximately 87% of the total variation associated with milk yield (kg) per milking (R^2 = 0.8647; RMSE = 1.7588; P < 0.0001; Figure 3.2). Lastly, the estimation equations for milk protein (% per milking) and MUN (mg/dL per milking) contained the following terms: DIM, DIM^2, time (AM vs. PM), milk yield per milking (**Milk**), and the interaction between time and milk. The milk protein and MUN estimations accounted for approximately 92.8% (R^2 = 0.9277; RMSE = 07028; P < 0.0001; Figure 3.3) and 84.1% (R^2 = 0.8411; RMSE = 1.22304; P < 0.0001; Figure 3.4) of the total variations associated with milk protein percentage and MUN, respectively.

The results of the estimation equations for the aforementioned production variables are similar to the results of previously published estimation equations for these parameters. Franco et al. (2017) evaluated 6 published equations that predicted BW in growing Holstein heifers based on several body measurements (heart girth, body length, wither height, hip height, and hip width) and reported that these equations accounted for approximately 84.6 – 93.4% of total variation associated with BW which is similar to the variation explained (98.5%) by the BW estimation equation reported in the current study. In regard to milk yield, Otwinowska-Mindur et al. (2015) compared 6 equations that estimated milk yield based on time (AM vs. PM milking), milking interval, DIM, and parity. The authors reported that these equations accounted for approximately 81.0 – 86.5% and 82.8 – 88.4% of the total variation associated with milk yield in the morning and evening milkings, respectively (Otwinowska-Mindur et al., 2015). These results are congruent with the current study in which the milk yield estimation equation accounted for approximately 86.5% of the total variation associated with milk yield (kg/milking). Klopčič et al. (2003) compared 8 equations that estimated milk protein percentage using the following parameters: time (AM vs. PM), milking interval, breed, DIM, and parity. The protein percentage prediction equations accounted for approximately 95.6 and 97.6% of the total variation associated with milk protein percentage in the morning and evening milkings, respectively (Klopčič et al., 2003). The milk protein percentage estimation equation in the current study accounted for approximately 92.8% of the total variation associated with milk protein (%) which are similar to the results of the aforementioned publication. Lastly, the MUN estimation equation in the current study accounted for approximately 84.1% of the total variation association with MUN. Although MUN has become a useful, non-invasive management tool in the dairy industry to assess protein and energy balance of cows within a herd, very little work has been done to develop equations to predict or estimate MUN on an individual cow basis (Hof et al., 1997; Schepers and Meijer, 1998). Therefore, the estimation of MUN based on DIM, milk yield, and time (AM vs. PM) is a novel component of this study.

In conclusion, the estimation equations for BW, milk yield, milk protein (%), and MUN developed in this study adequately estimated each production parameter and missing values in the dataset were replaced by estimated values such that each cow had a complete daily MY record prior to model development and validation.

Estimation of DMI on an Individual Cow Basis

The estimated coefficients and their respective SE for the 8 novel DMI estimation equations as well as the original and modified Jonker equations are presented in Table 3.3. In addition, the results of the regression analyses between measured DMI and estimated DMI values are presented in Table 3.4.

The least successful DMI estimation equation was the original Jonker equation (Eq. 9) which only explained approximately 29.9% of the total variation in DMI ($R^2 = 0.299$; RMSE = 2.759; $P < 0.0001$; Table 3.3; Table 3.4). Several evaluations of this equation have determined that significant mean biases arise when using this method to estimate DMI (Kauffman and St-Pierre, 2001; Kohn et al., 2002; Sannes et al., 2002). In the equation, Jonker et al. (1998) used a coefficient (12.54) multiplied by MUN to estimate UN output. The dataset used to develop the Jonker equation had a range of 12 to 16 mg/dL MUN; however, most herds currently have a MUN range of 8 to 12 mg/dL (Kohn et al., 2002). The use of the incorrect MUN concentration range occurred because this equation was developed prior to the discovery of a hardware malfunction in MUN analyzers being used in various laboratories across the United States and this malfunction resulted in measured MUN concentrations that were much higher than the actual concentration of urea N in milk samples (Kohn et al., 2002). Once the hardware issue was resolved, MUN concentrations were found to be much lower on farms than previously reported (Kohn et al., 2002). As a result of this hardware malfunction, the UN component of the Jonker equation (UN = MUN x 12.54) does not accurately estimate UN when MUN concentrations are below the target range of 12 to16 mg/dL (Kohn et al., 2002). The average MUN concentration of the modeling dataset was 11.79 mg/dL; therefore, it is no surprise that the original Jonker equation was least successful at estimating DMI on an individual cow basis.

As discussed previously, Kohn et al. (2002) evaluated the original Jonker equation and found that the estimate of UN was inaccurate based on the current range (8 to 12 mg/dL) of MUN concentrations on typical dairy farms. Kohn et al. (2002) evaluated several methods to estimate UN and proposed that the best approach to estimate UN was to multiply MUN by BW and a coefficient of 0.0259 proposed by Kauffman and St-Pierre (2001). Using this new term to estimate UN, a modified-Jonker equation was also evaluated in this study (Kauffman and St-Pierre, 2001; Kohn et al., 2002). Although the modified-Jonker proved to be more successful than the original Jonker equation, it still did not accurately estimate DMI on an individual cow basis as it only accounted for approximately 38.1% of the total variation associated with DMI ($R^2 = 0.3809$; RMSE = 2.5940; $P < 0.0001$; Table 3.3; Table 3.4). Meyer et al. (2012) evaluated the 3 following equations to estimate MUN: 1) MUN = UN ÷ 12.54 (Jonker et al., 1998; Model 1), 2) MUN = UN ÷ 17.6 (Kauffman and St-Pierre, 2001; Model 2), and 3) MUN = UN ÷ (BW × 0.0259) (Kauffman and St-Pierre, 2001; Model 3). Meyer et al. (2012) reported that all 3 models had significant mean biases which indicated a lack of accuracy for each model. Specifically, Models 1, 2, and 3 overestimated MUN by 50%, 7%, and 10%, respectively (Meyer et al., 2012). In regard to linear biases, all 3 models had negative linear biases which indicates that bias was highest when MUN values were lowest. These results were as expected as these models were generated using inflated MUN concentrations (Kohn et al., 2002; Meyers et al., 2012). Overall, Model 1 had the least precision and Model 3 had the most precision; therefore, Model 1 (Jonker et al., 1998) was the least successful equation as it had the lowest accuracy and precision compared to both models proposed by Kauffman and St-Pierre (2001). The presence of both mean and linear biases as well as limitations in accuracy and precision in the UN estimation equations proposed by Jonker et al. (1998) and Kauffman and St-Pierre (2001) may explain why Equations 9 and 10 did not successfully estimate DMI on an individual cow basis in the current study.

It is important to note that the DMI estimation Equation 1 is fundamentally identical to the modified-Jonker equation (Eq. 10) except that the coefficient multiplied by MUN and BW to estimate UN is estimated using PROC NLIN (SAS 9.4) instead of being held at the constant value of 0.0259 (Kauffman and St-Pierre, 2001; Table 3.3). Although Equation 1 explained more variation (47.8%) compared to the modified-Jonker equation (38.1%), the estimated coefficient was 0.031 which is analogous to the coefficient (0.026) proposed by Kauffman and St-Pierre (2001) (Table 3.4). Due to similarities between these 2 equations, it is possible that the limitations seen by Meyer et al. (2012) in regard to the UN estimation equation proposed by Kauffman and St-Pierre (2001) may also explain why this DMI estimation equation was the least successful equation developed within this study.

As shown in Table 3.4, DMI estimation Equations 4, 5, 7, and 8 were moderately successful in estimating DMI on an individual cow basis as these equations explained 59.6. 62.6, 60.1, and 57.0% of the total variation associated with DMI, respectively (Table 3.3; Table 3.4). Although Equations 4, 5, 7, and 8 included similar model terms as the 3 most successful DMI estimation equations (Eq. 2, 3, and 6), these 4 less successful equations did not include BW as a model parameter which was the case in the 3 most successful equations. It is well known that BW is highly correlated with DMI as BW dictates a cow's maintenance requirement and drives feed intake (VandeHaar, 2016; VandeHaar et al., 2016). In fact, a majority of published DMI equations include some iteration of BW (or metabolic BW; $BW^{0.75}$) as a term in the model as BW tends to explain a substantial amount of variation associated with feed intake (Connor, 2015; Halachmi et al., , 2004; Roseler et al., 1997). Therefore, it may be possible that Equations 4, 5, 7, and 8 explained a moderate amount of variation in DMI based on the N balance of N intake (as a function of diet N) with the N outputs of milk N, UN, and fecal N; however, accounting for endogenous N outputs using BW as a parameter in the equation may result in a more successful estimation of DMI on an individual cow basis.

The 3 most successful DMI estimation equations in this study were Equations 2, 3, and 6 as these equations accounted for approximately 65.3, 63.9, and 68.2% of the total variation associated with DMI (Table 3.3, Table 3.4, and Figure 3.5). All 3 equations included milk N, a coefficient (B) associated with BW, BW, and an estimate of available dietary N. In regard to UN estimates in the equation, only Equation 2 included a coefficient (C) multiplied by MUN to estimate UN while Equations 3 and 6 did not include an estimation of UN as a portion of the equation. Lastly, Equation 6 allowed for dietary N digestibility and MFN to be estimated during data analysis in PROC NLIN (SAS 9.4) while Equations 2 and 3 had constant values assigned to dietary N digestibility and MFN which were 83% and 3, respectively. Overall, Equation 6 proved to be the most successful equation developed in this current study to estimate DMI on an individual cow basis.

Determination of Mean and Linear Biases in the 3 Selected DMI Equations

As shown in Figure 3.6, mean and linear biases in each DMI estimation equation were determined by regressing residuals (measured – estimated DMI values) on centered

DMI estimates. Mean biases were not detected in any of the 3 selected DMI estimation equations ($P > 0.05$). Linear biases were detected in each of the 3 selected DMI estimation equations such that Equations 2, 3, and 6 had linear biases of -0.0522, -0.0587, and -0.0280, respectively ($P < 0.05$). The presence of negative linear biases in all 3 equations indicates that DMI is consistently being underestimated at low DMI and overestimated at high DMI. To quantify the magnitude of the linear biases, biases at the minimum and maximum estimated DMI values were determined for each equation (St-Pierre, 2003). For Equation 2, the magnitude of the linear bias translates to approximately 0.42 kg/d DMI at the minimum estimated DMI value (14.82 kg/d) and 0.53 kg/d at the maximum estimated DMI value (32.16 kg/d). For Equation 3, the magnitude of the linear bias translates to approximately 0.45 kg/d DMI at the minimum estimated DMI value (15.16 kg/d) and 0.54 kg/d at the maximum estimated DMI value (31.30 kg/d). For Equation 6, the magnitude of the linear bias translates to approximately 0.22 kg/d DMI at the minimum estimated DMI value (15.08 kg/d) and 0.26 kg/d at the maximum estimated DMI value (31.22 kg/d). As reported across 4 experimental studies conducted by our laboratory, the SE of DMI measurements ranged from 0.46 to 0.62 kg/d DMI (Abdelatty et al., 2017; Iwaniuk et al., 2015). The magnitudes of the linear biases in Equations 2, 3, and 6 are smaller than the maximum SE value (0.62 kg/d) reported in the aforementioned literature for DMI which suggests that these biases are minimal (Abdelatty et al., 2017; Iwaniuk et al., 2015; St-Pierre, 2003).

Evaluation of the 3 Selected DMI Estimation Equations

The descriptive statistics for the 4 validation datasets used to evaluate the 3 selected DMI estimation equations are presented in Table 3.5. To evaluate each DMI estimation equation, 2 different approaches were used. In the first approach, each DMI estimation equation was analyzed within each validation dataset using PROC NLIN (SAS 9.4) to generate new equation coefficients to estimate DMI (Table 3.6) and regression analyses were performed to compare measured and estimated DMI values (Table 3.7). In the second approach, each DMI estimation equation was analyzed within each validation dataset using the parameter coefficients generated during model development presented in Table 3.3 (Table 3.8). Model evaluation using the MES (Tedeschi, 2006) was completed for both evaluation methods and the results are presented in Tables 3.9 and 3.10.

The coefficients generated from each DMI estimation equation within each validation dataset using PROC NLIN (SAS 9.4) are presented in Table 3.6 and the results of the regression analyses between measured and estimated DMI from these analyses are shown in Table 3.7. With the exception of DMI estimation Equation 6 within Validation dataset #2, the results of the regression analyses between measured and estimated DMI values for all 3 DMI estimation equations within all 4 validation datasets were improved compared to the initial regression analyses from the modeling dataset presented in Table 3.4. In addition, the average RMSE is lower for all DMI equations in the validation datasets (Eq. 2, RMSE = 1.684; Eq. 3, RMSE = 1.725; Eq. 6, RMSE = 1.675) compared to the modeling dataset (Eq. 2, RMSE = 1.943; Eq. 3, RMSE = 1.982; Eq. 6, RMSE = 1.860). The improvement in the R^2 values and reduction in RMSE for the DMI estimation equations in the validation datasets may be attributed to that fact that random, individual cow variation was accounted for during the experimental design phase or data analysis and individual cow variation can account for a large portion of error in parameters such as DMI (Connor, 2015; Shonka and Spurlock, 2013; St-Pierre and Weiss, 2009). On average, the regression analyses using the validation datasets produced the following results: Equation 3 was least successful (R^2 = 0.704), Equation 2 was moderately successful (R^2 = 0.718), and Equation 6 was most successful (R^2 = 0.719) at explaining the total variation associated with DMI which mirrors the results of the regression analyses using the modeling dataset (Eq. 3, R^2 = 0.639; Eq. 2, R^2 = 0.653; and Eq. 6, R^2 = 0.682). Based on the results of this portion of the equation evaluations, the most successful equation developed to estimate DMI on an individual cow basis is still Equation 6.

The results of the evaluation conducted using the MES (Tedeschi, 2006) between measured and estimated DMI values using the 3 selected DMI equations within each validation dataset are presented in Table 3.9. On average across all validation datasets, the best DMI estimation equation was Equation 6. The average R^2 values for Equations 2, 3, and 6 were 0.718, 0.704, and 0.719, respectively. In addition to R^2 which tests accuracy, the CCC was also estimated as it is a measure of both accuracy and precision in which a value of 1.0 is indicative of a perfect agreement between measured and estimated values (Tedeschi, 2006). The average CCC values for Equations 2, 3, and 6 were 0.826, 0.819, and 0.828, respectively. As it had the highest value for both statistics compared to the other DMI estimation equations, Equation 6 was the most accurate and precise equation. Additionally, Equation 6 had the smallest, average mean bias (-0.007) compared to Equations 2 (-0.013) and 3 (-0.009) and this value indicates that Equation 6 underestimated DMI by 0.007 kg/d, on average. Equation 6 also had the smallest RMSEP (1.682 kg/d) compared to Equation 2 (1.687 kg/d) and Equation 3 (1.733 kg/d). When MSEP was decomposed, Equation 2 had the smallest mean bias (0.031%); however, Equation 6 had the smallest linear bias (3.430%). Lastly, Equation 6 had the largest portion of random error (96.54 %) as a component of MSEP as compared to the other equations which indicates that Equation 6 had less combined mean and linear biases (% SMEP) compared to the other equations. Overall, Equation 6 proved to be the best equation to estimate DMI on an individual cow basis compared to the other selected equations.

During the next phase of equation evaluation, DMI was estimated using each of the 3 selected equations within each validation dataset using the original coefficients generated during equation development as shown in Table 3.3. The results of the regression analyses between measured and estimated DMI values for each equation within each validation dataset are presented in Table 3.8. For Validation Datasets 1, 2, and 3, the results of the regression analyses (R^2 and RMSE) are very similar for this analysis as discussed in the previous analysis, which used the validation datasets to generate new parameter coefficients for each DMI estimation equation. However, in this analysis, Validation Dataset 4 had much lower success in estimating DMI in all 3 equations (Eq. 2, $R^2 = 0.445$, RMSE = 2.984; Eq. 3, $R^2 = 0.439$, RMSE = 2.999; Eq. 6, $R^2 = 0.441$; RMSE = 2.996). As shown in Table 3.5, the average DMI for Validation Dataset 4 was 23, 27, and 21% larger than Validation Datasets 1, 2, and 3, respectively. As discussed previously, there was a minimal, but detected linear bias associated with each DMI equation such that DMI was overestimated at high DMI values. Because DMI was substantially higher in Validation dataset 4 as compared to the other 3 validation datasets, it is possible that the effects of the linear biases were more profound in this dataset which reduced the DMI estimation performance in all 3 selected DMI equations. On average, Equations 2, 3, and 6 explained 65.2, 64.9, and 63.7% of the total variation associated with DMI. Therefore, the most successful equation developed to estimate DMI on an individual cow basis is Equation 2 based on this portion of the evaluation.

The results of the evaluation conducted using the MES (Tedeschi, 2006) between measured and estimated DMI values using the 3 selected DMI equations within each validation dataset are presented in Table 3.10. The average R^2 values for Equations 2, 3, and 6 were 0.652, 0.649, and 0.637, respectively; thus, Equation 2 had the strongest measure of accuracy as determined by R^2 values. Similarly, Equation 2 had the lowest mean bias (-0.261) as compared to Equation 3 (-0.308) and Equation 6 (0.710). However, Equation 6 had the highest CCC (0.709) and lowest RMSEP (2.163 kg/d) compared to Equation 2 (0.686; 2.318 kg/d) and Equation 3 (0.686; 2.325 kg/d), respectively. Looking at the MSEP decomposition, Equation 3 had the smallest linear bias (3.82%); however, Equation 6 had the smallest mean bias (14.91%). Lastly, the largest portion of random error (% MSEP) belonged to Equation 6 (81.04%) as compared to the other equations. We found that Equation 6 had the lowest combined mean and linear biases (% MSEP) compared to the other equations. Although Equation 2 had the highest R^2 and lowest mean bias values and Equation 3 had the smallest slope bias value, Equation 6 still proved to be the best equation to estimate DMI of individual cows compared to the other selected equations as it had the highest CCC value and lowest RMSEP, mean bias (% MSEP), and random error (% MSEP) values.

Although DMI estimation Equations 2 and 3 were strong candidates, Equation 6 was selected as the best equation to estimate DMI on an individual cow basis based on the performance of the equation during rigorous evaluation. The principal components of this equation include N outputs of milk N, endogenous N (B × BW), and the available concentration of dietary N (I × Diet N × MFN). It is interesting to note that this equation does not contain a UN component estimated by the multiplication of a coefficient (C) by MUN. The relationship between MUN and DMI was assessed using regression analyses within the modeling dataset and the relationship was quite poor (data not shown). Although MUN has been shown to be highly correlated to UN, the relationship between MUN and DMI has yet to be established (Jonker et al., 1998; Kauffman and St-Pierre, 2001; Kohn et al., 2002). Several factors have been shown to affect MUN such as diet composition, water intake, milking time, milking frequency, and breed (Ishler, 2017). Therefore, when used as a parameter in the equation to estimate DMI, MUN may not explain much of the total variation associated with DMI as the relationship between DMI and MUN may vary depending on additional dietary and production factors.

APPLICATIONS TO THE DAIRY INDUSTRY

Overall, Equation 6 proved to be the most successful developed equation used to estimate DMI on an individual cow basis. Equation 6 was the most simplistic DMI estimation equation developed during this study in regard to parameter inclusion and its simplicity may increase the likelihood that this equation would be used on-farm to estimate DMI as a dairy producer would only be required to record/calculate the following 3 parameters: 1) milk N based on milk yield and milk protein concentration on the individual cow, 2) BW of the individual cow, and 3) dietary N from the herd ration composition. These inputs are relatively straight-forward to measure; therefore, Equation 6 may be used as a simple, practical method to estimate DMI on an individual cow basis even if cows are fed in a group setting.

Dairy producers may be able to utilize the results of this study to calculate DMI, and subsequently FE, on an individual cow basis which has been a virtually impossible feat on standard dairy farms as cows are often fed in groups. The knowledge of an individual cow's FE status may help producers make more informed management decisions in their current herd as they will have the ability to select for more efficient cows which would increase profitability. In regard to future herd improvements, dairy producers can select highly efficient cows for genetic selection to improve the FE of future generations within the herd. Improving FE will result in increased profitability for dairy producers as well as a reduction in the environmental impact of dairy production.

CONCLUSIONS

The results of this study indicate that DMI can be successfully estimated on an individual cow basis using common, on-farm measurements. The results of this study can be utilized by dairy producers to estimate DMI, and subsequently FE, on an individual cow basis to select for more efficient cows in current and future herds. Future research should be completed that examines the relationship between the DMI estimated from each of the 3 selected DMI equations and measured DMI on farm in a controlled experiment. Additionally, the 3 selected DMI estimation equations developed in this study should be evaluated against any additional DMI equations that are currently being used in the dairy industry.

ACKNOWLEDGMENTS

The authors thank Amy Cannistraci (Lebanon County Conservation District) and Dr. Uzi Moallem (Agricultural Research Organization; Volcani Center) for permission to use their datasets as validation datasets to evaluate our proposed DMI equations.

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Table 3.1. Estimation equations for BW, milk yield[1], milk protein (%), and MUN[2].

Item	Model
BW, kg	= DIM[3] + DIMSq[4]
Milk Yield, kg/milking	= Time[5] + DIM + DIMSq
Milk Protein, %/milking	= Time + Milk[1] + Milk*Time[6] + DIM + DIMSq
MUN[1], mg/dL/milking	= Time + Milk + Milk*Time + DIM + DIMSq

[1]Milk Yield = Milk yield per milking (AM vs. PM).
[2]MUN = Milk urea N (mg/dL per milking).
[3]DIM = Days in Milk.
[4]DIMSq = DIM*DIM.
[5]Time = Time of milking (AM vs. PM).
[6]Interactive effect of milk yield (per milking) and time of milking (AM vs. PM).

Table 3.2. Descriptive statistics for the continuous variables used to estimate the individual DMI of lactating dairy cows.

Item[1,2]	Mean	SD[3]	Minimum	Maximum
DMI[4], kg/d	22.45	3.30	14.72	31.24
MilkN[5], g/d	212.32	32.63	137.35	303.28
Milk Yield[6], kg/d	43.98	7.30	27.56	64.32
Milk Protein, %	2.82	0.24	1.80	3.87
BW[7], kg	583.7	61.3	456.4	763.7
MUN[8], mg/dL	11.79	2.62	4.67	18.34
Dietary CP[9], %	16.59	0.73	14.70	18.50
Dietary N[10], g/d	26.55	1.16	23.52	29.60

[1]All continuous variables (except DMI) contain both actual and estimated values based on the estimation equations described in Table 3.1 and Figures 3.1 – 3.4.
[2]Sample size for each variable (n) = 8,081 means averaged weekly on an individual cow basis.
[3]SD = standard deviation.
[4] DMI = Dry matter intake.
[5]MilkN = (Protein yield (g/d)/6.25)/(0.93).
[6]Milk yield (kg/d) = AM Milk (kg/d) + PM Milk (kg/d).
[7]BW = Body weight.
[8]MUN = Milk urea N.
[9]CP = Crude protein (% DM basis).
[10]Dietary N = (Dietary CP (%)/6.25) × 10.

Table 3.3. Equations used to estimate individual cow DMI based on common on-farm measurements of bi-weekly dietary composition and production data means.

		Model	Estimate	
Eq.	DMI[1] Estimation Equations	Terms	Coeff.	SE
1	DMI (kg/d) = (MilkN[2] + (A[3] × BW[4] × MUN[5]))/(0.83[6] × DietN[7] - 3[8])	A	0.031	0.000
2	DMI (kg/d) = (MilkN + (B[9] × BW) + (C[10] × MUN))/(0.83 × DietN - 3)	B	0.355	0.001
		C	0.504	0.042
3	DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3)	B	0.367	0.000
4	DMI (kg/d) = (MilkN + D[11] + (C × MUN))/(0.83 × DietN + 5[12] – E[13])	D	167.7	1.898
		C	0.394	0.058
		E	9.659	0.093
5	DMI (kg/d) = (MilkN + (C × MUN) + (F[14] × Milk[15] × MUN))/(DietN - (I[16] × DietN) – MFN[17])	C	5.424	0.038
		F	-0.095	0.001
		I	1.006	0.007
		MFN	-10.271	0.191
6	DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN)	B	0.373	0.002
		I	-0.046	0.012
		MFN	-20.348	0.329
7	DMI (kg/d) = (MilkN + (C × MUN))/(I × DietN - MFN)	C	3.145	0.035
		I	-0.012	0.008
		MFN	-11.510	0.218
8	DMI (kg/d) = (D × (MilkN + MUN))/(0.83 × DietN - 3)	D	1.895	0.001
9	DMI[18] (kg/d) = ((MilkN + (MUN × 12.54[19]) + 97[20])/(0.83))/(CP[21]/10)	--[22]	--	--
10	DMI[23] (kg/d) = ((MilkN + (MUN × BW × 0.0259[24]) + 97)/(0.83))/(CP/10)	--	--	--

[1]DMI = Dry matter intake (kg/d).

[2]MilkN = (Milk protein yield (g/d)/6.25)/(0.93).

[3]A = coefficient used to estimate N output based on changes in body weight (ΔBW; kg) and milk urea N (ΔMUN; mg/dL).
[4]BW = Body weight (kg).
[5]MUN = Milk urea N (mg/dL).
[6]0.83 = constant used to estimate the digestibility of dietary N.
[7]DietN = Dietary N = (Dietary crude protein (%)/6.25) × 10.
[8]3 = constant used to estimate metabolic fecal N (MFN).
[9]B = coefficient used to estimate N output based on ΔBW.
[10]C = coefficient used to estimate N output based on ΔMUN.
[11]D = intercept used for estimated UN output.
[12]5 = constant used to estimate metabolic fecal N (MFN).
[13]E = adjustment in differences in diet N availability and MFN.
[14]F = coefficient used to estimate N output based on ΔMilk and ΔMUN.
[15]Milk = total milk yield (kg/d).
[16]I = coefficient used to estimate digestibility of dietary N.
[17]MFN = coefficient used to estimate metabolic fecal N (g/d).
[18]DMI estimation equation proposed by Jonker et al. (1998).
[19]12.54 = slope based on the relationship between MUN and UN excretion (Jonker et al., 1998).
[20]97 = estimate of endogenous N (Jonker et al., 1998).
[21]CP = Crude protein (% DM basis).
[22]DMI estimation equations did not have estimated model coefficients or SEs.
[23]DMI estimated using a modified- Jonker equation proposed by Kohn et al. (2002).
[24]0.0259 = coefficient proposed by Kohn et al. (2002) to estimate UN based on MUN and BW.

Table 3.4. Regression relationships between observed and estimated DMI for the proposed DMI equations.

Eq.	Slope	SE	*P*-value	Int.	SE	*P*-value	R^2	RMSE[1]	*P*-value
1[2]	0.645	0.008	<.0001	8.183	0.168	<.0001	0.478	2.382	<.0001
2[3]	0.948	0.008	<.0001	1.191	0.174	<.0001	0.653	1.943	<.0001
3[4]	0.941	0.008	<.0001	1.341	0.178	<.0001	0.639	1.982	<.0001
4[5]	1.000	0.009	<.0001	0.008	0.207	0.9679	0.596	2.095	<.0001
5[6]	0.923	0.008	<.0001	1.758	0.180	<.0001	0.626	2.017	<.0001
6[7]	0.972	0.007	<.0001	0.638	0.167	0.0001	0.682	1.860	<.0001
7[8]	0.797	0.007	<.0001	4.626	0.163	<.0001	0.601	2.083	<.0001
8[9]	0.738	0.007	<.0001	5.987	0.161	<.0001	0.570	2.161	<.0001
9[10]	0.802	0.014	<.0001	5.794	0.285	<.0001	0.299	2.759	<.0001
10[11]	0.704	0.010	<.0001	6.847	0.223	<.0001	0.381	2.594	<.0001

[1]RMSE = root mean square error.
[2]DMI (kg/d) = (MilkN + (A × BW × MUN))/(0.83 × DietN - 3).
[3]DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[4]DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[5]DMI (kg/d) = (MilkN + D + (B × MUN))/(0.83 × DietN + 5 – E).
[6]DMI (kg/d) = (MilkN + (C × MUN) + (F × Milk × MUN)/(DietN - (I × DietN) – MFN).
[7]DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).
[8]DMI (kg/d) = (MilkN + (C × MUN))/(I × DietN - MFN).
[9]DMI (kg/d) = (D × (MilkN + MUN))/(0.83 × DietN - 3).
[10]DMI (kg/d) = ((MilkN + (MUN × 12.54) + 97)/(0.83))/(CP/10).
[11]DMI (kg/d) = ((MilkN + (MUN × BW × 0.0259) + 97)/(0.83))/(CP/10).

Table 3.5. Descriptive statistics for continuous variables of 4 experiments used to validate the 3[1,2,3,4] selected DMI estimation equations.

	Validation Dataset 1[5]		Validation Dataset 2[6]		Validation Dataset 3[7]		Validation Dataset 4[8]	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DMI[9], kg/d	23.0	3.0	22.2	2.7	23.2	3.0	28.2	4.0
MilkN[10], g/d	197.8	30.0	191.3	29.5	177.7	22.6	251.7	31.2
Milk Yield, kg/d	39.2	6.7	37.3	6.5	34.7	5.1	45.1	6.6
Milk Protein, %	2.95	0.23	3.01	0.26	3.00	0.23	3.26	0.20
BW[11], kg	635	53	640	77	672	70	672	58
MUN[12], mg/dL	13.8	2.0	15.1	2.0	12.5	1.9	14.3	3.3
CP[13], %	15.5	0.2	15.9	0.0	16.0	0.1	16.5	0.0
DietN[14], g/d	24.8	0.3	25.4	0.0	25.5	0.2	26.4	0.0
NI[15], g/d	569	74	564	69	593	77	745	106

[1]Based on the results of the DMI estimation equation evaluation shown in Table 3.4.
[2]Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[3]Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[4]Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).
[5]Validation Dataset 1 = Iwaniuk et al., 2015 (n = 80; Exp. 2).
[6]Validation Dataset 2 = Iwaniuk et al., 2015 (n = 80; Exp. 3).
[7]Validation Dataset 3 = Weidman et al., 2018 (unpublished data; n = 52).
[8]Validation Dataset 4 = Moallem et al., 2014 (unpublished data; n = 407).
[9]DMI = Dry matter intake (kg/d).
[10]MilkN = (Milk protein yield (g/d)/6.25)/(0.93).
[11]BW = Body weight (kg).
[12]MUN = Milk urea N (mg/dL).
[13]CP = Crude protein (% DM basis).
[14]DietN = Dietary N = (Dietary crude protein (%)/6.25) × 10.
[15]NI = N intake (g/d).

Table 3.6. Parameter estimates for the 3 selected DMI estimation equations developed from the 4 validation datasets.

DMI Eq.	VD[1]	Model Terms	Estimate Coefficient	SE[2]
2[3]	1[4]	B[5]	0.371	0.035
		C[6]	-2.144	1.584
	2[7]	B	0.279	0.031
		C	2.100	1.293
	3[8]	B	0.350	0.028
		C	0.646	1.501
	4[9]	B	0.251	0.179
		C	6.549	6.544
3[10]	1	B	0.325	0.005
	2	B	0.328	0.005
	3	B	0.362	0.005
	4	B	0.428	0.021
6[11]	1	B	0.399	0.097
		I[12]	1.945	0.687
		MFN[13]	28.598	16.307
	2	B	0.340	0.069
		I	0.884	0.079
		MFN	4.000	.[14]
	3	B	0.356	0.091
		I	2.079	0.885
		MFN	35.035	21.577
	4	B	0.827	1.514
		I	1.266	1.223
		MFN	6.000	.[14]

[1]VD = validation dataset.
[2]SE = standard error of each individual coefficient.
[3]Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[4]Validation Dataset 1 = Iwaniuk et al., 2015 (n = 80; Exp. 2).
[5]B = coefficient used to estimate N output based on ΔBW.
[6]C = coefficient used to estimate N output based on ΔMUN.
[7]Validation Dataset 2 = Iwaniuk et al., 2015 (n = 80; Exp. 3).
[8]Validation Dataset 3 = Weidman et al., 2018 (unpublished data; n = 52).
[9]Validation Dataset 4 = Moallem et al., 2014 (unpublished data; n = 407).
[10]Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[11]Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).
[12]I = coefficient used to estimate digestibility of dietary N.
[13]MFN = coefficient used to estimate MFN.
[14]SE was not estimated for these coefficients.

Table 3.7. Regression relationships between observed and estimated DMI for the proposed DMI equations developed using estimated DMI coefficients generated from the 4 validation datasets[1].

DMI Eq.	VD[2]	Slope	SE[3]	*P*-value	Int.	SE	*P*-value	R^2	RMSE[4]	*P*-value
2[5]	1[6]	1.066	0.083	<.0001	-1.540	1.907	0.4218	0.681	1.708	<.0001
	2[7]	0.934	0.076	<.0001	1.480	1.685	0.3824	0.662	1.580	<.0001
	3[8]	1.209	0.078	<.0001	-4.875	1.818	0.0099	0.827	1.235	<.0001
	4[9]	0.973	0.032	<.0001	0.768	0.900	0.394	0.700	2.214	<.0001
3[10]	1	1.084	0.085	<.0001	-1.971	1.967	0.3193	0.675	1.724	<.0001
	2	0.886	0.072	<.0001	2.547	1.613	0.1185	0.658	1.590	<.0001
	3	1.183	0.078	<.0001	-4.268	1.815	0.0227	0.821	1.256	<.0001
	4	0.964	0.034	<.0001	1.017	0.973	0.2967	0.662	2.328	<.0001
6[11]	1	1.120	0.084	<.0001	-2.795	1.950	0.1558	0.693	1.676	<.0001
	2	0.889	0.073	<.0001	2.494	1.619	0.1274	0.657	1.590	<.0001
	3	1.159	0.075	<.0001	-3.708	1.744	0.0384	0.827	1.236	<.0001
	4	0.986	0.032	<.0001	0.387	0.914	0.6723	0.699	2.198	<.0001

[1]Estimated DMI values were developed using new coefficients generated from PROC NLIN (SAS 9.4, SAS Institute, Cary, NC) using the 4 validation datasets.
[2]VD = validation dataset.
[3]SE = standard error.
[4]RMSE = root mean square error.
[5]Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[6]Validation Dataset 1 = Iwaniuk et al., 2015 (n = 80; Exp. 2).
[7]Validation Dataset 2 = Iwaniuk et al., 2015 (n = 80; Exp. 3).
[8]Validation Dataset 3 = Weidman et al., 2018 (unpublished data; n = 52).
[9]Validation Dataset 4 = Moallem et al., 2014 (unpublished data; n = 407).
[10]Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[11]Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).

Table 3.8. Regression relationships between observed and estimated DMI for the 3 selected DMI equations developed using estimated DMI coefficients generated from the modeling dataset using the 4 validation datasets[1].

DMI Eq.	VD[2]	Slope	SE[3]	*P*-value	Int.	SE	*P*-value	R^2	RMSE[4]	*P*-value
2[5]	1[6]	1.049	0.082	<.0001	-2.693	2.011	0.1844	0.678	1.717	<.0001
	2[7]	0.852	0.070	<.0001	2.117	1.645	0.202	0.659	1.587	<.0001
	3[8]	1.199	0.078	<.0001	-4.750	1.818	0.0118	0.826	1.240	<.0001
	4[9]	1.279	0.071	<.0001	-5.39	1.871	0.0042	0.445	2.984	<.0001
3[10]	1	1.046	0.081	<.0001	-2.675	1.994	0.1837	0.681	1.708	<.0001
	2	0.840	0.069	<.0001	2.409	1.631	0.1438	0.656	1.594	<.0001
	3	1.174	0.077	<.0001	-4.284	1.816	0.0223	0.821	1.256	<.0001
	4	1.260	0.071	<.0001	-4.96	1.869	0.0083	0.439	2.999	<.0001
6[11]	1	1.111	0.092	<.0001	-2.180	2.097	0.3019	0.650	1.789	<.0001
	2	0.882	0.072	<.0001	2.394	1.634	0.1469	0.655	1.595	<.0001
	3	1.220	0.086	<.0001	-4.149	1.931	0.0365	0.801	1.325	<.0001
	4	1.270	0.071	<.0001	-5.09	1.872	0.0068	0.441	2.996	<.0001

[1]Estimated DMI values were developed using the initial coefficients generated from PROC NLIN (SAS 9.4, SAS Institute, Cary, NC) using modeling dataset (Table 3.3).
[2]VD = validation dataset.
[3]SE = standard error.
[4]RMSE = root mean square error.
[5]Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[6]Validation Dataset 1 = Iwaniuk et al., 2015 (n = 80; Exp. 2).
[7]Validation Dataset 2 = Iwaniuk et al., 2015 (n = 80; Exp. 3).
[8]Validation Dataset 3 = Weidman et al., 2018 (unpublished data; n = 52).
[9]Validation Dataset 4 = Moallem et al., 2014 (unpublished data; n = 407).
[10]Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[11]Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).

Table 3.9. Evaluation of the 3 selected equations used to estimate DMI on an individual cow basis using estimated DMI coefficients generated from the 4 validation datasets[1].

						MSEP decomposition[5] (%)		
DMI Eq.	VD[2]	R^2	Mean bias	CCC[3]	RMSEP[4] (kg/d)	Mean bias	Slope bias	Random error
2[6]	1[7]	0.681	-0.022	0.799	1.693	0.026	0.814	99.169
	2[8]	0.662	0.008	0.806	1.568	0.002	0.980	99.017
	3[9]	0.827	-0.042	0.874	1.296	0.104	12.476	87.431
	4[10]	0.700	0.002	0.827	2.190	0.000	0.181	99.819
3[11]	1	0.675	-0.031	0.791	1.713	0.033	1.243	98.724
	2	0.658	0.028	0.808	1.594	0.032	3.064	96.904
	3	0.821	-0.037	0.875	1.298	0.083	9.873	90.044
	4	0.662	0.004	0.802	2.326	0.000	0.271	99.729
6[12]	1	0.693	-0.026	0.797	1.677	0.024	2.542	97.434
	2	0.657	0.030	0.807	1.594	0.036	2.918	97.047
	3	0.827	-0.036	0.883	1.265	0.080	8.214	91.706
	4	0.699	0.006	0.825	2.193	0.001	0.044	99.956

[1]Estimated DMI values were developed using new coefficients generated from PROC NLIN (SAS 9.4, SAS Institute, Cary, NC) using the 4 validation datasets.
[2]VD = validation dataset.
[3]CCC = concordance correlation coefficient.
[4]RMSEP = root mean squared errors of prediction.
[5]MSEP = mean squared errors of prediction.
[6]Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[7]Validation Dataset 1 = Iwaniuk et al., 2015 (n = 80; Exp. 2).
[8]Validation Dataset 2 = Iwaniuk et al., 2015 (n = 80; Exp. 3).
[9]Validation Dataset 3 = Weidman et al., 2018 (unpublished data; n = 52).
[10]Validation Dataset 4 = Moallem et al., 2014 (unpublished data; n = 407).
[11]Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[12]Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).

Table 3.10. Evaluation of the 3 selected equations used to estimate DMI on an individual cow basis using estimated DMI coefficients generated from the modeling dataset using the 4 validation datasets[1].

						MSEP decomposition[5] (%)		
DMI Eq.	VD[2]	R^2	Mean bias	CCC[3]	RMSEP[4] (kg/d)	Mean bias	Slope bias	Random error
2[6]	1[7]	0.678	-1.502	0.692	2.268	43.888	0.253	55.859
	2[8]	0.659	-1.355	0.715	2.106	41.391	3.201	55.408
	3[9]	0.826	-0.127	0.874	1.299	0.949	11.442	87.609
	4[10]	0.445	1.938	0.462	3.599	28.984	2.607	68.409
3[11]	1	0.681	-1.542	0.690	2.287	45.422	0.227	54.351
	2	0.656	-1.358	0.714	2.119	41.04	3.815	55.145
	3	0.821	-0.225	0.874	1.312	2.945	8.89	88.165
	4	0.439	1.892	0.465	3.582	27.903	2.33	69.767
6[12]	1	0.650	0.333	0.761	1.814	3.369	1.762	94.870
	2	0.655	-0.254	0.803	1.621	2.457	3.215	94.328
	3	0.801	0.774	0.816	1.584	23.889	8.829	67.282
	4	0.441	1.987	0.457	3.633	29.908	2.406	67.686

[1]Estimated DMI values were developed using the initial coefficients generated from PROC NLIN (SAS 9.4, SAS Institute, Cary, NC) using modeling dataset (Table 3.3).
[2]VD = validation dataset.
[3]CCC = concordance correlation coefficient.
[4]RMSEP = root mean squared errors of prediction.
[5]MSEP = mean squared errors of prediction.
[6]Equation 2: DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3).
[7]Validation Dataset 1 = Iwaniuk et al., 2015 (n = 80; Exp. 2).
[8]Validation Dataset 2 = Iwaniuk et al., 2015 (n = 80; Exp. 3).
[9]Validation Dataset 3 = Weidman et al., 2018 (unpublished data; n = 52).
[10]Validation Dataset 4 = Moallem et al., 2014 (unpublished data; n = 407).
[11]Equation 3: DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3).
[12]Equation 6: DMI (kg/d) = (MilkN + (B × BW))/(I × DietN - MFN).

Figure 3.1. Relationship between observed and estimated values for BW. [BW (kg) = 1.000x + 0.0000; intercept P = 1.0000; intercept SE = 1.01; slope P = < 0.0001, slope SE = 0.00171, R^2 = 0.985; root mean (predicted) standard error (**RMSE**) = 8.23; n = 5116].

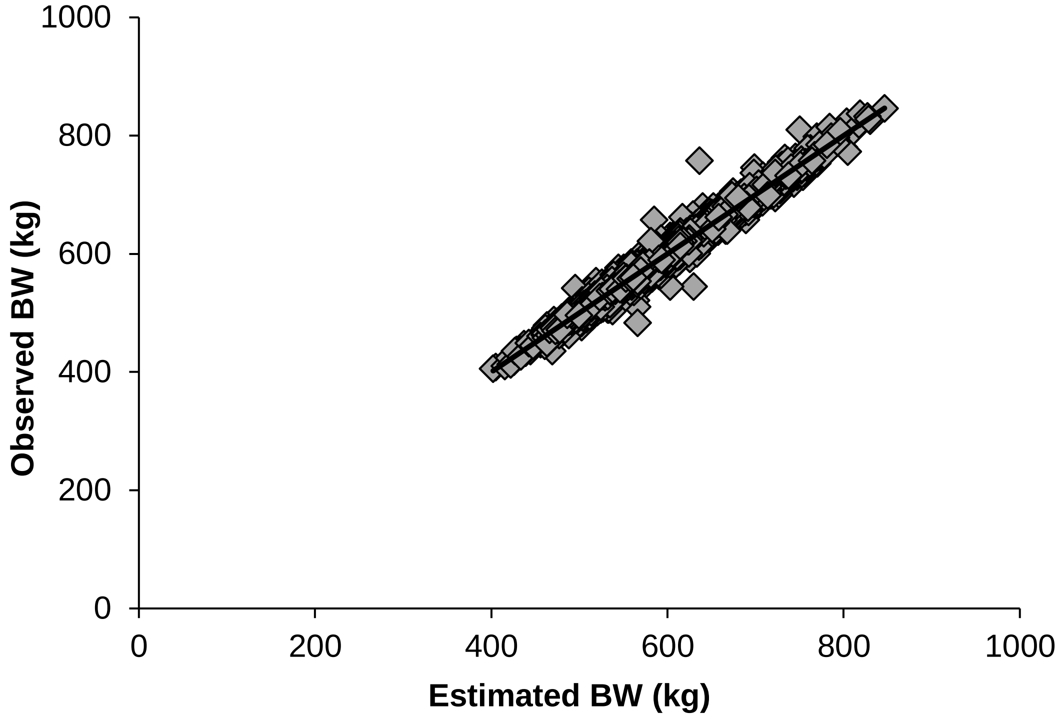


Figure 3.2. Relationship between observed and estimated values for milk yield. [MY (kg/milking) = 1.0000x + 0.0002; intercept P = 1.0000; intercept SE = 0.024; slope P = < 0.0001, slope SE = 0.001, R^2 = 0.865; RMSE = 1.76; n = 140,101].

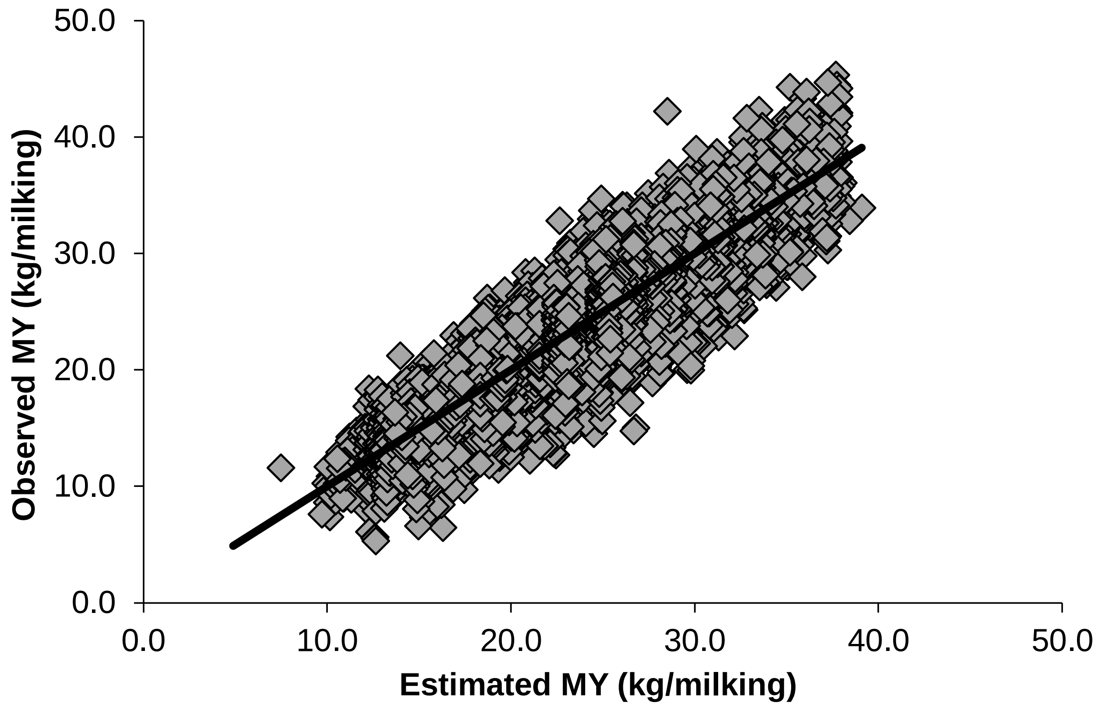


Figure 3.3. Relationship between observed and estimated values for milk protein percent. [Milk protein (% per milking) = 1.000x + 0.0000; intercept P =1.0000; intercept SE = 0.008; slope P = <0.0001, slope SE = 0.003, R^2 = 0.928; RMSE = 0.070; n = 9,915].

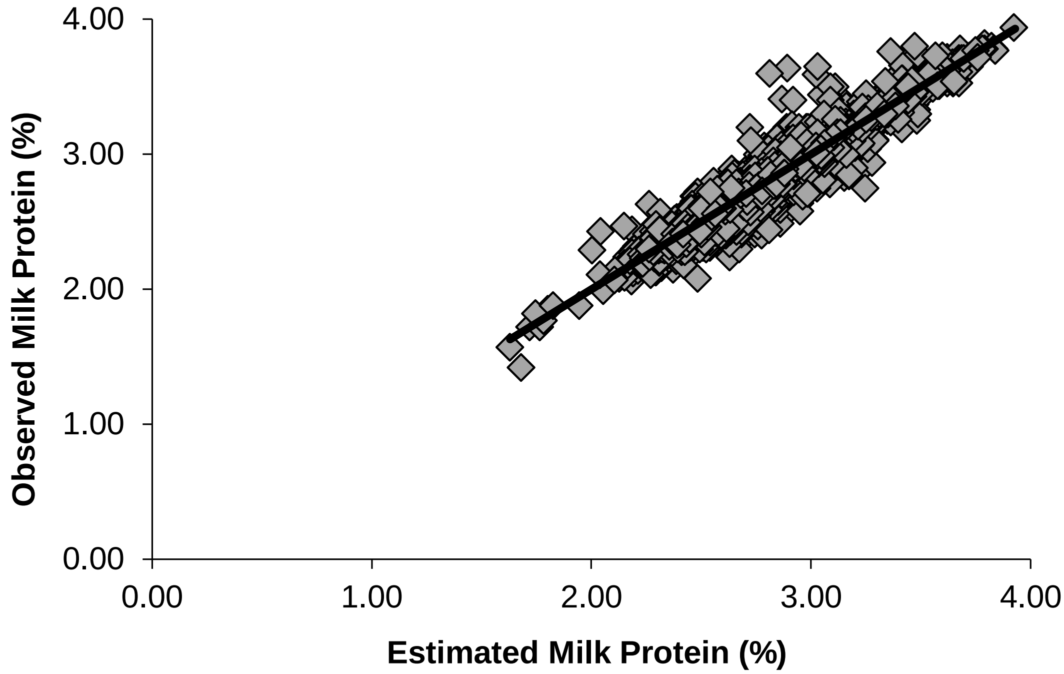


Figure 3.4. Relationship between observed and estimated values for MUN. [MUN (mg/dL per milking) = 1.000x + 0.0000; intercept P = 1.0000; intercept SE = 0.057; slope P = < 0.0001, slope SE =0.005, R^2 = 0.841; RMSE = 1.22; n = 8670].

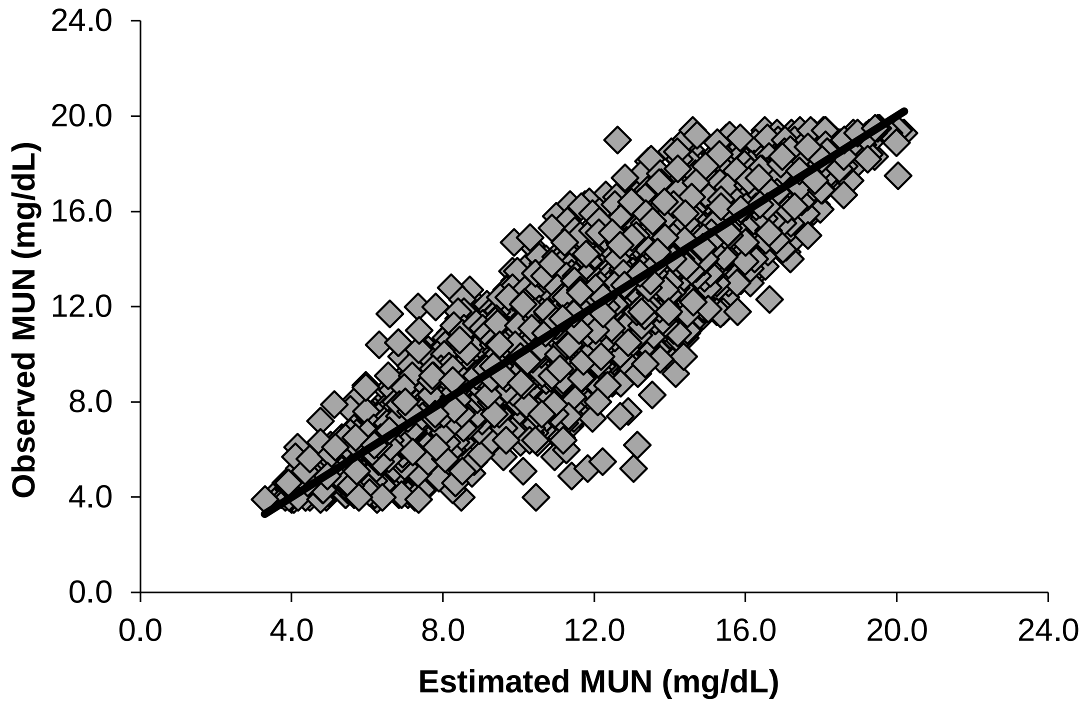


Figure 3.5. Relationship between observed and estimated DMI values for the 3 selected DMI equations. (A) Equation 2 [DMI (kg/d) = 0.948x + 1.191; intercept P < 0.0001; intercept SE = 0.174; slope P < 0.0001; slope SE = 0.008; R^2 = 0.653; RMSE = 1.943; P < 0.0001], (B) Equation 3 [DMI (kg/d) = 0.941x + 1.341; intercept P < 0.0001; intercept SE = 0.178; slope P < 0.0001; slope SE = 0.008; R^2 = 0.639; RMSE = 1.982; P < 0.0001], and (C) Equation 6 [DMI (kg/d) = 0.972x + 0.638; intercept P < 0.0001; intercept SE = 0.167; slope P < 0.0001; slope SE = 0.007; R^2 = 0.682; RMSE = 1.860; P < 0.0001].

(A) (B)

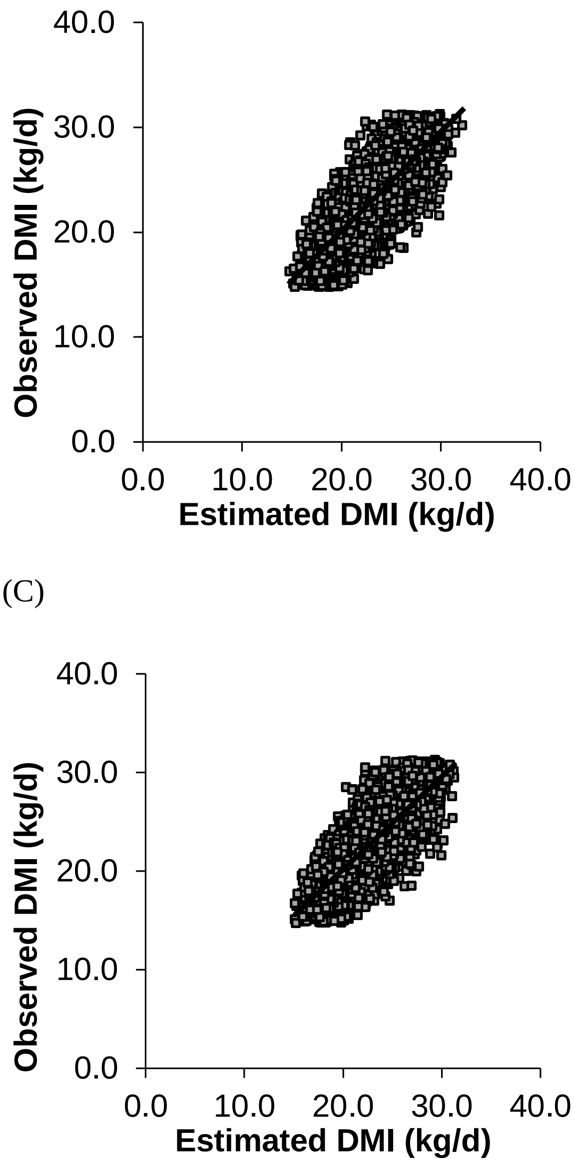
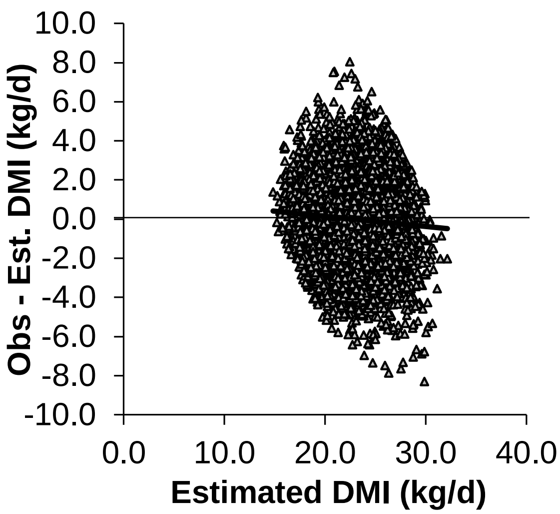
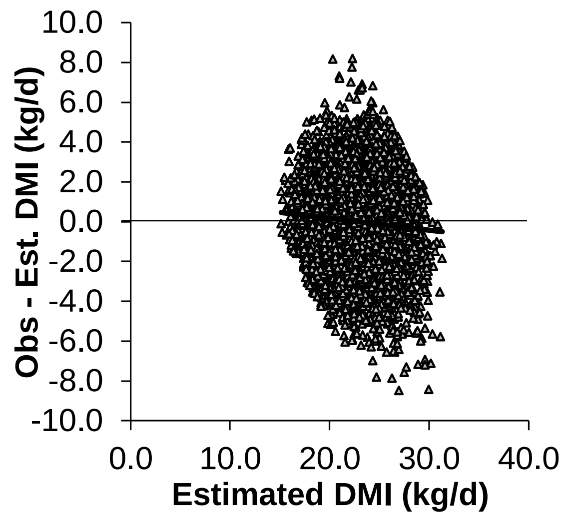


Figure 3.6. Plots of residuals (observed – estimated DMI values) regressed on centered estimated DMI values (each DMI estimated – mean of all DMI estimations) for the evaluation of mean and linear biases in the following 3 selected DMI estimation equations. (A) y = -0.05219(x - 22.4294) + 0.02073; intercept P = 0.3376; intercept SE = 0.02162; slope P < 0.0001; slope SE = 0.00769; R^2 = 0.0057; RMSE = 1.9432 (DMI Equation 2), (B) y = -0.05874(x - 22.4263) + 0.02383; intercept P = 0.2799; intercept SE = 0.0221; slope P < 0.0001; slope SE = 0.00788; R^2 = 0.0068; RMSE = 1.9823 (DMI Equation 3), and (C) y = -0.0280(x - 22.4403) + 0.00978; intercept P = 0.6363; intercept SE = 0.0207; slope P = 0.0002; slope SE = 0.0074; R^2 = 0.0018; RMSE = 1.8596 (DMI Equation 6). Although mean biases were not statistically significant for any of these equations, all 3 DMI estimation equations had statistically significant linear biases.

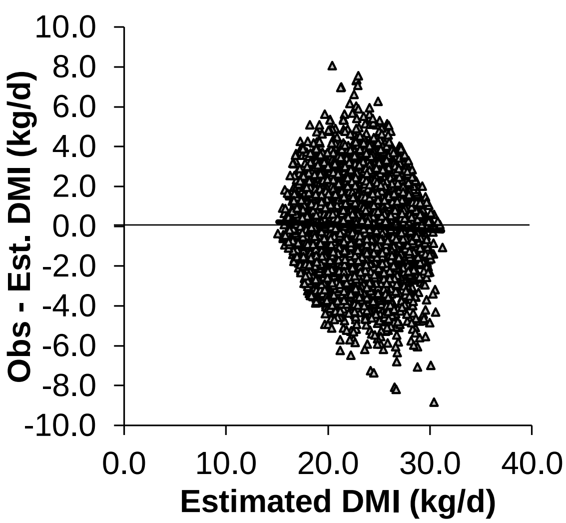
(A)



(B)



(C)



CHAPTER 4: EXPERIMENT 2

Determination of the relative discriminatory power of several biological, production, and dietary factors that affect the dairy FE ratio using 3 complementary discriminant analyses[1]

[1]Iwaniuk, M. E., E. E. Connor, and R. A. Erdman. Determination of the relative discriminatory power of several biological, production, and dietary factors that affect the dairy FE ratio using 3 complementary discriminant analyses. In preparation for submission to the Journal of Dairy Science.

INTERPRETIVE SUMMARY

Determination of the relative discriminatory power of several biological, production, and dietary factors that affect the dairy feed efficiency ratio using 3 complementary discriminant analyses. *Iwaniuk et al., page 000.* Using a dataset provided by the USDA, 3 complementary discriminant analyses (DAs) were conducted in order to determine the relative importance of biological, production, and dietary factors on dairy feed efficiency (**FE**), which was calculated as ECM per unit of DMI. The following variables were used to develop the discriminant function: BW, days in milk (DIM), calving month, parity, milk fat yield, milk protein yield, MUN, NE_L, CP, and NDF. If daily data were missing, BW, milk yield, milk fat, milk protein, and MUN were estimated using a generalized linear modeling technique. The results of the discriminant analyses indicated that cows can be successfully separated (≤ 10.04% error rate) into High (FE ≥ 2.12) and Low (FE ≤ 1.79) FE groups using biological, production, and dietary parameters in which milk fat yield, DIM, and BW were the 3 most important variables to consider when predicting FE group membership.

Determination of the relative discriminatory power of several biological, production, and dietary factors that affect the dairy FE ratio using 3 complementary discriminant analyses.

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ABSTRACT

Dairy feed costs account for approximately 50% of the total costs associated with milk production. In order to reduce feed costs per unit of milk produced, dairy producers are interested in selecting cows with high feed efficiency (**FE**). However, most cows are fed in a group setting such that the DMI of an individual cow basis is unknown. Thus, FE ratios for individual cows cannot be calculated on most dairy farms. Because several factors affect dairy FE, the hypothesis of this study was that dairy cows could be successfully separated into High and Low FE groups based on commonly-measured biological, production, and dietary parameters without requiring individual DMI to be measured. Therefore, the objective of this study was to differentiate between High and Low FE dairy cows based on several factors and to determine the relative discriminatory power of each factor on FE group assignment. The dataset for this study was provided by the United States Department of Agriculture and it contained 7,750 weekly production records averaged by cow for 522 cows across 334 weeks. Dairy FE was calculated for each weekly cow record and weekly cow means were classified into the following 4 equal quartiles based on their FE values: 1) FE $\leq$ 1.79, 2) 1.79 $<$ FE $\leq$ 1.94, 3) 1.94 $<$ FE $<$ 2.12, and 4) FE $\geq$ 2.12. Because most dairy producers only select cows that are either substantially above or below average, only the top (FE $\geq$ 2.12) and bottom (FE $\leq$ 1.79) 25% of weekly cow FE means were retained for the discriminant analyses resulting in 1,899 weekly cow records per FE group. A stepwise discriminant analysis (SDA) was used as a data reduction technique to reduce the number of variables used in the canonical (CDA) and basic discriminant analyses (DA). Nine variables were selected based on the SDA: body weight (**BW**; kg/), days in milk, calving month, parity, milk fat yield (g/d), milk urea nitrogen (**MUN**; mg/dL), net energy of lactation (**NE$_L$**; Mcal/kg), crude protein (**CP**; %), and neutral detergent fiber (**NDF**; %). After the SDA, the data were randomly split into a training data set (70.01%) which was used to develop the canonical (**CAN**) function and the test dataset (29.99%) which was used to assess if the High and Low FE groups were effectively separated by the CAN function. The CDA and DA were performed using all 9 original variables, and the results of these analyses indicated that cows can be successfully separated ($\leq$ 10.04% error rate) into High and Low FE groups using commonly-measured parameters. Once the performance of the full-model CAN function had been assessed, original variables were systematically removed from the CDA to generate 12 reduced CAN functions. Variables were removed in order of their increasing discriminatory power based on the partial R^2 results of the SDA. Milk fat yield, DIM, and BW had the most discriminatory power and using only these 3 variables to predict FE group membership resulted in a misclassification error rate of approximately 11.01%. The discriminatory power of milk fat yield, DIM, and BW was not equal. When used as the sole variable included in the CAN function, milk fat yield, DIM and BW has misclassification error rates of 22.30, 28.04, and 45.12%, respectively. In conclusion, dairy producers can successfully select between High and Low FE cows based on several commonly-measured parameters without requiring the costly and labor-intensive measurement of DMI.

Key Words: feed efficiency, discriminant analysis, milk fat yield, prediction

INTRODUCTION

Dairy feed costs represent the single largest expense associated with milk production on dairy farms (Beck and Ishler, 2016; Hardie et al., 2017; Valvekar et al., 2010). Currently, feed costs account for approximately 50% of total production costs for milk production (Beck and Ishler, 2016; USDA-ERS, 2018; Hardie et al., 2017). Because feed costs affect profitability, dairy producers are interested in calculating feed efficiency (**FE**) on an individual cow basis such that highly efficient cows can be selected for current and future herds through management and genetic selection (Erdman, 2011). Ultimately, selecting for high efficiency cows will reduce feed costs as well as the environmental impact of milk production while improving producer profitability and increasing milk production to meet the demands of the growing global population (Capper et al., 2009; VandeHaar et al., 2016).

One of the most common methods used to estimate dairy FE is to calculate the ratio of energy-corrected milk (**ECM**; kg/d) per unit of dry matter intake (**DMI**; kg/d) (DRMS, 2014). One major issue associated with calculating the FE ratio is that DMI is rarely measured on individual cows on most dairy farms as DMI measurements tend to be costly and labor intensive (Connor et al., 2013; Faverdin et al., 2017; Halachmi et al., 2004). Thus, it would be advantageous for dairy producers to be able to differentiate between high and low efficiency cows in their herds without measuring DMI,

Research has shown that several biological, production, and dietary factors affect dairy FE. For example, St-Pierre (2012) demonstrated that stage of lactation, or days in milk (**DIM**), significantly affects FE such that FE is highest during early lactation (~60 DIM) and steadily declines until a cow reaches the dry off period (305 DIM). Dairy FE changes throughout lactation because cows reach their peak milk production at approximately 60 DIM during which time their DMI has not yet peaked and they are mobilizing body tissue to support the demands of milk production such that FE is high (NRC, 2001). However, as lactation continues, milk production decreases while DMI increases until a plateau is reached which results in reduced FE values. Thus, FE is dependent on the stage of lactation in lactating dairy cows.

In addition, parity affects FE. Lee and Kim (2006) found that there was a significant linear increase in the average 305-d milk production from first (8,431 kg) to fourth (10,812 kg) lactation Holstein cows. The differences in milk production between primiparous and multiparous dairy cows can be attributed to the fact that primiparous cows are still growing; thus, a portion of their energy intake is partitioned to growth instead of milk production (NRC, 2001). In addition to partitioning nutrients towards growth, primiparous cows are also typically smaller in stature and BW compared to multiparous cows, which results in reduced milk yield and DMI. Lastly, research has shown that multiparous cows have increased metabolic activity of the secretory cells in the mammary gland compared to primiparous cows, especially in early lactation, and this may account for differences in milk production between parities (Miller et al., 2006).

Other biological factors affect FE such as calving month and BW. Research has shown that cows that calve during hot, summer months tend to have decreased DMI, milk yield, and milk component yields due to the negative effects of heat stress on production (Tao et al., 2018; Torshizi, 2016; Utrera et al., 2013). In addition, photoperiod has been shown to affect milk production such that cows exposed to long-day photoperiods (16 to 18 h of light/d) produced an average of 2.5 kg/cow/d more milk compared to cows exposed to short-day photoperiods (≤ 12 h of light/d) due to changes in endocrine mechanisms that regulate lactation (Dahl et al., 2000). Because calving month dictates the month in which a cow enters lactation, it is possible that cows that calve during months associated with long-day photoperiods (May to August) may have increased milk production, and subsequently FE, compared to cows that calve during months associated with short-day photoperiods (September to April; Dahl et al., 2000). In regard to BW, Linn (2006) compared the FE (3.5% FCM per unit of DMI) of smaller cows to larger cows and found that FE decreased from 1.52 to 1.30 as BW increased from 544 to 816 kg. The decreased FE is a result of increased DMI as the larger cows require more nutrients to meet maintenance requirements compared to smaller cows (Linn, 2006; NRC, 2001). Thus, increasing BW increases maintenance requirements which can result in increased feed intake and reduced FE, depending on the cow's milk production (Heinrichs et al., 2016).

In addition to biological factors, production factors also affect FE (Erdman, 2011; Heinrichs et al., 2016; Ishler, 2016). Because FE is calculated as the ratio of ECM per unit of DMI, cows that epigenetically have higher milk yields, milk fat concentrations, or milk protein concentrations, tend to have higher FE values as increases in these parameters result in increased in the numerator of the FE ratio (ECM). Other milk components, such as milk urea nitrogen (**MUN**; mg/dL) may affect FE; however, previous research on this topic is limited.

Lastly, it is well known that diet composition can have substantial effects on milk and milk component production as well as DMI and these effects may result in altered FE. Research has shown that increasing the net energy of lactation (**NE_L**; Mcal/kg) through fat supplementation can increase dairy FE (Onetti et al., 2001; Weiss and Pinos-Rodriguez, 2009; Zou et al., 2007). Similarly, increasing dietary crude protein concentration (**CP**; %) has been shown to increase milk yield and milk fat yield which may subsequently increase FE (Broderick et al., 2015; Kalscheur et al., 1999). Lastly, research has shown that decreasing neutral detergent fiber (**NDF**; %) results in increased milk fat concentration which can result in increased FE (Kellogg et al., 2009; Oba and Allen, 2009).

Because several factors have been shown to affect dairy FE, the hypothesis of this study was that commonly-measured biological, production, and dietary parameters could be used to differentiate High and Low FE cows without requiring DMI measurements. Therefore, the objective of this study was to develop and assess a discriminant function that utilizes these commonly-measured parameters to distinguish between High and Low FE cows. This objective was completed using 3 complementary discriminant analyses (**DA**). The results of this study can be utilized dairy producers to select for High FE cows within their herd to reduce feed costs, increase production, and improve profitability using commonly recorded measurements.

MATERIALS AND METHODS

Initial Database

The data used for this modeling project were obtained from the laboratory of Dr. Erin Connor at the United States Department of Agriculture (**USDA**; Beltsville, Agricultural Research Center, Beltsville, MD). All data collection involving animals was approved by the Northeast Area Animal Care and Use Committee. The initial dataset contained production records for 529 lactating Holstein cows, which resulted in 95,633 daily production observations. To remove natural variation associated with production parameters for cows in the transition period as well as late lactation, individual cow observations with days in milk (**DIM**) less than or equal to 21 DIM or greater than or equal to 150 DIM were removed from the dataset. Removing individual cow observations based on DIM resulted in an initial dataset that contained production records for 529 lactating Holstein cows and 70,672 daily production observations.

Estimation Equations and Outlier Removal for Key Production Variables

To be included in the final dataset, each daily individual cow production record was required to have the following parameters: DMI (kg/d), body weight (**BW**; kg/d), milk yield (**MY**; kg/d), milk fat (%), milk protein (%), and MUN (mg/dL). If a daily production record was missing DMI, the entire record was removed from the dataset. If a daily production record was missing BW, MY, milk fat (%), milk protein (%), and/or MUN, the parameters were individually estimated by cow and lactation number using PROC GLM (SAS 9.4, SAS Institute, Cary. NC) using the estimation equations shown in Table 4.1. Milk yield, milk fat (%), milk protein (%), and MUN were estimated per milking (2X/d; AM vs. PM). To determine the success of the estimation equation, measured parameter values were regressed on estimated parameter values using PROC REG (SAS 9.4) and estimations were evaluated based on the following criteria: coefficient of determination (**R^2**), root-mean-square error (**RMSE**), and *P*-value as shown in Figures 4.1 – 4.5. During the regression analysis, outliers for each parameter were removed if the R-Studentized residual was less than -3 or greater than +3. If a parameter had a missing value (either inherently missing or removed during outlier detection), these values were replaced with the estimated values generated using PROC GLM (SAS 9.4). The use of estimated values in this dataset was particularly critical for the BW, milk fat (%), milk protein (%), and MUN variables as these parameters were only measured biweekly with milk components determined during alternate morning and evening milkings each week whereas DMI and MY (AM and PM) were measured and recorded daily. After the estimation equations and outliers were removed for the key production variables, the dataset contained 70,175 observations which contained a daily measured DMI and either measured or estimated values for BW, MY, milk fat (%), milk protein (%), and MUN for each cow.

Data Management and Weekly Cow Means

Individual daily cow production records were averaged by cow by week. Individual weekly cow means were removed from the dataset if an individual cow had fewer than 5 out of 7 daily production records per week. This data removal reduces variation within the dataset and ensures that weekly means have relatively similar weighting. After weekly production means were calculated for each cow and data were removed, the dataset contained 10,089 weekly mean observations.

Final Outlier Removal for Key Variables Used in the Discriminant Analyses

A final procedure was performed to remove any outliers that may have been generated from the estimations of BW, MY, milk fat (%), milk protein (%), or MUN. Outlier removal was performed using PROC UNIVARIATE (SAS 9.4, Cary, NC) such that any values greater than the 99% quantile or less than the 1% quantile for each variable were removed. After these outliers were removed from the dataset, the dataset contained 8,742 weekly cow mean observations.

Grouping the Data into 2-wk Intervals

The last data management step conducted prior to the discriminant analyses involved grouping the individual cow weekly means data into 2-wk intervals. If a 2-wk interval had fewer than 30 weekly cow means observations, then that 2-wk interval was removed from the dataset in order to reduce variation and ensure that each 2-wk interval had similar weighting. The final complete dataset contained 7,750 weekly cow mean observations and 167 2-wk intervals. The descriptive statistics for the final dataset are presented in Table 4.2. It is important to note that this procedure was conducted so that the dataset contained 2-wk intervals which were used to predict DMI for RFI calculations completed in Chapter 5. Because the discriminant analyses as well as the dataset for Chapters 4 and 5 were identical, these 2 chapters should be viewed as companion studies.

Categorizing Cows into High and Low FE Groups

Prior to conducting the discriminant analyses, FE was calculated for each weekly cow mean based on the ratio of ECM per unit of DMI (DRMS, 2014; Tyrrell and Reid, 1965). Outlier removal was performed using PROC UNIVARIATE (SAS 9.4) such that any FE values greater than the 99% quantile or less than the 1% quantile were removed. Using PROC UNIVARIATE and PROC FREQ in SAS (SAS 9.4), weekly cow means were classified into the following 4 equal quartiles based on their FE values: 1) FE $\leq$ 1.79, 2) 1.79 $<$ FE $\leq$ 1.94, 3) 1.94 $<$ FE $<$ 2.12, and 4) FE $\geq$ 2.12.

Weekly cow means within the second and third quartiles (1.79 $<$ FE $<$ 2.12) were removed from the dataset such that only the weekly cows means within the 25% highest and 25% lowest FE groups remained in the dataset. The final dataset contained 1,899 weekly cow means for each FE group and the descriptive statistics for each group are presented in Table 4.3.

Discriminant Analyses

Discriminant analysis (**DA**) is a multivariate statistical technique that utilizes several continuous variables within a dataset to develop a discriminant function that can effectively discriminate between 2 or more known, categorical groups (Fisher, 1936; Martínez Marín et al., 2012; McLachlan, 2004). After the discriminant function is derived from a modeling dataset, the discriminant function can then be subsequently applied to new data with unknown groupings such that group membership can be predicted based on several continuous variables (Conte et al., 2018; Martínez Marín et al., 2012; McLachlan, 2004). Three complementary Das were conducted to discriminate between High and Low FE lactating dairy cows: stepwise DA (**SDA**), canonical DA (**CDA**), and DA.

The STEPDISC procedure (SAS 9.4) was used to a conduct a SDA to select a subset of a continuous, quantitative variables that have potential discriminatory power to distinguish between the 2 known FE groups using a series of Wilks' lambda tests to determine if variables should enter, remain in, or be removed from the model (Jennrich, 1977; Klecka, 1980). The SDA was applied to the following 10 variables and the most discriminant variables were selected for the CDA and DA: milk fat yield (g/d), milk protein yield (g/d), MUN (mg/dL), BW (kg), dietary CP (%), dietary NDF (%), dietary NE_L (Mcal/kg), DIM, parity, and calving month (Conte et al., 2018). The significance levels to enter and stay in the model were both set at 0.15 (Constanza and Afifi, 1979).

After the variables with the most discriminatory power to separate High and Low FE cows were selected using SDA, the dataset was divided into 2 groups: a training dataset (70.01%) that was used to develop the discriminant functions and a test dataset (29.99%) to evaluate the predictive performance of the discriminant functions. A random value was assigned to each weekly cow mean using the PROC RANNOR (SAS 9.4) and then cows were randomly assigned to the training or test datasets.

Once training and test datasets were established, CDA was conducted on the training dataset using PROC CANDISC (SAS 9.4) with prior probabilities proportional to sample sizes and a parametric, linear classification structure. The CDA is a dimension-reduction multivariate technique that utilizes a set of continuous, quantitative variables and a classification variable (FE group) to derive a canonical function (**CAN**) that provides maximal separation between the known groupings (Conte et al., 2018). The CAN is a new, linear combination of the original continuous, quantitative variables in the dataset. The function consists of canonical coefficients (**CC**; c_i) which are derived from methodology similar to that of multivariate analysis of variance (MANOVA) and scores of the original variables (Xi; Conte et al., 2018). The weight of the CC reflects the weighted contribution of each original variable within the CAN (Conte et al., 2018). An example of the CAN function is written in Equation 1:

$$\text{CAN} = c_1\text{X}_1 + c_2\text{X}_2 + c_3\text{X}_3 + c_4\text{X}_4 + \ldots + c_n\text{X}_n \tag{1}$$

As previously mentioned, the primary goal of the CAN function is to provide the maximum amount of separation between the known groupings using a new linear combination of the original variables (Conte et al., 2018). The number of CANs extracted from an analysis depends on the number of known groupings (***k***) as CANs extracted are always $k - 1$. In this study, there were 2 known groups (High FE and Low FE); thus, the number of CANs extracted was one (Conte et al., 2018).

To determine if the High and Low FE groups were effectively separated by the CAN function, the Mahalanobis distance was assessed (Conte et al., 2018; De Maesschalck et al., 2000). Mahalanobis distance measures the distance in standard deviations of a data point from the mean of a distribution. In this CDA, the Mahalanobis distance was calculated as follows: 1) the CAN function was applied to each weekly cow production record such that each record has a calculated discriminant score (**DS**), 2) the centroids (multivariate means) of the High and Low FE groups were calculated, 3) the distance of each DS to the 2 centroids was measured in standard deviations, and 4) each DS was assigned to either the Low or High FE group based on the smallest distance to the that group's centroid (Conte et al., 2018; Mardia et al., 2000). Once weekly cow means were assigned to a FE group, the accuracy of group separation was assessed using error rate calculations in the DA (PROC DISCRIM; SAS 9.4). Additionally, Hotelling's T-square test was utilized to determine the efficacy of the CDA as this statistical test is synonymous with Student's t-test in that it compares the multivariate distributions of the High and Low FE groups (Conte et al., 2018). Significance was declared at $P \leq 0.05$.

To evaluate the performance of the CDA, resubstitution and cross-validation misclassification error rates were examined (Braga-Neto et al., 2004). Essentially, the resubstitution method uses all data (sample size, n) in the training dataset to generate a CAN function and then measures the misclassification of all data points in the training dataset (Zollanvari et al., 2010). Conversely, the cross-validation method uses a series of $n - 1$ datasets within the training dataset to generate parameter estimates in the CAN function and then uses the average of all of the parameter estimates to develop the final CAN function for the training dataset (Efron and Stein, 1981). Error rates tend to be lower using the resubstitution method as all "tested" data points are used in model development whereas cross-validation (or jack-knifing) omits one data point per iteration (Braga-Neto et al., 2004). Both methods are reported in this study for the training dataset.

In addition, the CAN function developed from the training dataset was applied to the test dataset to predict population membership of individual weekly cow means using resubstitution error rate methods (Huberty, 1994; Braga-Neto et al., 2004). Cross-validation error rates are not reported for the test dataset as the test dataset was not used to develop the CAN function.

It is important to note that this CAN function contained terms for all 9 original variables that were selected during the SDA: milk fat yield (g/d), BW (kg), DIM, NE_L (Mcal/kg), MUN (mg/dL), calving month, parity, CP (%), and NDF (%). Once the performance of the full-model CAN function was assessed, original variables were systematically removed from the CDA to generate 12 reduced CAN functions. Variables were removed in order of their increasing discriminatory power based on the partial R^2 results of the SDA. Performance evaluation of the reduced CAN functions to predict population membership of individual weekly cow means was assessed in the training dataset using resubstitution and cross-validation error rate methods and in the test dataset using the resubstitution method (Huberty, 1994; Braga-Neto et al., 2004). Based on the assessments of the reduced CAN functions, original variables were ranked in relative importance based on their discriminatory power to predict High or Low FE cows.

RESULTS AND DISCUSSION

Estimation of 5 Key Production Parameters

Prior to conducting a series of discriminant analyses to determine factors that can effectively distinguish between Low and High FE cows, 5 key production parameters were estimated on a daily, individual cow basis to fill in missing data points within the dataset. As presented in Table 4.1., estimation equations were developed for BW (kg), milk yield (kg/d), milk fat concentration (% per milking), milk protein concentration (% per milking), and MUN (mg/dL). To assess the performance of each equation, regression analyses were conducted between measured and estimated production parameters, as shown in Figures 4.1 to 4.5. Individual cow BW (kg/d) was estimated using DIM and DIM^2 as the equation parameters and these parameters accounted for approximately 98.5% of the total variation in BW measurements ($R^2 = 0.98$; RMSE = 8.24; $P < 0.0001$; Figure 4.1.). Similar to the BW estimation equation, the estimation equation for MY (kg/milking) also contained DIM and DIM^2 as equation parameters as well as time (AM vs. PM) as the MY variable was expressed in kilograms per milking and cows were milked 2X daily. The MY estimation equation accounted for approximately 86.5% of the total variation associated with milk yield (kg) per milking ($R^2 = 0.865$; RMSE = 1.76; $P < 0.0001$; Figure 4.2.). Lastly, the estimation equations for milk fat percentage, milk protein percentage, and MUN (mg/dL) contained the following terms: DIM, DIM^2, time (AM vs. PM), milk yield per milking (**Milk**), and the interaction between time and milk. The milk fat and protein percentage estimations accounted for approximately 85.2% ($R^2 = 0.852$; RMSE = 0.222; $P < 0.0001$; Figure 4.3.) and 92.8% ($R^2 = 0.928$; RMSE = 0703; $P < 0.0001$; Figure 4.4.) of the total variation associated with milk fat and protein concentrations, respectively. Lastly, MUN estimations accounted for approximately 84.1% ($R^2 = 0.841$; RMSE = 1.22; $P < 0.0001$; Figure 4.5) of the total variation associated with MUN.

The results of the estimation equations for the aforementioned production variables were similar to the results of previously published estimation equations for these parameters. Franco et al. (2017) evaluated 6 published equations that predicted BW in growing Holstein heifers based on several body measurements (heart girth, body length, wither height, hip height, and hip width) and reported that these equations accounted for approximately 84.6 – 93.4% of total variation associated with BW which is similar to the variation explained (98.5%) by the BW estimation equation reported in the current study (Figure 4.1.). In regard to milk yield, Otwinowska-Mindur et al. (2015) compared 6 equations that estimated milk yield based on time (AM vs. PM milking), milking interval, DIM, and parity. The authors reported that these equations accounted for approximately 81.0 – 86.5% and 82.8 – 88.4% of the total variation associated with milk yield in the morning and evening milkings, respectively (Otwinowska-Mindur et al., 2015). These results are congruent with the current study in which the milk yield estimation equation accounted for approximately 86.5% of the total variation associated with milk yield (kg/milking; Figure 4.2.). Liu et al. (2000) developed and validated 6 models that estimated milk yield as well as milk fat and protein yields at morning and evening milkings. The authors reported that the accuracy (R^2 in percentage) of predictions ranged between 75.6 and 83.0% in estimating milk fat yield across all lactations (Liu et al., 2000). Although milk fat was estimated as a percentage in this study, the accuracy of the estimation (R^2 = 0.852; Figure 4.3.) was very similar to the best model (R^2 = 0.830) developed by Liu et al. (2000). Klopčič et al. (2003) compared 8 equations that estimated milk protein percentage using the following parameters: time (AM vs. PM), milking interval, breed, DIM, and parity. The protein percentage prediction equations accounted for approximately 95.6 and 97.6% of the total variation associated with milk protein percentage in the morning and evening milkings, respectively (Klopčič et al., 2003). The milk protein percentage estimation equation in the current study accounted for approximately 92.8% of the total variation associated with milk protein (%) which are similar to the results of the aforementioned publication (Figure 4.4.). Lastly, the MUN estimation equation in the current study accounted for approximately 84.1% of the total variation association in MUN. Although MUN has become a useful, non-invasive management tool in the dairy industry to assess protein and energy balance of cows within a herd, very little work has been done to develop equations to predict and/or estimate MUN in individual cows (Hof et al., 1997; Schepers and Meijer, 1998). Therefore, the estimation of MUN based on DIM, milk yield, and time (AM vs. PM) is a novel component of this study (Figure 4.5).

In conclusion, the estimation equations for BW, milk yield, milk fat (% per milking) milk protein (% per milking), and MUN developed in this study adequately estimated each production parameter; thus, missing values in the dataset were replaced by estimated values such that each cow had a complete daily production record prior to the DAs.

SDA of High and Low FE Cows Using Biological, Production, and Dietary Variables

As shown in Table 4.4., the SDA selected the following 9 variables based on discriminatory power (partial R^2): milk fat yield (R^2 = 0.3820), BW (R^2 = 0.2152), DIM (R^2 = 0.1648), NEL (R^2 = 0.0614), MUN (R^2 = 0.0157), calving month (R^2 = 0.0127), parity (R^2 = 0.0089), CP (R^2 = 0.0055), and NDF (R^2 = 0.0012). Of the 10 original variables, only milk protein yield (g/d) was eliminated from the analysis as a weak predicator variable of dairy FE groups.

One of the goals of SDA is to develop a discriminant function that successfully distinguishes between known groupings of a classification variable by selecting for the fewest number of predicator variables that contribute the most discriminatory power towards accurate group assignments (Munita et al., 2006). Because milk fat yield and milk protein yield are both calculated using the same milk yield value for each individual cow record, it is possible that these 2 terms "compete" in the discriminant model as they share redundant information (Munita et al., 2006). It is well understood that milk fat concentration is the most variable component of milk whereas milk protein concentration is relatively constant (Bauman et al., 2011; Varga and Ishler, 2010). It is possible that milk fat yield was selected over milk protein yield in the discriminant model because milk fat yield experiences larger variations which may be more impactful on changes in dairy FE as compared to the smaller fluctuations observed in milk protein yields. In addition, it is also possible that milk fat remained in the SDA as it contains a higher energy concentration compared to milk protein which would result in a larger effect on ECM. Due to its lack of discriminatory power, milk protein yield was not used in the CDA to develop the full-model or reduced CAN functions.

CDA of High and Low FE Cows Using Biological, Production, and Dietary Variables

The CDA was conducted using the 9 aforementioned selected variables in the training dataset (70.01%) to create one CAN function that successfully discriminated between High and Low FE cow groups (*P*-value for Mahalanobis Distance < 0.0001; *P*-value for Hotelling's *t*-test < 0.0001). The CAN function that was produced explained approximately 64.0% of the total variation between High and Low FE cow groups and a visualization of separation based on CAN function is presented in Figure 4.6. The canonical coefficients and canonical structure (correlations between individual variables and the canonical scores) for each of the nine original variables are presented in Table 4.7. The CAN function was positively correlated with milk fat yield (r = 0.775), NE_L (r = 0.425), MUN (r = 0.086), calving month (r = 0.051), and parity (r = 0.186), but negatively correlated with BW (r = -0.212), DIM (r = -0.624), CP (r = -0.080), and NDF (r = -0.224). According to class means, the CAN function is positively correlated with increasing FE (Low FE = -1.337; High FE = +1.328). Therefore, it can be concluded that FE is positively correlated with increasing milk fat yield, dietary NE_L concentration, MUN, calving month, and parity, but negatively correlated with increasing BW, DIM, and dietary CP and NDF concentrations.

Once the CAN function was developed, its ability to differentiate between High and Low FE cows in the test dataset was assessed in the training dataset using resubstitution and cross-validation methods (Tables 4.8. and 4.9.). Using the resubstitution method, 85 (of 1,325) Low FE weekly cow means were misclassified as High FE weekly cows means and 142 (of 1,334) High FE weekly cow means were misclassified as Low FE weekly cow means, resulting in a combined misclassification error rate of 8.54%. Similarly, the cross-validation method resulted in 89 (of 1,325) Low FE weekly cow means that were misclassified as High FE weekly cow means and 143 (of 1,334) High FE weekly cow means that were misclassified as Low FE weekly cow means, resulting in a combined misclassification error rate of 8.73%. When the CAN function was applied to the test dataset, 41 (of 574) Low FE weekly cow means were misclassified as High FE weekly cow means and 73 (of 565) High FE weekly cow means were misclassified as Low FE weekly cow means, resulting in a combined misclassification error rate of 10.04%. Based on these results, it can be concluded that the CAN function successfully differentiates between High and Low FE cows as the error rates of misclassification were fairly low (≤ 10.04% error). In regard to practical application, these results suggest that dairy producers can confidently select High and Low FE cows within their herd using commonly measured biological, production, and dietary parameters without requiring a costly and labor-intensive measurements of DMI.

After the full-model CAN function was developed and assessed, reduced CAN functions were systematically developed and evaluated as described above to determine the relative discriminatory power of each variable in the CAN function. These results are presented in Tables 4.8 and 4.9.

Variables with Low Discriminatory Power

In both the training and test datasets, removing dietary NDF (%), dietary CP (%), parity, calving month, MUN (mg/dL), and dietary NE_L (Mcal/kg) from the CAN functions did not have a significant impact on misclassification error rates (training error ≤ 8.84%;

test error: ≤ 10.30%). These results suggest that these parameters do not have significant discriminatory power to distinguish between High and Low FE dairy cows.

Parity and Calving Month

Including parity and calving month in the CAN function did not significantly contribute to the overall discriminatory power of the function (Tables 4.8 and 4.9). In this study, parity was weakly, but positively correlated with FE ($r = 0.186$) which is consistent with previous research suggesting that primiparous cows are less feed efficient compared to multiparous cows as primiparous cows are smaller, consume less DMI, sanction a portion of their intake energy towards growth, and have reduced metabolic activity of milk secretory cells in the mammary gland (Lee and Kim, 2006; Miller et al., 2006; NRC, 2001). However, parity did not contribute a significant amount of discriminatory power to the CAN function. Because FE was measured as the ratio of ECM per unit of DMI, it is possible that primiparous and multiparous cows had similar ratios, despite primiparous cows consuming less feed or producing less milk. Additionally, BW was found to have high discriminatory power (discussed below); therefore, it may be possible that parity effects on FE were minimized and attributed to BW as BW and age (parity) are strongly correlated.

Calving month also lacked significant discriminatory power to distinguish between High and Low FE cows when included in the CAN function. Calving month has been shown to affect FE as heat stress in warm months can reduce DMI, milk yield, and milk component yield (Tao et al., 2018; Torshizi, 2016; Utrera et al., 2013). In addition, calving month is also associated with photoperiodic effects on lactation such that cows exposed to long-day photoperiods (16 to 18 h of light/d; May to August) produced an average of 2.5 kg/cow/d more milk compared to cows exposed to short-day photoperiods (≤ 12 h of light/d; September to April; Dahl et al., 2000). However, calving month in this study did not significantly contribute to differences in High and Low FE cow assignments and lacked a strong correlation with FE ($r = 0.051$). It is possible that calving month has been shown to affect FE as calving month is indirectly related to heat stress or photoperiod; thus, heat stress or photoperiod, not calving month (indicated on a 1 to 12 scale), may have more discriminatory power in the CAN function. However, heat stress or photoperiod were not measured in this dataset so their effects could not be determined.

Milk Urea N

In addition to the biological parameters, MUN concentration also did not have significant discriminatory power to distinguish between High and Low FE cows (Tables 4.8 and 4.9). Currently, dairy producers utilize MUN concentrations in the milk to estimate the overall protein status of the cow as blood urea concentration (BUN; mg/dL) is indicative of protein metabolism efficiency and MUN is strongly correlated to BUN (Ishler, 2016; Kohn, 2007; Roseler et al., 1997). Because MUN is indicative of a cow's protein status and her protein status affects her FE, it was hypothesized that MUN concentration may be associated with FE status. However, the results of this study indicated that MUN lacked a strong correlation to FE ($r = 0.086$) such that MUN did not have significant discriminatory power to differentiate between High and Low FE dairy cows. Future research should be conducted that explores the relationship between protein status as indicated by MUN concentrations and dairy FE.

Dietary Variables

In regard to the dietary variables, it is well known that diet composition can affect production variables which subsequently alter dairy FE. For example, research has shown that increasing dietary CP and NE_L concentrations have resulted in increased milk yield and milk fat yield which may translate into improved ECM and FE (Broderick et al., 2015; Kalscheur et al., 1999; Onetti et al., 2001; Weiss and Pinos-Rodriguez, 2009; Zou et al., 2007). Increasing CP and NE_L concentrations in the ration results in improved milk and milk component yield as additional dietary protein and energy can be utilized for milk and component production purposes (Onetti et al., 2001; Weiss and Pinos-Rodriguez, 2009; Zou et al., 2007). Similarly, research showed that decreasing dietary NDF concentration results in increased milk yield or milk fat concentration which can translate into improved FE (Kendall et al., 2009; Oba and Allen, 2009; Ruiz et al., 1995). Because NDF is less digestible than other non-fiber carbohydrate sources, increasing dietary NDF decreases energy intake which could be sanctioned to milk and component production (NRC, 2001). Thus, it was hypothesized that varying dietary CP, NE_L, and NDF concentrations may be associated with different levels of FE in dairy cows.

As shown in Table 4.7, the results of this study indicated that dietary NEL was moderately and positively correlated with FE (r = 0.425) which supports previous research conclusions. In addition, dietary NDF concentrations exhibited a weak, negative correlation with FE (r = -0.224) which also supports previous research. In regard to CP, the results of this study indicated that CP is negatively correlated with FE (r = -0.080) which is inconsistent with previously published literature, but it is important to note that this correlation is very weak. Overall, the results of this study suggest that these dietary factors do not hold significant discriminatory power to discern between High and Low FE cows. All cows within this dataset received similar dietary treatments that were formulated to meet or exceed NRC (2001) requirements; thus, large variations of these dietary parameters were not present in this dataset as shown in Table 4.2, 4.3, 4.5, and 4.6. It is possible that because High and Low FE cows received similar dietary treatments, the discriminatory power of these dietary variables were reduced. A meta-analysis should be conducted using experiments with large variations in dietary parameters to further assess the effects of each dietary parameter on dairy FE using DAs.

Variables with High Discriminatory Power

Days in Milk

As presented in Tables 4.8 and 4.9, the CAN7 function containing milk fat yield, BW, and DIM resulted in misclassification error rates of 10.57, 10.64, and 11.01% using the resubstitution method in the training dataset, the cross-validation method in the training dataset, and the resubstitution method in the test dataset, respectively. Removing DIM from the CAN function (CAN8) in both the training and test datasets resulted in an approximate 4.0-5.0% increase in misclassification error rates, as presented in Tables 4.8 and 4.9. These results suggest that DIM contributes a significant portion of discriminatory power to the function. In fact, when included as the only variable in the CAN function (CAN11), the misclassification error rates are less than 28.04% in both the training and test datasets. In this scenario, using DIM as the only discriminatory variable accurately predicts FE group membership for 71.96% of the cows.

In CAN9, DIM was coupled with milk fat yield in the CAN function and the combined misclassification error rates for the training and test datasets were less than 14.70% (Tables 4.8 and 4.9; Figure 4.7). Thus, dairy producers could use these 2 variables to predict cow FE and correctly predict FE group membership for 85.30% of High and Low FE cows. Similarly, CAN10 contained only DIM and BW as discriminatory variables and the combined misclassification error rates in the training and test datasets were less than 27.96% (Tables 4.8 and 4.9; Figure 4.8). This error rate is similar to using only DIM as the discriminatory variable in the function so including BW in this model does not appear to be advantageous to correct group assignments.

The results of this study indicate that DIM is negatively correlated with dairy FE (r = -0.624; Table 4.7) which is to be expected because of the decline in milk production as DIM increases. This also supports previous literature that suggests FE decreases as stage of lactation increases due to the nature of milk yield and DMI curves (NRC, 2001; St-Pierre, 2012).

Body Weight

As presented in Tables 4.8 and 4.9, the CAN function containing milk fat yield and BW had misclassification error rates less than 15.46% for the both training and test datasets (Figure 4.9). Thus, dairy producers could accurately assignment FE group membership to 84.54% of High and Low FE cow using only BW and milk fat yield as discriminatory variables. On its own in the CAN function (CAN12), BW did not have significant discriminatory power as the misclassification error rates were 43.74 and 45.12% in the training and test datasets, respectively. Thus, it would not be recommended to base FE group assignment on BW alone as only approximately 54.88% of High and Low FE cows would be assigned correctly.

The results of this study indicate that BW is negatively correlated with dairy FE (r = -0.212; Table 4.7) which supports previous literature that stated that increasing BW decreases FE as a larger body size requires more nutrients to be used for maintenance instead of production (Linn, 2006; Heinrichs et al., 2016; NRC, 2001). This research further supports the concept that larger cows should not be selected in hopes of improving dairy FE (VandeHaar et al., 2016). Dairy cows have gotten larger over time. Pott's et al. (2017) reported that mean BW for Holstein cows increased by 1.8 kg over a 44-yr period from 1970 to 2014. In part, this may be due to genetic selection of cattle that was based primarily on milk yield without respect to body size. In general, larger cows will produce more milk but they also will eat more.

Milk Fat Yield

The results of this study indicate there is a strong, positive correlation between milk fat yield and dairy FE (r = 0.775) which are consistent with previous studies (Table 4.7). The CAN function (CAN13) that utilizes milk fat yield as the sole discriminatory variable resulted in misclassification error rates of 22.60 and 22.30 for the training and test datasets, respectively (Tables 4.8 and 4.9). Thus, accurate FE group assignment could be made for approximately 77.40% of High and Low FE cows when milk fat yield is used as the predicator variable. As indicated earlier, milk fat concentration is the most variable milk component (Bauman et al., 2011). The numerator of the dairy FE equation is ECM (kg/d) which requires milk yield, milk fat yield, and milk protein yield for its calculation (DRMS, 2014). Thus, it is no surprise that the variable with the most individual discriminatory power is milk fat yield as it is a major component in the calculation of dairy FE. However, the novel discovery of this study is that producers can adequately (77.40%) predict FE group membership in Holstein dairy cattle solely based on this variable. Fat yield and DIM are routinely recorded on dairy farms, such that a dairy producer could successfully assign FE group membership to High and Low FE cows 85.30% without requiring any additional labor-intensive and costly measurements, such as measuring DMI (Figure 4.7).

CONCLUSIONS

The results of this study suggest that commonly measured biological, production, and dietary variables can be utilized to successfully discriminate between High and Low FE dairy cows. Variables with low discriminatory power included: dietary NDF (%), dietary CP (%), parity, calving month, MUN (mg/dL), and dietary NE_L (Mcal/kg). The variables with the most discriminatory power included DIM, BW, and milk fat yield d) with DIM and milk fat yield being the most powerful discriminatory variables. The variable DIM was negatively correlated to FE (r = -0.624) while milk fat yield was positively correlated to FE (r = 0.775). The CAN function that contained only DIM and milk fat yield resulted in misclassification error rates less than 14.70%. Thus, it can be concluded that a dairy producer can successfully assign FE group membership to 85.30% of High and Low FE cows using milk fat yield and DIM as the sole discriminatory variables.

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Table 4.1. Estimation equations for BW[1], milk yield[2], milk fat (%), milk protein (%), and MUN[3].

Item	Model
BW[1], kg	= DIM[4] + DIMSq[5]
Milk Yield[2], kg/milking	= Time[6] + DIM + DIMSq
Milk Fat, %/milking	= Time + Milk[1] + Milk*Time[7] + DIM + DIMSq
Milk Protein, %/milking	= Time + Milk + Milk*Time + DIM + DIMSq
MUN[3], mg/dL/milking	= Time + Milk + Milk*Time + DIM + DIMSq

[1]BW = Body weight.
[2]Milk Yield = Milk yield per milking (AM vs. PM).
[3]MUN = Milk urea N (mg/dL per milking).
[4]DIM = Days in milk.
[5]DIMSq = DIM*DIM.
[6]Time = Time of milking (AM vs. PM).
[7]Interactive effect of milk yield (per milking) and time of milking (AM vs. PM).

Table 4.2. Descriptive statistics for the complete dataset prior to FE group[1] and dataset[2] assignment.

Item[3,4]	Mean	SD[5]	Minimum	Maximum
DMI[6], kg/d	22.5	3.3	14.7	31.2
Milk yield[7], kg/d	44.0	7.3	27.6	64.3
Milk fat, %	3.54	0.45	2.17	4.74
Milk fat yield, g/d	1554	297	758	2693
Milk protein, %	2.82	0.23	1.80	3.87
Milk protein yield, g/d	1234	190	798	1762
ECM[8], kg/d	44.0	7.0	27.0	67.3
BW[9], kg	583	61	456	764
MUN[10], mg/dL	11.8	2.6	4.7	18.3
Dietary CP[11], %	16.6	0.7	14.7	18.5
Dietary NDF[12], %	32.0	2.4	26.4	40.7
Dietary NE_L[13], Mcal/kg	0.77	0.02	0.73	0.84
Days in Milk (DIM)	65.9	27.24	23	142
Parity[14]	1.44	0.50	1	2
Calving Month[15]	7.30	3.26	1	12
FE (ECM/DMI)	1.97	0.24	1.44	2.70

[1]Weekly cow means were either assigned to Low or High FE groups.
[2]The data was divided into training (70.01%) and test (29.99%) datasets.
[3]The following continuous variables contain both actual and estimated values based on the estimation equations described in Table 4.1 and Figures 4.1 – 4.5: milk yield (kg/d), milk fat (%), milk protein (%), BW (kg) and MUN (mg/dL).
[4]Sample size for each variable (n) = 7,750 means averaged weekly on an individual cow basis.
[5]SD = standard deviation.
[6]DMI = Dry matter intake.
[7]Milk yield (kg/d) = AM Milk (kg/d) + PM Milk (kg/d).
[8]ECM = ((12.95 x lbs milk fat) + (7.65 x lbs milk protein) + (0.327 x lbs milk)/2.2) (DRMS, 2014).
[9]BW= Body weight.
[10]MUN = Milk urea N.
[11]CP = Crude protein (% DM basis).
[12]NDF = Neutral detergent fiber (% DM basis).
[13]NE_L = Net energy of lactation (Mcal/kg).
[14]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[15]Calving month ranges from January (1) to December (12).

Table 4.3. Descriptive statistics for the low (FE ≤ 1.79) and high (FE ≥ 2.12) FE groups prior to dataset[1] assignment and SDA.

Item[2]	Mean	SD[3]	Minimum	Maximum
Low FE Group (FE ≤ 1.79)				
DMI[4], kg/d	23.7	2.9	15.5	31.2
Milk yield[5], kg/d	39.9	5.8	27.6	59.0
Milk fat, %	3.46	0.48	2.17	4.72
Milk fat yield, g/d	1368	209	758	2058
Milk protein, %	2.92	0.24	2.25	3.87
Milk protein yield, g/d	1158	159	798	1662
ECM[6], kg/d	39.6	5.1	27.0	55.0
BW[7], kg	595	63	458	763
MUN[8], mg/dL	11.5	2.6	4.7	18.3
Dietary CP[9], %	16.6	0.7	14.7	18.5
Dietary NDF[10], %	32.4	2.3	26.4	40.7
Dietary NE_L[11], Mcal/kg	0.77	0.01	0.73	0.83
Days in Milk (DIM)	79.1	27.1	23.0	142.0
Parity[12]	1.38	0.49	1	2
Calving Month[13]	7.17	3.13	1	12
Dairy FE (ECM/DMI)	1.67	0.09	1.44	1.79
High FE Group (FE ≥ 2.12)				
DMI[4], kg/d	21.1	3.1	14.7	30.1
Milk yield[5], kg/d	47.7	7.2	30.0	64.3
Milk fat, %	3.72	0.39	2.41	4.74
Milk fat yield, g/d	1764	289	1010	2694
Milk protein, %	2.72	0.20	1.80	3.75
Milk protein yield, g/d	1297	197	800	1763
ECM[6], kg/d	48.4	7.1	32.6	67.3
BW[7], kg	575	59	456	764
MUN[8], mg/dL	11.9	2.6	5.0	18.3
Dietary CP[9], %	16.5	0.8	14.7	18.5
Dietary NDF[10], %	31.5	2.4	26.4	40.7
Dietary NE_L[11], Mcal/kg	0.78	0.02	0.73	0.84
Days in Milk (DIM)	51.1	22.6	23.0	139.0
Parity[12]	1.52	0.50	1	2
Calving Month[13]	7.37	3.57	1	12
Dairy FE (ECM/DMI)	2.30	0.14	2.12	2.70

[1]The data was divided into training (70.01%) and test (29.99%) datasets.
[2]Sample size for each variable (n) = 1,899 means averaged weekly on an individual cow basis per group.
[3]SD = standard deviation.

[4]DMI = Dry matter intake.
[5]Milk yield (kg/d) = AM Milk (kg/d) + PM Milk (kg/d).
[6]ECM = ((12.95 x lbs milk fat) + (7.65 x lbs milk protein) + (0.327 x lbs milk)/2.2) (DRMS, 2014).
[7]BW = Body weight.
[8]MUN = Milk urea N.
[9]CP = Crude protein (% DM basis).
[10]NDF = Neutral detergent fiber (% DM basis).
[11]NE_L = Net energy of lactation (Mcal/kg).
[12]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[13]Calving month ranges from January (1) to December (12).

Table 4.4. Ranking of the original variables based discriminatory power calculated during the SDA.

Original Variable[1]	Partial R^2	F Value	*Pr* > F	Wilks' Lambda	*Pr* < Lambda	ASCC[2]	*Pr* > ASCC
Milk fat yield, g/d	0.382	2346	<.0001	0.618	<.0001	0.382	<.0001
BW[3], kg	0.215	1041	<.0001	0.485	<.0001	0.515	<.0001
DIM[4], d	0.164	748	<.0001	0.405	<.0001	0.595	<.0001
NE_L[5], Mcal/kg	0.061	248	<.0001	0.380	<.0001	0.620	<.0001
MUN[6], mg/dL	0.016	60.5	<.0001	0.374	<.0001	0.626	<.0001
Calving month[7]	0.013	48.6	<.0001	0.370	<.0001	0.631	<.0001
Parity[8]	0.009	33.9	<.0001	0.366	<.0001	0.634	<.0001
CP[9], %	0.006	21.0	<.0001	0.364	<.0001	0.636	<.0001
NDF[10], %	0.001	4.42	0.0355	0.364	<.0001	0.636	<.0001
Milk protein yield[11], g/d	-	-	-	-	-	-	-

[1]Sample size for each variable (n) = 1,899 means averaged weekly on an individual cow basis per group.
[2]Average squared canonical correlation (ASCC).
[3]BW = Body weight.
[4]DIM = Days in milk.
[5]NE_L = Net Energy of Lactation.
[6]MUN = Milk urea N.
[7]Calving month ranges from January (1) to December (12).
[8]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[9]CP = Crude Protein (% DM basis).
[10]NDF = Neutral Detergent Fiber (% DM basis).
[11]Milk protein yield (g/d) was removed during the SDA from the list of original variables to be included in the CDA and DA as it lacked sufficient discriminatory power ($P > 0.15$).

Table 4.5. Descriptive statistics for original variables in the CAN function for the Low (FE ≤ 1.79) and High (FE ≥ 2.12) FE groups in the training dataset (70.01%).

Item	Mean	SD[1]	Minimum	Maximum
Low FE Group (FE ≤ 1.79)[2]				
Milk fat yield, g/d	1365	212	758	2004
BW[3], kg	596	63	458	763
MUN[4], mg/dL	11.6	2.6	4.7	18.3
Dietary CP[5], %	16.6	0.7	14.7	18.5
Dietary NDF[6], %	32.4	2.3	27.4	40.7
Dietary NE_L[7], Mcal/kg	0.77	0.01	0.73	0.83
Days in Milk (DIM)	78.9	26.9	23.0	142.0
Parity[8]	1.38	0.49	1	2
Calving Month[9]	7.20	3.13	1	12
High FE Group (FE ≥ 2.12)[10]				
Milk fat yield, g/d	1766	289	1051	2651
BW[3], kg	575	59	456	764
MUN[4], mg/dL	12.0	2.6	5.1	18.3
Dietary CP[5], %	16.5	0.8	14.7	18.5
Dietary NDF[6], %	31.5	2.4	26.4	40.7
Dietary NE_L[7], Mcal/kg	0.78	0.02	0.73	0.84
Days in Milk (DIM)	50.5	22.2	23.0	139.0
Parity[8]	1.53	0.50	1	2
Calving Month[9]	7.47	3.53	1	12

[1]SD = Standard deviation.
[2]Sample size for each variable (n) = 1,325 means averaged weekly on an individual cow basis.
[3]BW = Body weight.
[4]MUN = Milk urea N.
[5]CP = Crude protein (% DM basis).
[6]NDF = Neutral detergent fiber (% DM basis).
[7]NE_L = Net energy of lactation (Mcal/kg).
[8]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[9]Calving month ranges from January (1) to December (12).
[10\8]Sample size for each variable (n) = 1,334 means averaged weekly on an individual cow basis.

Table 4.6. Descriptive statistics for original variables in the CAN function for the Low (FE ≤ 1.79) and High (FE ≥ 2.12) FE groups in the test dataset (29.99%).

Item	Mean	SD[1]	Minimum	Maximum
Low FE Group (FE ≤ 1.79)[2]				
Milk fat yield, g/d	1375	200	836	2058
BW[3], kg	595	61	463	758
MUN[4], mg/dL	11.3	2.6	4.8	18.2
Dietary CP[5], %	16.6	0.7	14.7	18.4
Dietary NDF[6], %	32.4	2.3	26.4	40.7
Dietary NE_L[7], Mcal/kg	0.77	0.01	0.73	0.83
Days in Milk (DIM)	79.7	27.7	23.0	142.0
Parity[8]	1.40	0.49	1	2
Calving Month[9]	7.13	3.13	1	12
High FE Group (FE ≥ 2.12)[10]				
Milk fat yield, g/d	1760	289	1010	2694
BW[3], kg	576	60	456	748
MUN[4], mg/dL	11.8	2.6	5.0	18.1
Dietary CP[5], %	16.5	0.7	14.7	18.5
Dietary NDF[6], %	31.5	2.4	26.4	40.7
Dietary NE_L[7], Mcal/kg	0.78	0.02	0.73	0.84
Days in Milk (DIM)	52.7	23.5	23.0	138.0
Parity[8]	1.51	0.50	1	2
Calving Month[9]	7.17	3.67	1	12

[1]SD = Standard deviation.
[2]Sample size for each variable (n) = 574 means averaged weekly on an individual cow basis.
[3]BW = Body weight.
[4]MUN = Milk urea N.
[5]CP = Crude protein (% DM basis).
[6]NDF = Neutral detergent fiber (% DM basis).
[7]NE_L = Net energy of lactation (Mcal/kg).
[8]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[9]Calving month ranges from January (1) to December (12).
[10]Sample size for each variable (n) = 565 means averaged weekly on an individual cow basis.

Table 4.7. Total sample standardized canonical coefficients and pooled within canonical structure for the CDA conducted on the training dataset (70.01%)[1,2].

Original Variables[3]	Standardized Canonical Coefficients[4,5]	Pooled Within Canonical Structure[6]
Milk fat yield, g	1.253	0.775
BW[7], kg	-0.522	-0.212
DIM[8], d	-0.687	-0.624
NE_L[9], Mcal/kg	0.429	0.425
MUN[10], mg/dL	0.202	0.086
Calving month[11]	-0.128	0.051
Parity[12]	-0.144	0.186
CP[13], %	-0.076	-0.080
NDF[14], %	0.075	-0.224
Eigenvalue	1.778	-
Canonical Correlation	0.800	-
Variance Explained	64.005	-
Class Means		
Low FE Group	-1.337	-
High FE Group	1.328	-
RS Error Counts[15], %	8.54	-
CV Error Counts[16], %	8.73	-

[1]Sample size (n) = 1,325 means averaged weekly on an individual cow basis for the Low FE group.
[2]Sample size (n) = 1,334 means averaged weekly on an individual cow basis for the High FE group.
[3]Milk protein yield (g/d) was removed during the SDA from the list of original variables to be included in the CDA and DA as it lacked sufficient discriminatory power ($P > 0.15$).
[4]Canonical coefficients are the weighted contribution of each original variable to the CAN function.
[5]CAN = ((1.253 x milk fat yield (g/d)) + (-0.522 x BW (kg)) + (-0.687 x DIM) + (0.429 x NEL (Mcal/kg)) + (0.202 x MUN (mg/dL)) + (-0.128 x calving month) + (-0.144 x parity) + (-0.076 x CP (%)) + (0.075 x NDF (%)).
[6]Canonical structure is calculated as the correlation between the canonical function and each original variable.
[7]BW = Body weight.
[8]DIM = Days in milk.
[9]NE_L = Net Energy of Lactation (Mcal/kg).
[10]MUN = Milk urea N.
[11]Calving month ranges from January (1) to December (12).
[12]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[13]CP = Crude Protein (% DM basis).
[14]NDF = Neutral Detergent Fiber (% DM basis).
[15]Error rates (%) calculated using the resubstitution method.
[16]Error rates (%) calculated using the cross-validation method.

Table 4.8. Resubstitution and cross-validation error rates in the training dataset for the full-model[1] and reduced CAN functions.

CAN Function[2]	Error Rate (%) RS	Error Rate (%) CV
CAN1 = (c_1MFY[3]) + (c_2BW[4]) + (c_3DIM[5]) + ($c_4$$NE_L$[6]) + ($c_5$MUN[7]) + ($c_6$CM[8]) + ($c_7$P[9]) + ($c_8$CP[10]) + ($c_9$NDF[11])	8.54	8.73
CAN2 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN) + ($c_6$CM) + ($c_7$P) + ($c_8$CP)	8.57	8.91
CAN3 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN) + ($c_6$CM) + ($c_7$P)	8.57	8.69
CAN4 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN) + ($c_6$CM)	8.61	8.69
CAN5 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN)	8.50	8.61
CAN6 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$)	8.76	8.84
CAN7 = (c_1MFY) + (c_2BW) + (c_3DIM)	10.57	10.64
CAN8 = (c_1MFY) + (c_2BW)	14.10	14.18
CAN9 = (c_1MFY) + (c_3DIM)	14.37	14.40
CAN10 = (c_2BW) + (c_3DIM)	26.29	26.33
CAN11 = (c_3DIM)	27.08	27.08
CAN12 = (c_2BW)	43.74	43.74
CAN13 = (c_1MFY)	22.60	22.60

[1]Full model (CAN1) includes the nine variables selected during the SDA.
[2]c^i are the canonical coefficients applied to each term in the CAN function.
[3]MFY = milk fat yield (g/d).
[4]BW = Body weight (kg).
[5]DIM = Days in milk.
[6]NE_L = Net Energy of Lactation (Mcal/kg).
[7]MUN = Milk urea N (mg/dL).
[8]CM = Calving month which ranges from January (1) to December (12).
[9]P = Parity (cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2)).
[10]CP = Crude Protein (% DM basis).
[11]NDF = Neutral Detergent Fiber (% DM basis).

Table 4.9. Resubstitution error rates[1] in the test dataset for the full-model[2] and reduced CAN functions.

CAN Function[3]	Error Rate (%)[1]
CAN1 = (c_1MFY[4]) + (c_2BW[5]) + (c_3DIM[6]) + ($c_4$$NE_L$[7]) + ($c_5$MUN[8]) + ($c_6$CM[9]) + ($c_7$P[10]) + ($c_8$CP[11]) + ($c_9$NDF[12])	10.04
CAN2 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN) + ($c_6$CM) + ($c_7$P) + ($c_8$CP)	9.86
CAN3 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN) + ($c_6$CM) + ($c_7$P)	9.78
CAN4 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN) + ($c_6$CM)	9.87
CAN5 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$) + ($c_5$MUN)	10.04
CAN6 = (c_1MFY) + (c_2BW) + (c_3DIM) + ($c_4$$NE_L$)	10.30
CAN7 = (c_1MFY) + (c_2BW) + (c_3DIM)	11.01
CAN8 = (c_1MFY) + (c_2BW)	15.46
CAN9 = (c_1MFY) + (c_3DIM)	14.70
CAN10 = (c_2BW) + (c_3DIM)	27.96
CAN11 = (c_3DIM)	28.04
CAN12 = (c_2BW)	45.12
CAN13 = (c_1MFY)	22.30

[1]Cross-validation error rates were not reported for the test dataset as this method does not apply.
[2]Full model (CAN1) includes the nine variables selected during the SDA.
[3]c^i are the canonical coefficients applied to each term in the CAN function.
[4]MFY = milk fat yield (g/d).
[5]BW = Body weight (kg).
[6]DIM = Days in milk.
[7]NE_L = Net Energy of Lactation (Mcal/kg).
[8]MUN = Milk urea N (mg/dL).
[9]CM = Calving month which ranges from January (1) to December (12).
[10]P = Parity (cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2)).
[11]CP = Crude Protein (% DM basis).
[12]NDF = Neutral Detergent Fiber (% DM basis).

Figure 4.1. Relationship between observed and estimated values for BW. [BW (kg) = 1.000x + 0.0000; intercept P = 1.0000; intercept SE = 1.01; slope P = < 0.0001, slope SE = 0.00171, R^2 = 0.985; root mean (predicted) standard error (**RMSE**) = 8.23; n = 5,116].

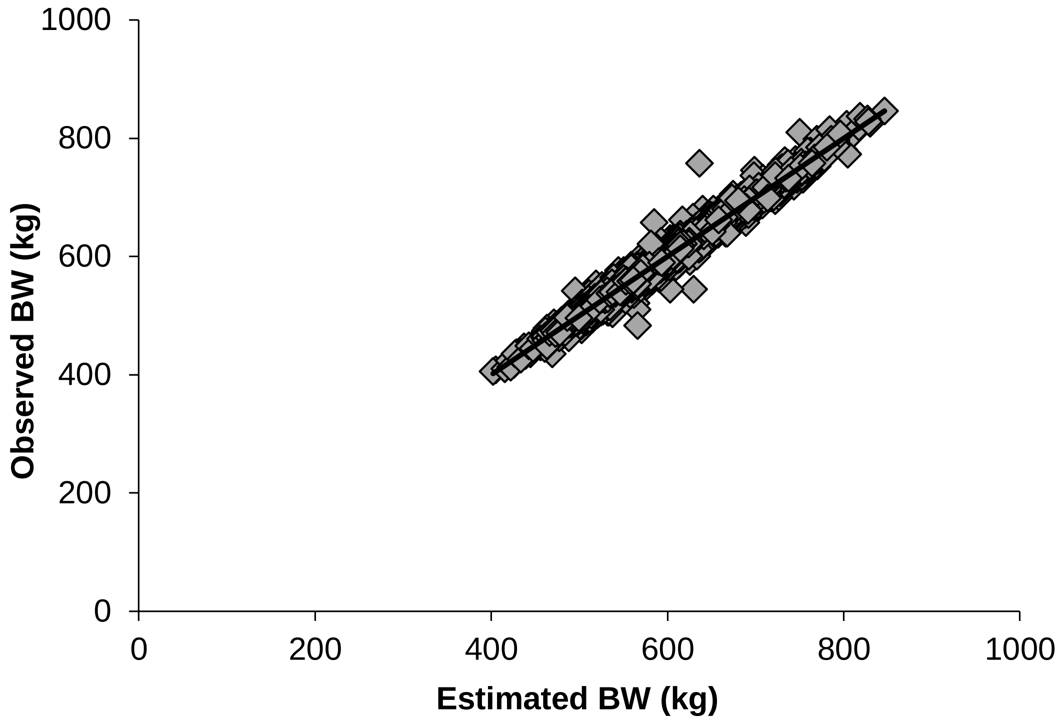


Figure 4.2. Relationship between observed and estimated values for milk yield. [MY (kg/milking) = 1.0000x + 0.0002; intercept P = 1.0000; intercept SE = 0.024; slope P = < 0.0001, slope SE = 0.001, R^2 = 0.865; RMSE = 1.76; n = 140,101].

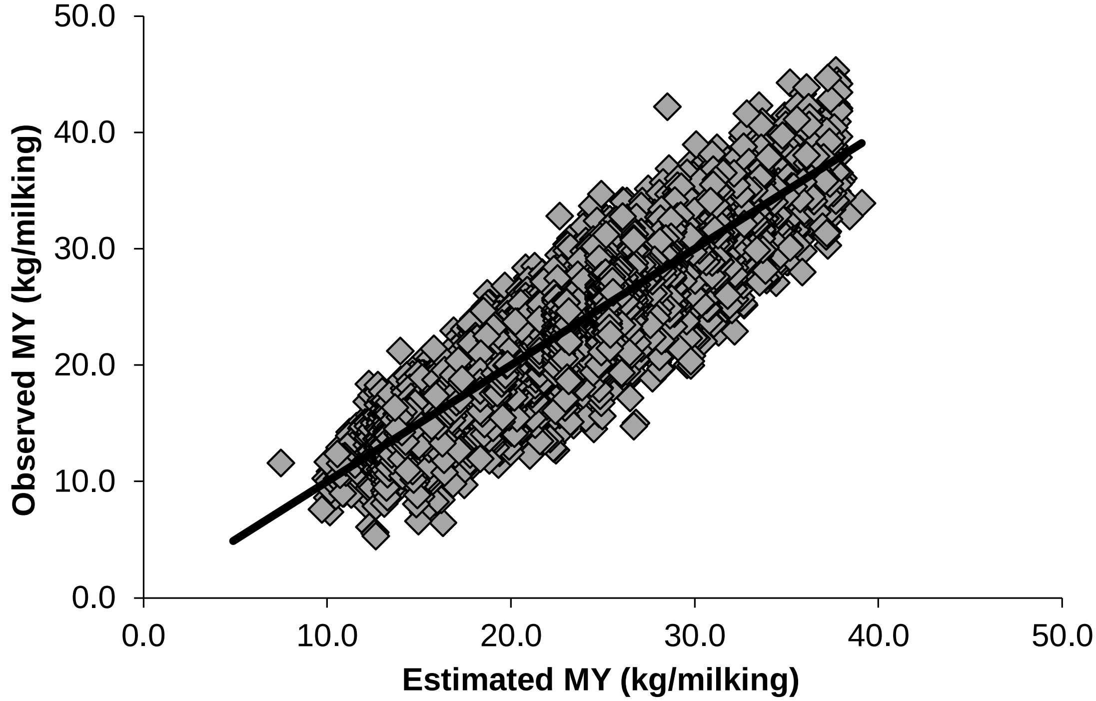


Figure 4.3. Relationship between observed and estimated values for milk fat percent. [Milk fat % = 1.0000x + 0.0003; intercept P = 1.0000; intercept SE = 0.016; slope P = < 0.0001, slope SE = 0.004, R^2 = 0.852; RMSE = 0.222; n = 8,943].

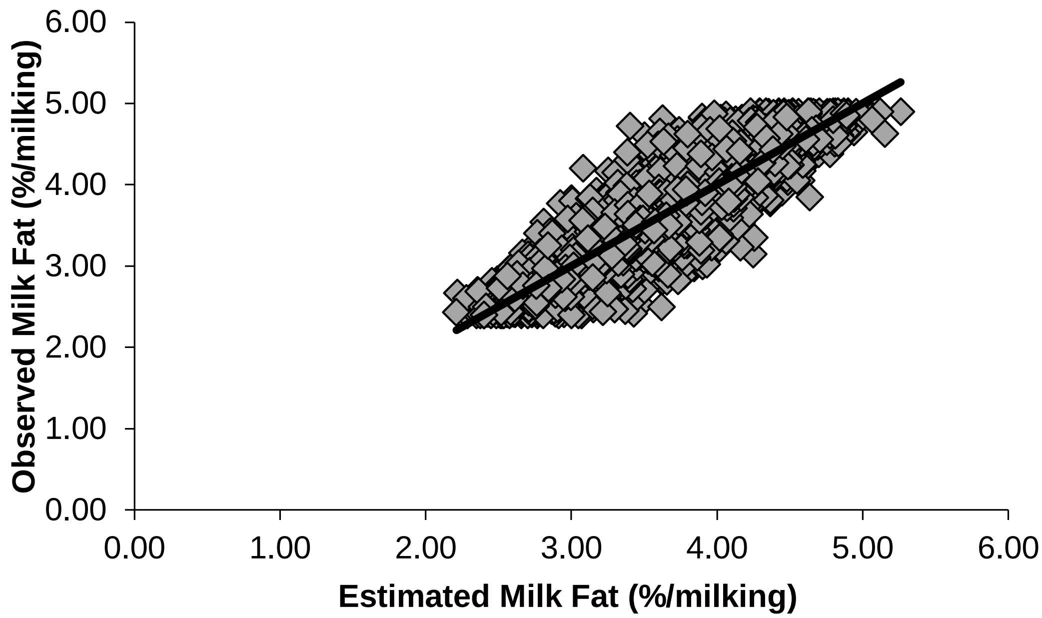


Figure 4.4. Relationship between observed and estimated values for milk protein percent. [Milk protein (% per milking) = 1.000x + 0.0000; intercept P =1.0000; intercept SE = 0.008; slope P = <0.0001, slope SE = 0.003, R^2 = 0.928; RMSE = 0.070; n = 9,915].

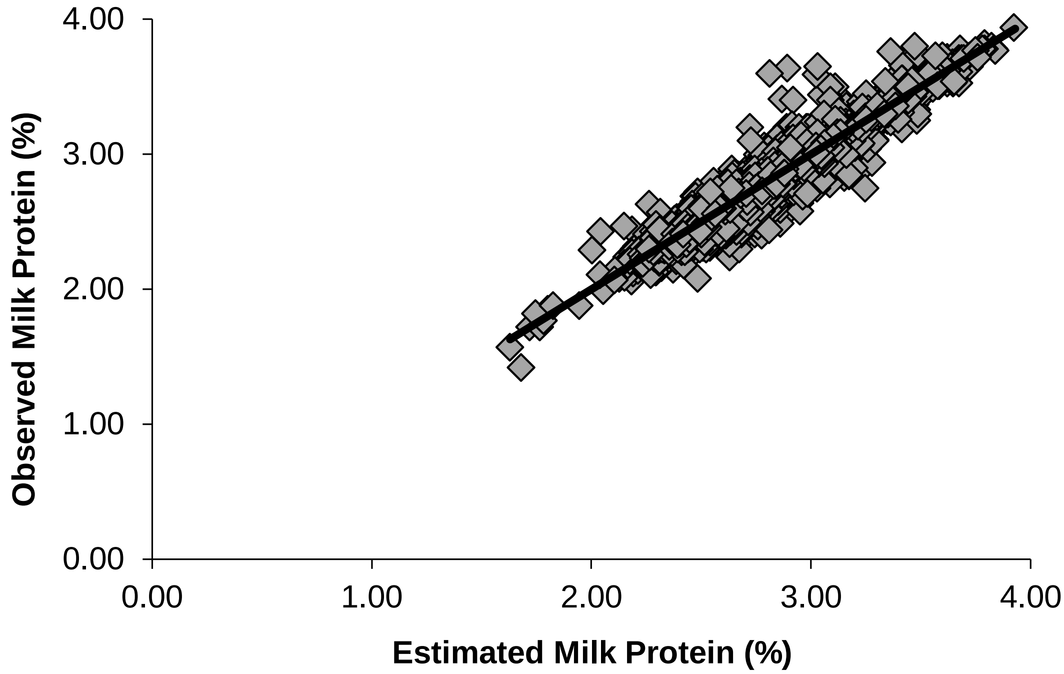


Figure 4.5. Relationship between observed and estimated values for MUN. [MUN (mg/dL per milking) = 1.000x + 0.0000; intercept P = 1.0000; intercept SE = 0.057; slope P = < 0.0001, slope SE =0.005, R^2 = 0.841; RMSE = 1.22; n = 8670].

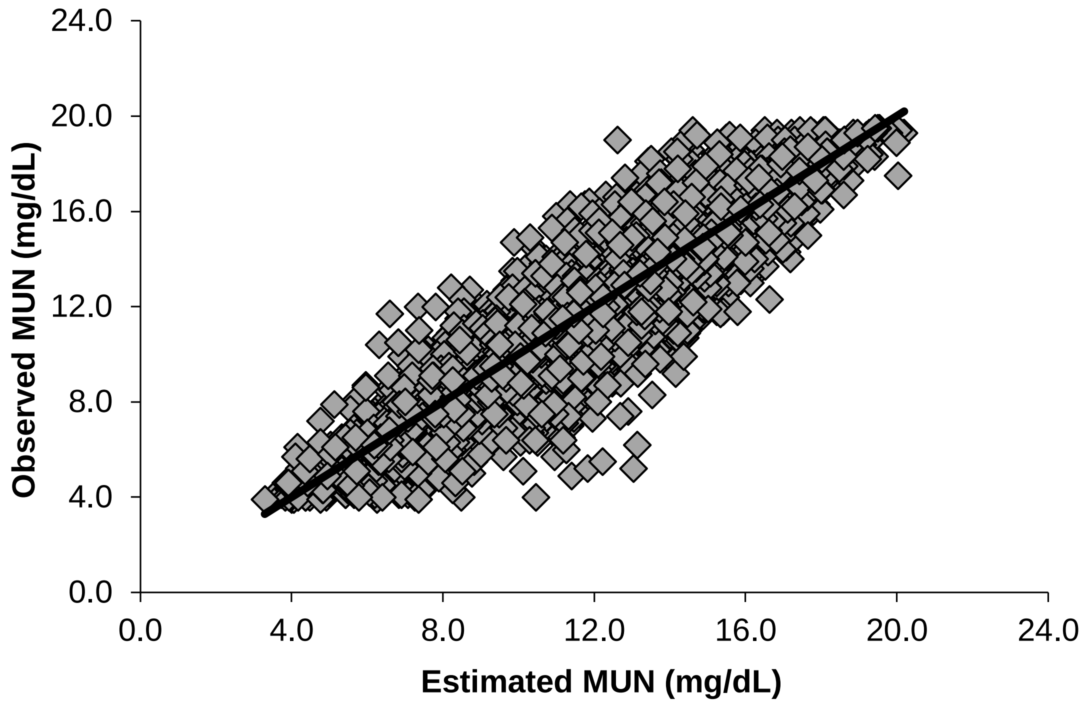


Figure 4.6. Graph of the canonical (CAN) function and canonical frequency distribution for the High and Low FE. The class means for the High and Low FE groups are -1.337 and 1.328, respectively. Positive and negative positions on the x-axis are dictated by positive and negative canonical coefficients.

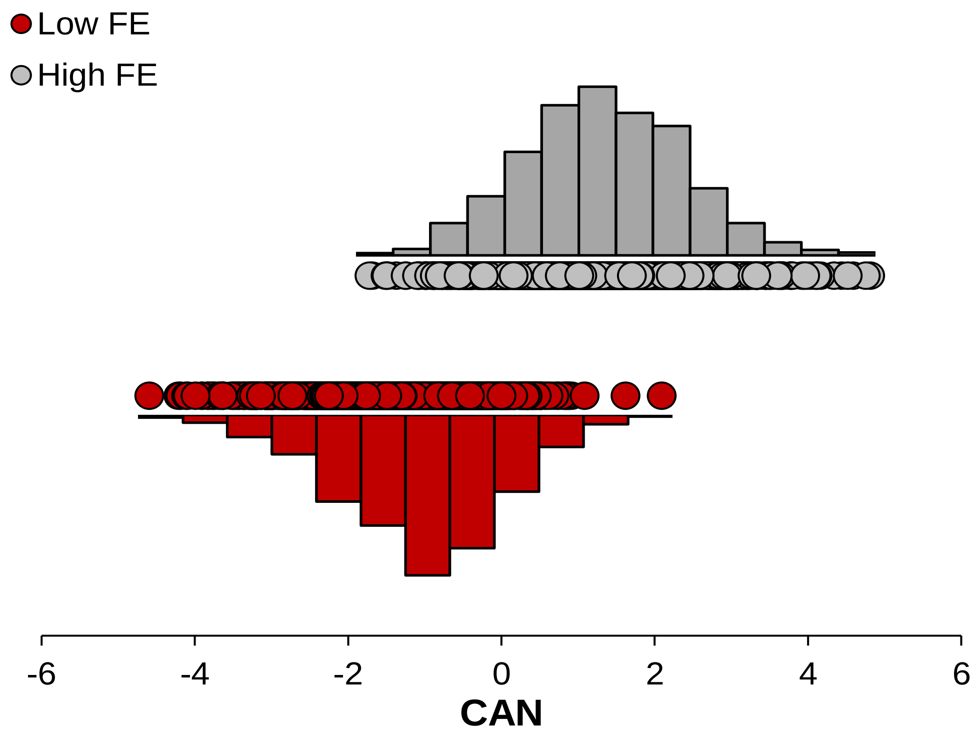


Figure 4.7. Discrimination between High and Low FE groups based on DIM and milk fat yield (g/d). Error rates of misclassification in the training dataset were 14.4% and 14.4% for re-substitution and cross-validation methods, respectively. Resubstitution misclassification error rate in the test dataset was 14.70%

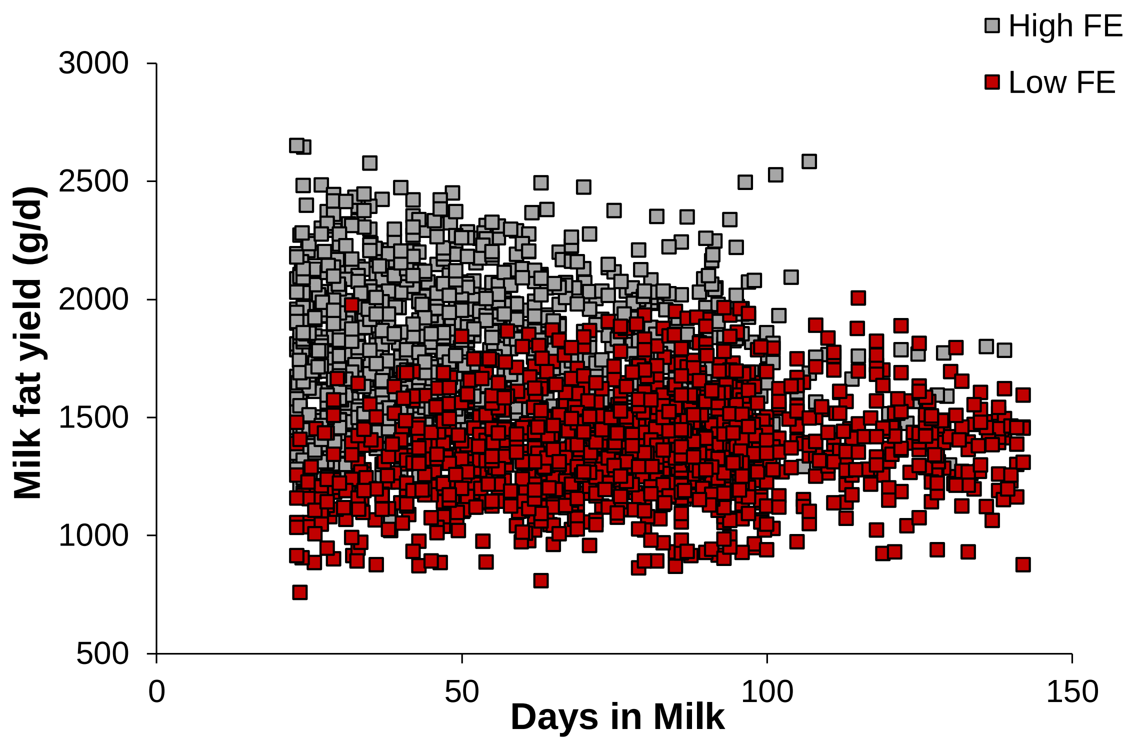


Figure 4.8. Discrimination between High and Low FE groups based on DIM and BW (kg). Error rates of misclassification in the training dataset were 26.3% and 26.3% for resubstitution and cross-validation methods, respectively. Resubstitution misclassification error rate in the test dataset was 28.0%

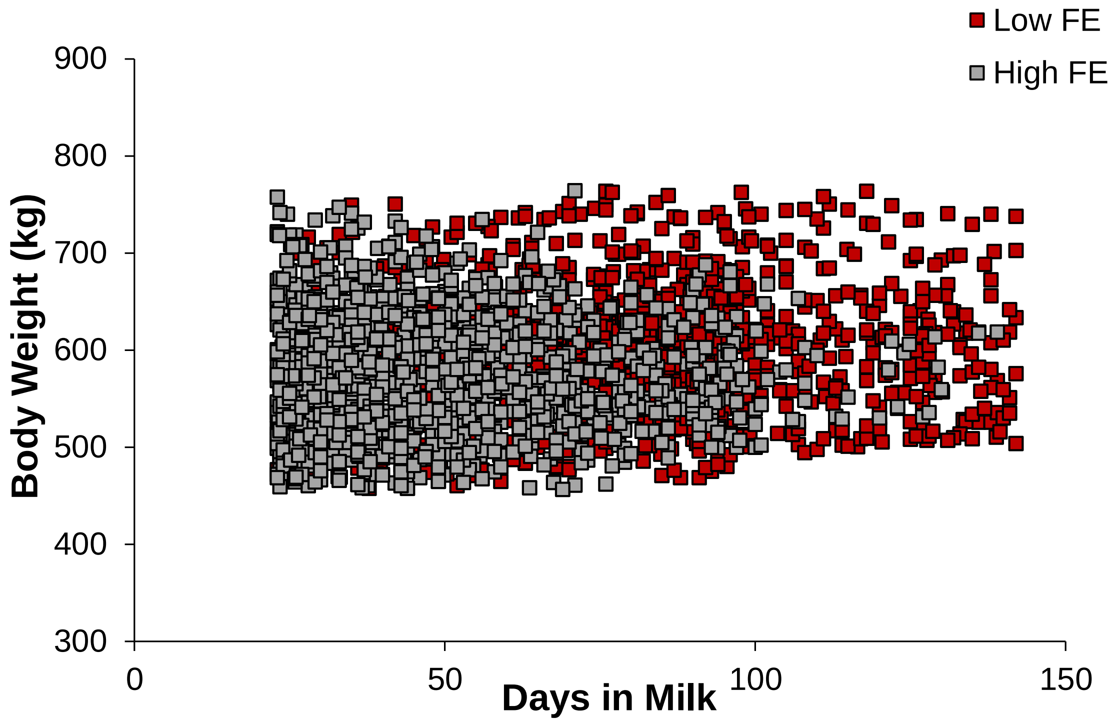
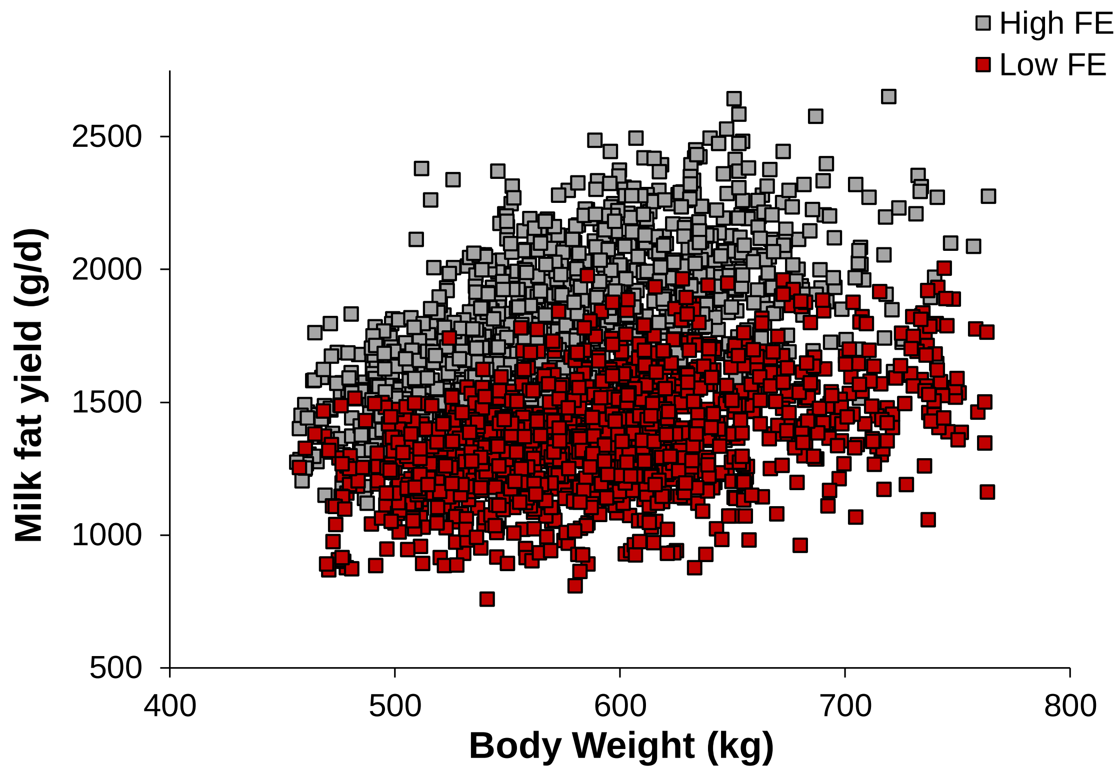


Figure 4.9. Discrimination between High and Low FE groups based on milk fat yield (g/d) and BW (kg). Error rates of misclassification in the training dataset were 14.10% and 14.18% for resubstitution and cross-validation methods, respectively. Resubstitution misclassification error rate in the test dataset was 15.5%



CHAPTER 5: EXPERIMENT 3

Determination of the relative discriminatory power of several biological, production, and dietary factors that affect residual feed intake using 3 complementary discriminant analyses[1]

[1]Iwaniuk, M. E., E. E. Connor, and R. A. Erdman. Determination of the relative discriminatory power of several biological, production, and dietary factors that affect residual feed intake using 3 complementary discriminant analyses. In preparation for submission to the Journal of Dairy Science.

INTERPRETIVE SUMMARY

Determination of the relative discriminatory power of several biological, production, and dietary factors that affect residual feed intake using 3 complementary discriminant analyses. *Iwaniuk et al., page 000.* Using a dataset provided by the USDA, 3 complementary discriminant analyses were conducted to determine the relative discriminatory power of biological, production, and dietary factors on residual feed intake. Residual feed intake is calculated as the difference in expected feed intake of a cow based on her maintenance and production requirements and her actual feed intake. A discriminant analysis using cow's production record, parity, days in milk and body size characteristics identified cows with either positive ($\geq$ 1.13 kg/d) and negative ($\leq$ -1.06 kg/d) residual feed intakes with an accuracy of 70.1%. A cow's days in milk had the most discriminatory power (69.5%) of all characteristics investigated.

Determination of the relative discriminatory power of several biological, production, and dietary factors that affect residual feed intake using 3 complementary discriminant analyses.

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ABSTRACT

Residual feed intake (**RFI**) has been shown to be a promising tool to identify dairy cows that have greater feed efficiency (**FE**). RFI is calculated as the difference between a cow's actual dry matter intake (**DMI**; kg/d) and her predicted DMI which is estimated from production parameters such as energy-corrected milk (**ECM**; kg/d), metabolic body weight (**MBW**; $BW^{0.75}$), and average daily gain (**ADG**; g/d). Research has suggested that RFI is phenotypically-independent of several production parameters and it is repeatable within and across lactations, diets, and climates. However, research has yet to be conducted to determine if group assignments based on RFI (-RFI vs. +RFI) can be differentiated based on biological, production, and dietary parameters. Thus, the objective of this study was to develop a discriminant function that can successfully differentiate between +RFI and -RFI cows and to determine the relative discriminatory power of each variable on RFI group assignment. The dataset for this study contained cow 7,750 weekly cow production records for 522 cows across 334 wk and was provided by the United States Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD. The DMI was predicted for each weekly cow record using the equation proposed by Connor et al. (2013) which included parity, MBW, ADG, and ECM in the model. Regression analysis between actual and predicted DMI indicated that the DMI equation explained 72.0% of the total variation in DMI. After DMI for each cow was predicted, RFI was calculated for each weekly cow record and -RFI (RFI ≤ -1.06) and +RFI (RFI ≥ 1.13) groups were determined. Stepwise, canonical, and basic discriminant analyses were conducted using the following 10 variables to discriminate between RFI groups: days in milk (**DIM**), milk protein yield (g/d), milk fat yield (g/d), BW (kg), milk urea nitrogen (mg/dL), parity, calving month,

dietary net energy of lactation concentration (Mcal/kg DM), dietary crude protein concentration (%), and dietary neutral detergent fiber concentration (%). The results of these analyses suggested that all variables except DIM lacked sufficient discriminatory power to differentiate between +RFI and -RFI cows. When DIM was included as the sole discriminatory variable in a reduced canonical (**CAN**) function, the misclassification error rate of cows to the incorrect RFI group was approximately 30.48%; thus, RFI group membership was successfully assigned at a rate of 69.52% based on DIM alone. Most -RFI cows tended to be in early lactation (low DIM) where most +RFI cows tended to be later in lactation. This suggested that the DMI equation used in calculating RFI was not robust enough to take into account stage of lactation effects. Other parameters evaluated lacked significant discriminatory power to differentiate RFI groups.

Key Words: residual feed intake; feed efficiency; discriminant analysis; stage of lactation

INTRODUCTION

It is well known that feed costs are the single, largest cost associated with milk production on U.S. dairy farms (Beck and Ishler, 2016; Hardie et al., 2017; Valvekar et al., 2010). To reduce feed costs and increase profitability, substantial research has been conducted to explore methods to estimate feed efficiency (**FE**) in individual cows so that dairy producers can select for the most feed-efficient cows within their herds (Connor, 2015).

Several methods have been developed to estimate dairy FE (Connor, 2015; Erdman, 2011). In particular, residual feed intake (**RFI**) has been shown to be a promising tool that may be used for the genetic selection of feed-efficient cows within a cohort as RFI has been shown to be indicative of differences in nutrient metabolism independent of differences in production or diet composition (Connor, 2015; Potts et al., 2015; VandeHaar et al., 2016). RFI is calculated as the difference between the observed dry matter intake (**DMI**; kg/d) of an individual cow and her predicted DMI (Connor, 2015). Several different DMI prediction equations have been published for dairy cows; however, substantial RFI research has been conducted using the DMI prediction equation proposed by Connor et al. (2013) which includes the following production parameters: parity, metabolic body weight (**MBW**; $BW^{0.75}$; kg), average daily gain (**ADG**; g/d), and energy-corrected milk (**ECM**; kg/d). Once DMI has been predicted for each cow, the RFI of an individual cow is estimated by subtracting the cow's predicted DMI from its observed DMI (Berry and Crowley, 2013; Connor, 2015; Potts et al., 2015). If a cow consumes more feed than predicted, she will have a positive (+) RFI and is considered to have low FE compared to cows of a similar body size and production level. Conversely, if a cow consumes less feed than predicted, she will have a negative (-) RFI and is considered to have high FE compared to cows of a similar body size and production level. (Connor, 2015; Potts et al., 2015).

Assuming that there is substantial variation in RFI values between individual cows within a target population, RFI is a great candidate for a genetic selection tool to select for cows with high FE as it is moderately heritable (h^2 = 0.17 – 0.36), repeatable across and within lactations, and is phenotypically independent of production parameters used for its calculation (Connor, 2015; Connor et al., 2013; Tempelman et al., 2015.

Although several advantages for RFI exist, there are also several major disadvantages to using RFI to estimate FE status of lactating dairy cows. First, actual DMI measurements are required for the RFI calculation and DMI tends to be labor-intensive and costly to measure in o individual cows (Connor et al., 2013; Faverdin et al., 2017; Halachmi et al., 2004). Secondly, predicting DMI requires the use of complex statistical modeling with large, robust datasets which makes this approach relatively impractical on most commercial dairy farms (Connor, 2015; VandeHaar et al., 2016). Lastly, RFI is calculated as the statistical error term in the regression analysis between actual and predict DMI; therefore, it is possible that RFI contains true variation associated with metabolism-related differences, but it also contains random error associated with inaccurate DMI measurements or predictions (VandeHaar et al., 2016). This chapter aims to address the first 2 aforementioned issues regarding RFI while Chapter 6 of this dissertation addresses the third aforementioned issue surrounding RFI.

Previous research has shown that various biological, production, or dietary parameters affect dairy FE when FE is calculated as ECM per unit of DMI (Erdman, 2011; Heinrichs et al., 2016). For example, FE is negatively correlated to stage of lactation as milk yield decreases while DMI increases throughout lactation (St-Pierre, 2012). Parity has been shown to be positively correlated with FE as multiparous cows are able to consume more feed and divert more energy towards milk production compared to smaller, primiparous cows that are still growing (Lee and Kim, 2006). In addition, research suggests that multiparous cows have higher milk production compared to primiparous cows due to differences in the metabolic activity of milk secretory cells in the mammary gland (Miller et al., 2006). Lastly, calving month has been shown to alter the FE of dairy cows as it is indirectly confounded with the effects of heat stress and photoperiod on production (Dahl et al., 2000; Torshizi, 2016). Research has shown that cows that calve during hot, summer months that may be predisposed to heat stress which decreases DMI, milk yield, and milk component production, ultimately lowering FE (Torshizi, 2016). In addition, cows that calve during months with short-day photoperiods (≤ 12 h of light/d) may produce significantly less milk per day compared to cows that enter lactation during months with long-day photoperiods (16 to 18 h of light/d) (Dahl et al., 2000).

In addition to biological parameters, FE can also be altered by changes in production parameters such as milk yield, milk composition, and BW (Erdman, 2011; Heinrichs et al., 2016; Lin, 2006). Research has shown that high genetic potential cows in well-managed herds that have higher milk yields or milk component yields (fat and protein) tend to have higher FE values (Erdman, 2011; Heinrichs et al., 2016). Body weight has been shown to be negatively correlated with FE as larger cows require more energy for maintenance compared to smaller cows (Linn, 2006).

Lastly, substantial research has shown that altering the composition the diet may affect dairy FE. Increasing dietary energy concentration (**NE_L**) through fat supplementation has been shown to increase milk and milk component yields, resulting in increased FE (Onetti et al., 2001; Weiss and Pinos-Rodriguez, 2009; Zou et al., 2007). Similarly, increasing dietary crude protein (**CP**) concentration (%) has been shown to increase milk and milk fat yields which can increase FE (Broderick et al., 2015; Kalscheur et al., 1999). Decreasing dietary neutral detergent fiber (**NDF**) concentrations (%) has been shown to increase milk fat yield which subsequently increases dairy FE (Kendall et al. 2009; Oba and Allen, 2009; Ruiz et al., 1995).

A companion study was conducted and presented in Chapter 4 of this dissertation which aimed to determine if high (ECM/DMI $\geq$ 2.12) and low (ECM/DMI $\leq$ 1.79) cows could be differentiated using the following variables: days in milk (**DIM**), parity, calving month, milk fat yield, milk protein yield, BW, dietary NE_L, dietary CP, and dietary NDF concentrations. Based on the results of 3 complementary discriminant analyses (**DA**), Iwaniuk et al. (2019; unpublished) found that High and Low FE cows could be successfully differentiated at a rate of 88.99% using milk fat yield, DIM, and BW. In particular, milk fat yield had the strongest discriminatory power (77.70% success rate) to separate cows based on FE status. Thus, it was concluded that dairy producers could successfully select between High and Low FE cows based solely on milk fat yield, without requiring the costly and labor-intensive measurement of DMI.

Based on the results in the previous chapter of this dissertation, the objective of the current study was to determine if biological, production, or dietary variables can be used to discriminate between +RFI and –RFI cows. If these variables can be used to differentiate between +RFI and -RFI cows, the results of this study would allow dairy producers to select cows based on RFI without requiring the costly and laborious measurement of DMI or complex statistical modeling to calculate RFI.

MATERIALS AND METHODS

Database

The data used for this project were obtained from the laboratory of Dr. Erin Connor at the United States Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD. All data collection involving animals was approved by the Northeast Area Animal Care and Use Committee. A detailed description of the initial database as well as the procedures associated with data management, production parameter estimations, and outlier removal are presented in Chapter 4 of this dissertation. The final dataset contained 7,750 weekly cows mean observations for 522 cows and 167 2-wk intervals. The descriptive statistics for the final dataset are presented in Table 5.1.

Calculating RFI

In order to calculate RFI, DMI was predicted using the following equation proposed by Connor et al. (2013):

$$\text{DMI (kg/d)} = b_0 + (b_1 \text{ x Parity}) + (b_2 \text{ x MBW}) + (b_3 \text{ x ADG}) + (b_4 \text{ x ECM}) + \text{RFI} \qquad (1)$$

where b_0 is the intercept, b_1 is the partial regression coefficient of intake on parity, b_2 is the partial regression coefficient of intake on MBW (kg), b_3 is the partial regression coefficient of intake on ADG (g/d), b_4 is the partial regression coefficient of intake on ECM (kg/d), and RFI is the statistical residual error. The DMI was predicted by 2-wk intervals using PROC REG (SAS 9.4; SAS Institute, Cary, N.C.). Regression analysis using PROC REG was conducted to examine the relationship between predicted DMI and actual DMI and the results of the analysis are presented in Figure 5.1. Once DMI was predicted, RFI values were calculated as the difference between observed DMI and predicted DMI for each weekly cow record.

Categorizing Cows into +RFI and –RFI Groups

Prior to conducting the discriminant analyses, outlier removal was performed using PROC UNIVARIATE in SAS (SAS 9.4) such that any RFI values greater than the 99% quantile or less than the 1% quantile were removed resulting in 7,596 weekly cow observations for 520 cows across 167 2-wk intervals. Using PROC UNIVARIATE and PROC FREQ (SAS 9.4) weekly cow means were classified into the following 4 equal quartiles based on RFI values: 1) RFI $\geq$ 1.13, 2) 0.03 $\leq$ RFI < 1.13, 3) -1.06 $\leq$ RFI < 0.03, and 4) RFI $\leq$ -1.06. In this method, groups were ranked from 1 to 4 in decreasing order of RFI.

Weekly cow means within the second and third quartiles were removed from the dataset such that only the 25% highest RFI and 25% lowest RFI remained in the dataset. Thus, only the top and bottom 25% of weekly cow RFI means were retained for the discriminant analyses. For the remaining portion of this chapter, cows with positive RFI values (RFI $\geq$ 1.13) will be referred to as +RFI cows whereas cows with negative RFI values (RFI $\leq$ -1.06) will be referred to as -RFI cows.

Discriminant Analyses

Three complementary DAs were conducted to determine if biological, production, or dietary parameters could be used to successfully separate individual cows based on RFI groupings. The following 3 complementary DAs were conducted to differentiate +RFI and -RFI lactating dairy cows: 1) stepwise DA (**SDA**), canonical DA (**CDA**), and discriminant analysis (**DA**).

A detailed description of the materials and methods used in these analyses is presented in Chapter 4 of this dissertation. Essentially, the methodology of the discriminate analyses in Chapters 4 and 5 are identical; the only difference between experiments is the classification variable used to construct the discriminant function. In Chapter 4, the discriminant analyses utilized FE ratios (ECM per unit of DMI) to establish High (FE $\geq$ 2.12) and Low (FE $\leq$ 1.79) classification groups. Conversely, the discriminant analyses in the present chapter utilized RFI to establish +RFI and -RFI classification groups.

RESULTS AND DISCUSSION

RFI Calculation

Predicted DMI used to calculate RFI was estimated using the equation proposed by Connor et al. (2013) which included parity, MBW ($BW^{0.75}$), ADG (g/d), and ECM (kg/d) in the model. The results of the regression analysis performed between actual DMI and predicted DMI is presented in Figure 5.1. As shown, the DMI estimation equation accounted for approximately 72.0% of the total variation associated with DMI. This result mirrors the amount of variation explained (72.0%) by Connor et al. (2013) using the same equation in their dairy cattle dataset, which was a subset of the current dataset. Similar success has been shown in the literature using other models to predict DMI to calculate RFI. Using a DMI estimation model that included milk yield (kg/d) and live weight (kg) in dairy cattle, Shetty et al. (2017) also reported that 72.0% of the total variation associated with DMI could be explained by their proposed intake model. Manafiazar et al., (2013) reported that 68% of total variation associated with DMI in dairy cattle was accounted for when MBW, empty BW, and milk production energy requirements were included in the DMI prediction equation. The results of the regression analysis of observed versus predicted DMI in this study were similar to those of previously published results. Thus, it can be concluded that the predicted DMI and calculated RFI values in this study reflect values previously observed for early to mid-lactation dairy cows.

Descriptive Statistics of the Dataset prior to SDA

The descriptive statistics for the entire dataset as well as the +RFI and –RFI datasets prior to SDA are presented in Tables 5.1 and 5.2, respectively. It is important to note that the following 5 variables contain both actual and estimated measurements as described in Chapter 4: milk yield (kg/d), milk fat concentration (%), milk protein concentration (%), BW (kg), and MUN (mg/dL).

In regard to NDF concentration, Potts et al. (2015) conducted an experiment to determine if RFI values for an individual cow were affected by dietary starch concentrations using 2 dietary treatments: high starch which contained 26% NDF and 30% starch or low starch which contained 40% NDF and 14% starch. Potts et al. (2015) reported that RFI was not affected by dietary treatment; the correlation between RFI values for individual cows receiving either dietary treatment was approximately 0.70 which was similar to the correlation among different RFI for individual cows receiving no dietary changes. Thus, the authors concluded that RFI is repeatable across varying dietary NDF and starch concentrations. The results of the current study support the conclusions reported by Potts et al. (2015) as the SDA revealed that NDF did not have sufficient power to differentiate between cows based on RFI values ($P > 0.15$).

In addition to NDF concentration, CP concentration was also removed from the SDA as it lacked sufficient discriminatory power to differentiate between +RFI and –RFI groups ($P > 0.15$). Research that aims to specifically assess the effects of dietary CP concentration on RFI has yet to be conducted; however, current research suggests that RFI is repeatable across various dietary compositions (Potts et al., 2015; VandeHaar et al, 2016). Connor et al. (2015) and Tempelman et al. (2015) reported that the repeatability of RFI across lactations was 0.56 and 0.77, respectively, and these repeatability values are higher compared to repeatability values for other production traits in dairy cattle such as milk yield (r = 0.34), milk fat yield (r = 0.35), and milk protein yield (r = 0.29; Roman et al., 2000). Because RFI was shown to be repeatable across lactations, it is possible that RFI is repeatable across different diets as diets tend to fluctuate within and across lactations (Connor et al., 2013; Tempelman et al., 2015; VandeHaar et al., 2016). However, more research needs to be conducted to determine the effects of specific dietary concentrations (e.g., CP) on RFI values (Connor, 2015).

In summary, the results of this study suggest that RFI is not dependent on dietary NDF or CP concentrations such that NDF and CP (%) were removed from the study during the SDA.

CDA of +RFI and –RFI Cows Using Biological, Production, and Dietary Variables

The 8 discriminatory variables selected during the SDA were subsequently used to develop the canonical (**CAN**) function to differentiate between +RFI and –RFI cows utilizing the training dataset (70.01%). The CAN function successfully discriminated between +RFI and -RFI cows groups based on the Mahalanobis Distance ($P < 0.0001$) and Hotelling's *t*-test ($P < 0.0001$; Rencher, 1992). However, the CAN function only explained 25.67% of the total variation between the 2 RFI groups which is shown graphically in Figure 5.2. The canonical coefficients and canonical structure (correlations between individual variables and the canonical scores) for the 8 original variables selected during the SDA are presented in Table 5.6. The CAN function was positively correlated with DIM (r = 0.904), milk protein yield (r = 0.225), BW (r = 0.075), MUN (r = 0.082), parity (r = 0.073), and calving month (r = 0.026). Conversely, the CAN function was negatively correlated with milk fat yield (r = 0.058) and dietary NE_L concentration (r = -0.036). Based on the class means, the CAN function is negatively correlated with decreasing RFI. Therefore, it can be concluded that RFI is positively correlated with increased milk fat yield and NE_L concentrations, but negatively correlated with increased DIM, milk protein yield, BW, MUN, parity, and calving month.

To assess the ability of the CAN function to discriminate between -RFI and +RFI cows, the resubstitution and cross-validation methods were used to calculate misclassification error rates in the training dataset. Using the resubstitution method, 339 (of 1,323) +RFI weekly cow means were incorrectly classified in the –RFI group, resulting in an error rate of 25.62%. Conversely, 358 -RFI weekly cow means were misclassified into the +RFI group, resulting in an error rate of 26.80%. Together, the combined error rate for the resubstitution method was 26.21%. Using the cross-validation method, 349 (of 1,323) +RFI weekly cow means were misclassified in the -RFI group while 366 -RFI weekly cow means were misclassified in the +RFI group, resulting in an overall misclassification error rate of 26.89%.

The CAN function derived from the training dataset (70.01%) was applied to the test dataset (29.99%) and misclassification error rates were calculated using the resubstitution method to examine the success rate of RFI group membership predictions based on the proposed discriminant function. When applied to the test dataset, the CAN function misclassified 29.92% of the total number (n = 1,139) of weekly cow observations.

Based on the results of the CDA, it can be concluded that +RFI (RFI ≥ 1.13) and -RFI (RFI ≤ -1.06) cows could be differentiated, but only at a rate of 70.08% based on the following parameters: DIM, milk protein yield, milk fat yield, BW, MUN, parity, calving month, and dietary NE_L concentration. It is important to note that the misclassification error rate is dependent on the cutoff values for RFI group membership. Thus, it is possible to alter the misclassification error rate by altering the cutoff values for RFI group membership. Misclassification error rates would likely decrease if RFI group membership became more strict. Future research should explore the effect of RFI group assignments on misclassification error rates.

After the full-model CAN function was developed using the 8 variables selected from the SDA and assessed using misclassification error rates, 11 reduced CAN functions were systematically developed and evaluated as described in Chapter 4 of this dissertation to determine the relative discriminatory power of each variable in the CAN function. These results are presented in Tables 5.7 and 5.8.

Variables with Low Discriminatory Power

Removing the following 5 variables from the CAN function did not have a significant impact on the misclassification error rates in the training dataset or the test dataset: dietary NE_L concentration, calving month, parity, MUN, or BW. In the training dataset, removing these 5 variables increased the resubstitution and cross-validation misclassification error rates from 26.21 and 26.89% (CAN1) to 27.94 and 28.02% (CAN6), respectively. These results suggest that these 5 variables only added 1.13 to 1.73% discriminatory power when included in the CAN function which is a relatively small amount of power. In the test dataset, the resubstitution error rate actually decreased from 29.92 to 29.74% when these 5 variables were removed. Thus, dietary NE_L concentration, calving month, parity, MUN, and BW were removed systematically from the CAN function (CAN1 – CAN6).

Dietary NE_L Concentration

As shown in Table 5.6, the results of this study indicate that NE_L was negatively correlated with RFI (r = -0.036) such that that increasing dietary NE_L decreased RFI. Research has shown that increasing dietary energy concentrations (typically through fat supplementation) results in increased milk and milk component yield as more energy is consumed and allocated towards production purposes (Onetti et al., 2001; Weiss and Pinos-Rodriguez, 2009; Zou et al., 2007). Thus, the results of this study are consistent with previously published research regarding the relationship between dietary energy concentrations and FE.

Although NE_L was included in the CAN function, it is important to note that the correlation between dietary NE_L and RFI was fairly weak (r = -0.036). When NE_L was removed from the CAN1 function, the error rate in the test dataset decreased by 0.10% suggesting that NE_L lacked any significant discriminatory power to differentiate between +RFI and –RFI dairy cows (Tables 5.7 and 5.8).

As previously discussed, current research suggests that RFI values are repeatable across varying dietary compositions (Potts et al., 2015; VandeHaar et al., 2016). In regard to dietary energy, Williams et al. (2019) examined the effects of dietary energy density (High vs. Low) and RFI groups (+RFI vs. –RFI) on growing dairy heifer FE and reported that DMI (kg/d), metabolizable energy intake (Mcal/d), net energy of maintenance intake (Mcal/d), and net energy of gain intake (Mcal/d) were not significantly affected by dietary energy density, RFI group, or the interactive effect of dietary energy density by RFI group. Thus, RFI divergent heifers from their study consumed similar energy intakes regardless of RFI status so dietary energy intake would not be a powerful discriminatory factor to differentiate between +RFI and –RFI heifers. Research suggests that there is a strong correlation between heifer RFI and subsequent RFI calculated during lactation (r = 0.58; Macdonald et al., 2014; Nieuwhof et al., 1992). Therefore, it appears that dietary energy intake is also a weak discriminatory variable to differentiate between +RFI and –RFI lactating dairy cows (Williams et al., 2019).

Calving Month

As shown in Table 5.6, calving month had a weak, positive correlation (r = 0.027) with the CAN function used to differentiate between +RFI and –RFI cows. Additionally, removing calving month from the CAN2 function resulted in a 0.11% increase in error rate in the test dataset; thus, calving month did not contribute much power to the CAN function to separate +RFI and –RFI dairy cows (Tables 5.7 and 5.8).

To the knowledge of the authors, this is the first study that has examined the relationship between RFI and month of calving. Previous research has shown that calving month affects FE (ECM/DMI) as heat stress in warm months can reduce production parameters linked to FE such as DMI, milk yield, and milk component yield (Tao et al., 2018; Torshizi, 2016; Utrera et al., 2013). In addition, calving month may be indirectly related to photoperiodic effects on lactation as photoperiod lengths vary throughout the year such that cows produce more milk during months with longer day lengths (May to August; Dahl et al., 2000). Because RFI was shown to be phenotypically independent of production traits, it is possible that calving month did not have much discriminatory power in this analysis as RFI is robust in regard to changes in production parameters (Connor, 2015; Mujibi et al., 2010; VandeHaar et al., 2016). Research has shown that season of testing RFI may affect RFI values; however, future research is required to further explore the effects of both season of RFI measurement as well as season of calving on RFI (Mujibi et al., 2010).

Parity

Parity had a weak, positive correlation (r = 0.073) with RFI (Table 5.6). As shown in Tables 5.7 and 5.8, removing parity from CAN3 actually decreased the error rate in the test dataset by 0.12%. Therefore, it can be concluded that parity lacks discriminatory power to differentiate between +RFI and –RFI cows.

In a preliminary analysis conducted prior to DMI estimation, Connor et al. (2013) reported that parity had a significant effect on energy intake ($P < 0.0001$). Thus, Connor et al. (2013) added a term to account for the effects of parity on intake in the equation used to predict DMI to calculate RFI. In the current study, DMI was predicted on an individual cow basis using the DMI estimation equation proposed by Connor et al. (2013). Because this equation contains a model term to account for the effects of parity on intake, it is no surprise that parity lacked sufficient discriminatory power to differentiate between +RFI and -RFI cows as RFI (Connor, 2015; Mujibi et al., 2010; Potts et al., 2015). Residual feed intake is theoretically robust across parameters that are used to predict DMI such that difference in RFI can be attributed to metabolic differences (Connor, 2015; Mujibi et al., 2010).

Milk Urea N

As shown in Table 5.6, MUN concentration had a weak, positive correlation (r = 0.082) with the CAN function developed to discriminate between +RFI and –RFI cows which suggested that MUN was lower in cows with -RFI (RFI ≤ -1.06). These results are consistent with previously published literature that found that MUN concentrations were significantly lower in cows with high FE (ECM/DMI; Xi et al., 2016). Because MUN concentration is indicative of protein metabolism status of the dairy cow, it is possible that lower MUN concentrations for cows with -RFI suggests that these cows may utilize dietary protein more efficiently (Garcia et al., 1997; Jonker and Kohn, 2001; Xi et al., 2016).

When MUN concentration was removed from the CAN5 function, the misclassification error rate in the test dataset increased by 0.01%, suggesting that MUN lacked discriminatory power to discriminate between cows based on RFI status in this study. Jonker and Kohn (2001) reported that MUN concentration is inversely related to milk protein concentration. Because milk protein concentration is a component of the ECM calculation, it is possible that milk protein content is accounted for during the DMI prediction portion of the RFI calculation (DRMS, 2014). Furthermore, as MUN and milk protein concentrations are inherently linked, it is possible that accounting for milk protein concentration also accounts for MUN concentration, rendering RFI independent of MUN concentration (Jonker and Kohn, 2001).

Body Weight

Body weight had a weak, positive correlation (r = 0.075; Table 5.6) with the CAN function used to discriminate between RFI divergent cows. When BW was removed from the CAN5 function, misclassification error rates decreased by 0.01% in the test dataset (CAN6) which indicated that BW essentially lacked any discriminatory power in the CAN function to separate cows based on RFI status (Tables 5.7 and 5.8). As discussed previously, by definition, RFI values are phenotypically independent of production parameters used to estimate DMI in the RFI calculation (Connor, 2015; VandeHaar et al., 2016). Because MBW is calculated from traditional BW measurements and was used as a model term to predict DMI, it not surprising that BW lacked discriminatory power in the CAN function.

In addition, some studies have suggested that RFI is independent of body size or BW in heifers and cows (Connor et al., 2013; Williams et al., 2011; Xi et al., 2016). For example, Hardie et al. (2017) performed a genome-wide association study to examine the genomic basis of RFI in lactating dairy cows and found that RFI is genetically unrelated to energy consumption for milk production or maintenance requirements (MBW). Therefore, the results of the current study are congruent with previously published reports that RFI values are independent of BW (Connor et al., 2013; Williams et al., 2011; Xi et al., 2016).

Milk Fat Yield

After removing dietary NE_L concentration, calving month, parity, MUN, and BW from the CAN function, the 3 variables with the highest discriminatory power in the SDA were investigated in a step-wise fashion and these variables included: milk fat yield, milk protein yield, and DIM.

The results of this study indicated that milk fat yield was negatively correlated ($r = -0.056$; Table 5.6) with RFI; however, the correlation was fairly weak. In the training dataset, removing milk fat yield from the CAN function decreased the resubstitution and cross-validation misclassification error rates from 27.94 and 28.02% (CAN6) to 27.83 and 27.87% (CAN7), respectively. The resubstitution error rate in the test data resulted a small decrease from 29.74 (CAN6) to 29.00% (CAN7) when milk fat yield was removed from the CAN6 function. The discrimination between RFI groups based on DIM and milk protein yield (CAN7) is shown in Figure 5.3. When included as the only discriminatory variable in the CAN function, milk fat yield (CAN10) had misclassification error rates of 46.60 and 46.82% in the training dataset for the resubstitution and cross-validation methods, respectively. Similarly, an error rate of 47.36% was observed in the test dataset when milk fat yield was included in the CAN10 function as the sole discriminatory variable. The high rates of error suggest that milk fat yield did not have significant discriminatory power in the CAN function to differentiate between +RFI and –RFI cows.

The results observed in the current study are consistent with previously published studies (Connor et al., 2013; Xi et al., 2016). Xi et al. (2016) reported that there were no significant differences ($P > 0.05$) in overall milk yield (kg/d) or milk fat concentration (%) between RFI divergent cows (Low RFI $\leq$ -0.84; High RFI $\geq$ 0.86). Although milk fat yield was not reported, it could be hypothesized that RFI groupings would not significantly affect milk fat yield as it is a combination of the aforementioned variables (Xi et al., 2016). Because variation associated with milk fat yield is accounted for in the ECM term of the DMI prediction equation, it is not surprising that RFI is independent of milk fat yield in this study (Connor et al., 2013; Connor, 2015).

Milk Protein Yield

Similar to milk fat yield, milk protein yield did not exhibit high discriminatory power within the CAN function to differentiate between +RFI and –RFI cows groups. The results of the CDA indicated that milk protein yield was positively correlated (r = 0.225) with RFI (Table 5.6). Although the correlation between milk protein yield and RFI was the second strongest correlation, milk protein yield did not exhibit high discriminatory power to differentiate between +RFI and –RFI groups.

When milk protein yield was removed from CAN6 (variables: DIM, milk fat yield, and milk protein yield), the misclassification error rates for the resubstitution and cross-validation methods increased from 27.94 and 28.02% (CAN6) to 28.66% (CAN8) for both methods, respectively (Figure 5.4). The resubstitution error rate in the test data increased from 29.74 (CAN6) to 30.84% (CAN8) when milk protein yield was removed from the CAN6 function. Removing milk protein yield from the function only increased the error rate by approximately 1.10%; thus, it can be concluded that this variable did not contribute much power to the overall CAN function.

When included as the sole discriminatory variable in the CAN11 function, milk protein yield produced a misclassification error rate of 44.45% for both assessment methods. Similarly, an error rate of 44.83% was observed in the test dataset when milk protein yield was included as the sole discriminatory variable in the CAN11 function. These error rates are close to the expected error with random classification (50%). Based on these results, it was concluded that milk protein yield had low discriminatory power to differentiate between +RFI and –RFI cow groups.

The results in this study are consistent with previously published research regarding differences in milk protein content based on divergent RFI groups (Macdonald et al., 2014). As previously mentioned, ECM yield is used in the equation to predict DMI for RFI calculations and milk protein content is a component of the ECM equation (DRMS, 2014). Therefore, it is possible that milk protein content is accounted for in during the DMI prediction portion of the RFI calculation such that generated RFI values are independent of milk protein yield (Connor et al., 2013; Macdonald et al., 2014; Xi et al., 2016).

Variables with High Discriminatory Power

Days in Milk

The results of the CDA suggested that DIM had a strong, positive correlation with RFI (r = 0.9036). As presented in Tables 5.7 and 5.8, the variable that possessed the most discriminatory power to differentiate between +RFI and –RFI cow groups was DIM. When DIM was included as the only discriminatory variable in the CAN12 function, the misclassification error rate for both assessment methods was 28.24% in the training dataset and 30.48% in the test dataset. The misclassification error rates for the full-model CAN1 function were 26.21 and 26.89% in the training dataset for the resubstitution and cross-validation error rates, respectively, and 29.92% for the test dataset. Removing all variables except DIM only increased the misclassification error rates by 2.03% and 0.56% in the training and test datasets, respectively. Therefore, it can be concluded that the majority of the discriminatory power of CAN1 through CAN8 functions was solely attributed to the presence of DIM as a variable in the model. When DIM was removed from the CDA in CAN9, CAN10, and CAN11, these functions lacked sufficient discriminatory power (test dataset error rates ≥ 41.86%) to differentiate between +RFI and –RFI cow groups even though milk protein and fat yields were the second and third most powerful discriminatory variables in the SDA. The relatively weak discrimination between RFI groups based on milk protein and fat yields (CAN9) is shown in Figure 5.5. It can be concluded that DIM was the only variable that had high discriminatory power to differentiate between +RFI and –RFI dairy cows.

In a recently published article, Li et al. (2017) explored the effects of stage of lactation (or DIM) on RFI by comparing 2 RFI models: 1) RFI model with constant partial regression coefficients of intake on ECM, MBW, and change in BW (Δ**BW**) throughout lactation and 2) RFI model with partial regression coefficients of intake on ECM, MBW, and ΔBW that changed throughout lactation (11 periods; 4 weeks per period). Li et al. (2017) reported that the partial regression coefficients of intake on ECM, MBW, and ΔBW varied throughout lactation and the most variation occurred during early lactation (DIM ≤ 112). Thus, Li et al. (2017) concluded that stage of lactation significantly affects RFI values and these results are consistent with the results observed in the current study.

Energy metabolism for a dairy cow fluctuates throughout lactation (NRC, 2001; St-Pierre, 2012). In early lactation, cows tend to be in a negative energy balance (**NEB**) as milk production peaks at approximately 60 DIM and cows mobilize their body tissue stores (lose BW) in order to meet the high energy demands of milk production (NRC, 2001; St-Pierre, 2012). At approximately 120 DIM, cows begin to enter a physiological state of positive energy balance (**PEB**) as milk production decreases while DMI increases to replenish body stores to prepare for the subsequent lactation (NRC, 2001). As cows shift from NEB to PEB throughout lactation, shifts in ECM, MBW, and ΔBW (ADG) also occur as these parameters are closely associated with energy metabolism of dairy cows (Li et al., 2017). Because these production parameters are typically used to predict DMI to calculate RFI, it is not surprising to find that RFI is dependent on DIM as stage of lactation affects energy-related production parameters (Li et al., 2017). Thus, the results of the current study suggest that stage of lactation significantly affects RFI which is consistent with previous research.

To overcome this issue, it may be advantageous to utilize a DMI estimation equation that accounts for stage of lactation to predict DMI in order to generate robust RFI values that are not dependent on stage of lactation (Tempelman et al., 2015; Vallimont et al., 2011). For example, Tempelman et al. (2015) reported a correlation of 0.77 for RFI repeatability values within lactation for dairy cows between 50 and 200 DIM. This correlation coefficient is 0.30 units larger than the correlation coefficient ($r = 0.47$) reported by Connor et al. (2013) for RFI repeatability during the first 90 DIM. It is possible that the large difference in correlation coefficients could be due to the fact that stage of lactation was only accounted for in the model proposed by Tempelman et al. (2015). In addition, research has shown that RFI tends to be fairly consistent after the early lactation period (DIM > 120) so it also possible that the discrepancy in RFI repeatability correlation coefficients may be due to differences in stage of lactation of cows used in each respective study. Regardless, it is possible that RFI repeatability may be improved if DIM is accounted for in the DMI prediction equation such that generated RFI values are not dependent on stage of lactation. Future research should be conducted that explores the effect of stage of lactation on RFI of dairy cows.

CONCLUSIONS

The results of this study suggest that RFI is phenotypically independent of biological parameters such as parity and calving month, production parameters such as milk protein yield, milk fat yield, BW, and MUN, and dietary parameters such as NE_L, CP, and NDF concentrations. The only variable that had sufficient discriminatory power to differentiate between +RFI (RFI ≥ 1.13) and –RFI (RFI ≤ -1.06) dairy cows was DIM and it was positively correlated (r = 0.904) with RFI. However, even with DIM, misclassification rates were still relatively high. When DIM was used as the sole discriminatory variable in the CAN12 function, the misclassification error rate in the test dataset was 30.48% compared to 29.92% error which occurred when DIM, milk protein yield, milk fat yield, BW, MUN, parity, calving month, and dietary NE_L concentration were cumulatively included in the CAN1 function. Thus, DIM was the only variable in the current study that was able to significantly discriminate between +RFI and –RFI cows. Based on the results of this study, +RFI and –RFI cows cannot be successfully differentiated based on most biological, production, or dietary variables.

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Table 5.1. Descriptive statistics for the complete dataset prior to RFI group[1] and dataset[2] assignment.

Item[3]	Mean	SD[4]	Minimum	Maximum
DMI[5], kg/d	22.5	3.3	14.7	31.2
Milk yield[6], kg/d	44.0	7.2	27.6	64.3
Milk fat, %	3.54	0.45	2.17	4.74
Milk fat yield, g/d	1554	297	758	2694
Milk protein, %	2.82	0.23	1.80	3.87
Milk protein yield, g/d	1234	190	798	1763
ECM[7], kg/d	44.0	7.1	26.0	68.1
BW[8], kg	583	61	456	763
MBW[9], kg	118.6	9.3	98.7	145.3
ADG[10], kg/d	0.34	0.69	-6.66	6.15
MUN[11], mg/dL	11.8	2.6	4.7	18.3
Dietary CP[12], %	16.6	0.7	14.7	18.5
Dietary NDF[13], %	32.0	2.4	26.4	40.7
Dietary NE_L[14], Mcal/kg	0.77	0.02	0.73	0.84
DIM[15]	66	27	23	142
Parity[16]	1.44	0.50	1	2
Calving Month[17]	7.3	3.3	1	12

[1]Weekly cow means were either assigned to +RFI (RFI ≥ 1.13) or –RFI (RFI ≤ -1.06) groups.
[2]The data was divided into training (70.01%) and test (29.99%) datasets.
[3]Sample size for each variable (n) = 7,750 means averaged weekly on an individual cow basis.
[4]SD = Standard deviation.
[5]DMI = Dry matter intake.
[6]Milk yield (kg/d) = AM Milk (kg/d) + PM Milk (kg/d).
[7]ECM = ((12.95 x kg milk fat) + (7.65 x kg milk protein) + (0.327 x kg milk)/2.2) (DRMS, 2014).
[8]BW = Body weight.
[9]MBW = Metabolic body weight ($BW^{0.75}$).
[10]ADG = Average daily gain.
[11]MUN = Milk urea nitrogen.
[12]CP = Crude protein (% DM basis).
[13]NDF = Neutral detergent fiber (% DM basis).
[14]NE_L = Net energy of lactation (Mcal/kg DM).
[15]DIM = Days in milk.
[16]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[17]Calving month ranges from January (1) to December (12).

Table 5.2. Descriptive statistics for each RFI group prior to dataset[1] assignment and SDA.

Item[2]	Mean	SD[3]	Minimum	Maximum
+RFI (RFI ≥ 1.13)				
DMI[4], kg/d	24.8	2.8	16.1	31.2
Milk yield[5], kg/d	44.6	7.1	27.6	64.2
Milk fat, %	3.51	0.46	2.17	4.72
Milk fat yield, g/d	1553	282	787	2651
Milk protein, %	2.85	0.24	2.23	3.87
Milk protein yield, g/d	1264	187	813	1763
ECM[6], kg/d	44.4	6.8	26.0	66.2
BW[7], kg	589	60	457	764
MBW[8], kg	119.4	9.1	98.8	145.3
ADG[9], g/d	0.33	0.61	-4.43	3.30
MUN[10], mg/dL	11.9	2.7	4.7	18.3
Dietary CP[11], %	16.6	0.7	14.7	18.5
Dietary NDF[12], %	32.0	2.4	26.4	40.7
Dietary NE_L[13], Mcal/kg	0.77	0.02	0.73	0.84
DIM[14]	77.4	25.2	23	142
Parity[15]	1.48	0.50	1	2
Calving Month[16]	7.31	3.21	1	12
RFI[17]	2.04	0.70	1.13	4.00
-RFI (RFI ≤ -1.06)				
DMI[4], kg/d	20.5	2.9	14.7	30.2
Milk yield[5], kg/d	43.8	7.2	27.7	64.2
Milk fat, %	3.60	0.44	2.27	4.74
Milk fat yield, g/d	1577	321	836	2694
Milk protein, %	2.79	0.22	2.04	3.46
Milk protein yield, g/d	1217	192	798	1760
ECM[6], kg/d	44.0	7.5	27.0	68.1
BW[7], kg	586	64	456	764
MBW[8], kg	119.0	9.7	98.8	145.3
ADG[9], g/d	0.32	0.75	-3.65	5.95
MUN[10], mg/dL	11.7	2.8	4.7	18.3
Dietary CP[11], %	16.6	0.7	14.7	18.5
Dietary NDF[12], %	32.0	2.4	26.4	40.7
Dietary NE_L[13], Mcal/kg	0.77	0.02	0.73	0.84
DIM[14]	51.6	24.5	23	142
Parity[15]	1.45	0.50	1	2
Calving Month[16]	7.3	3.3	1	12
RFI[17]	-2.07	0.80	-4.30	-1.06

[1]The data were divided into training (70.01%) and test (29.99%) datasets.

[2]Sample size for each variable (n) = 1,899 means averaged weekly on an individual cow basis per group.
[3]SD = Standard deviation.
[4]DMI = Dry matter intake.
[5]Milk yield (kg/d) = AM Milk (kg/d) + PM Milk (kg/d).
[6]ECM = ((12.95 x kg milk fat) + (7.65 x kg milk protein) + (0.327 x kg milk)/2.2) (DRMS, 2014).
[7]BW = Body weight.
[8]MBW = Metabolic body weight ($BW^{0.75}$).
[9]ADG = Average daily gain.
[10]MUN = Milk urea nitrogen.
[11]CP = Crude protein (% DM basis).
[12]NDF = Neutral detergent fiber (% DM basis).
[13]NE_L = Net energy of lactation (Mcal/kg DM).
[14]DIM = Days in milk.
[15]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[16]Calving month ranges from January (1) to December (12).
[17]RFI = observed minus predicted DMI.

Table 5.3. Ranking of the original variables based discriminatory power calculated during the SDA.

Original Variable[1]	Partial R^2	F Value	*Pr* > F	Wilks' Lambda	*Pr* < Lambda	ASCC[2]	*Pr* > ASCC
DIM[3]	0.212	1021.73	<.0001	0.788	<.0001	0.212	<.0001
Milk protein yield, g/d	0.014	49.46	<.0001	0.778	<.0001	0.222	<.0001
Milk fat yield, g/d	0.014	53.82	<.0001	0.767	<.0001	0.233	<.0001
BW[4], kg	0.010	37.75	<.0001	0.759	<.0001	0.241	<.0001
MUN[5], mg/dL	0.005	17.46	<.0001	0.756	<.0001	0.244	<.0001
Parity[6]	0.003	12.14	0.0005	0.754	<.0001	0.247	<.0001
Calving month[7]	0.001	4.4	0.0360	0.753	<.0001	0.247	<.0001
Dietary NE_L[8]	0.001	3.65	0.0562	0.752	<.0001	0.248	<.0001
Dietary CP[9]	-	-	-	-	-	-	-
Dietary NDF[10]	-	-	-	-	-	-	-

[1]Sample size for each variable (n) = 1,899 means averaged weekly on an individual cow basis per group.
[2]Average squared canonical correlation (ASCC).
[3]DIM = Days in milk.
[4]BW = Body weight.
[5]MUN = Milk urea nitrogen.
[6]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[7]Calving month ranges from January (1) to December (12).
[8]NE_L = Net energy of lactation (Mcal/kg DM).
[9]CP = Crude protein (% DM basis).
[10]NDF = Neutral detergent fiber (% DM basis).

Table 5.4. Descriptive statistics for the original variables in the CAN function for each of the RFI groups in the training dataset (70.01%).

Item	Mean	SD[1]	Minimum	Maximum
+RFI (RFI ≥ 1.13)[2]				
DIM[3]	78.1	25.1	23	142
Milk protein yield, g/d	1266.4	185.1	813	1763
Milk fat yield, g/d	1560	278	787	2651
BW[4], kg	590	60	457	764
MUN[5], mg/dL	11.9	2.7	4.7	18.3
Parity[6]	1.49	0.50	1	2
Calving Month[7]	7.4	3.2	1	12
Dietary NE_L[8]	0.77	0.02	0.73	0.83
-RFI (RFI ≤ -1.06)[9]				
DIM[3]	51.7	24.6	23	142
Milk protein yield, g/d	1217	192.1	798	1760
Milk fat yield, g/d	1580	320	834	2694\
BW[4], kg	585	63	456	764
MUN[5], mg/dL	11.7	2.8	5.0	18.3
Parity[6]	1.45	0.50	1	2
Calving Month[7]	7.3	3.3	1	12
Dietary NE_L[8]	0.77	0.02	0.73	0.84

[1]SD = Standard deviation.
[2]Sample size for each variable (n) = 1,323 means averaged weekly on an individual cow basis.
[3]DIM = Days in milk.
[4]BW = Body weight.
[5]MUN = Milk urea nitrogen.
[6]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[7]Calving month ranges from January (1) to December (12).
[8]NE_L = Net energy of lactation (Mcal/kg DM).
[9]Sample size for each variable (n) = 1,336 means averaged weekly on an individual cow basis.

Table 5.5. Descriptive statistics for original variables in the CAN function for each of the RFI groups in the test dataset (29.99%).

Item	Mean	SD[1]	Minimum	Maximum
+RFI (RFI ≥ 1.13)[2]				
DIM[3]	75.8	25.2	23	140
Milk protein yield, g/d	1257	193	825	1753
Milk fat yield, g/d	1539	288	791	2420
BW[4], kg	584	60	459	762
MUN[5], mg/dL	11.7	2.7	4.9	18.1
Parity[6]	1.45	0.50	1.0	2
Calving Month[7]	7.12	3.26	1	12.
Dietary NE_L[8]	0.77	0.02	0.73	0.84
-RFI (RFI ≤ -1.06)[8]				
DIM[3]	51.5	24.3	23	142
Milk protein yield, g/d	1216	192	840	1733
Milk fat yield, g/d	1568	322	868	2605
BW[4], kg	588	66	457	763
MUN[5], mg/dL	11.7	2.7	4.7	18.2
Parity[6]	1.47	0.50	1	2
Calving Month[7]	7.2	3.3	1	12
Dietary NE_L[8]	0.77	0.02	0.73	0.84

[1]SD = Standard deviation.
[2]Sample size for each variable (n) = 576 means averaged weekly on an individual cow basis.
[3]DIM = Days in milk.
[4]BW = Body weight.
[5]MUN = Milk urea nitrogen.
[6]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[7]Calving month ranges from January (1) to December (12).
[8]NE_L = Net energy of lactation (Mcal/kg DM).
[9]Sample size for each variable (n) = 563 means averaged weekly on an individual cow basis.

Table 5.6. Total sample standardized canonical coefficients and pooled within canonical structure for the CDA conducted on the training dataset (70.01%)[1,2].

Original Variables[3]	Standardized Canonical Coefficients[4,5]	Pooled Within Canonical Structure[6]
DIM[7]	1.079	0.9036
Milk protein yield, g/d	0.567	0.2247
Milk fat yield, g/d	-0.368	-0.0579
BW[8], kg	-0.291	0.0745
MUN[9], mg/dL	-0.110	0.08820
Parity[10]	0.217	0.0733
Calving Month[11]	0.105	0.0265
Dietary NE_L[12]	-0.072	-0.0361
Eigenvalue	0.345	-
Canonical Correlation	0.507	-
Variance Explained, %	25.7	-
Class Means		
+RFI (RFI ≥ 1.13)	0.590	-
-RFI (RFI ≤ -1.06)	-0.585	-
RS Error Counts[13], %	26.21	-
CV Error Counts[14], %	26.89	-

[1]Sample size (n) = 1,323 means averaged weekly on an individual cow basis for the +RFI group.
[2]Sample size (n) = 1,336 means averaged weekly on an individual cow basis for the –RFI group.
[3]Dietary CP and NDF concentrations (% DM basis) were removed during the SDA from the list of original variables to be included in the CDA and DA as they lacked sufficient discriminatory power ($P > 0.15$).
[4]Canonical coefficients are the weighted contribution of each original variable to the CAN function.
[5]CAN = ((1.079 x DIM) + (0.567 x milk protein yield (g/d)) + (-0.368 x milk fat yield (g/d)) + (-0.291 x BW (kg)) + (-0.110 x MUN (mg/dL)) + (0.217 x parity) + (0.105 x calving month) + (-0.072 x NEL (Mcal/kg)).
[6]Canonical structure is calculated as the correlation between the canonical function and each original variable.
[7]DIM = Days in milk.
[8]BW = Body weight.
[9]MUN = Milk urea nitrogen.
[10]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[11]Calving month ranges from January (1) to December (12).
[12]NE_L = Net energy of lactation (Mcal/kg DM).
[13]Error rates (%) calculated using the resubstitution method.
[14]Error rates (%) calculated using the cross-validation method.

Table 5.7. Resubstitution and cross-validation error rates in the training dataset for the full-model[1] and reduced CAN functions.

CAN Function[2]	Error Rate (%) RS	Error Rate (%) CV
CAN1 = (c_1DIM[3]) + (c_2MPY[4]) + (c_3MFY[5]) + (c_4BW[6]) + (c_5MUN[7]) + (c_6Parity[8]) + (c_7CalvMon[9]) + (c_8NEL[10])	26.21	26.89
CAN2 = (c_1DIM[3]) + (c_2MPY[4]) + (c_3MFY[5]) + (c_4BW[6]) + (c_5MUN[7]) + (c_6Parity[8]) + (c_7CalvMon[9])	26.25	26.70
CAN3 = (c_1DIM[3]) + (c_2MPY[4]) + (c_3MFY[5]) + (c_4BW[6]) + (c_5MUN[7]) + (c_6Parity[8])	26.29	26.93
CAN4 = (c_1DIM[3]) + (c_2MPY[4]) + (c_3MFY[5]) + (c_4BW[6]) + (c_5MUN[7])	27.30	27.68
CAN5 = (c_1DIM[3]) + (c_2MPY[4]) + (c_3MFY[5]) + (c_4BW[6])	27.30	27.72
CAN6 = (c_1DIM[3]) + (c_2MPY[4]) + (c_3MFY[5])	27.94	28.02
CAN7 = (c_1DIM[3]) + (c_2MPY[4])	27.83	27.87
CAN8 = (c_1DIM[3]) + (c_3MFY[5])	28.66	28.66
CAN9 = (c_2MPY[4]) + (c_3MFY[5])	39.11	39.19
CAN10 = (c_3MFY[5])	46.60	46.82
CAN11 = (c_2MPY[4])	44.45	44.45
CAN12 = (c_1DIM[3])	28.24	28.24

[1]Full model (CAN1) includes the eight variables selected during the SDA.
[2]c^i are the canonical coefficients applied to each term in the CAN function.
[3]DIM = Days in milk.
[4]MPY = Milk protein yield (g/d).
[5]MFY = Milk fat yield (g/d).
[6]BW = Body weight (kg).
[7]MUN = Milk urea N (mg/dL).
[8]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[9]Calving month ranges from January (1) to December (12).
[10]NE_L = Net Energy of Lactation (Mcal/kg DM).

Table 5.8. Resubstitution error rates[1] in the test dataset for the full-model[2] and reduced CAN functions.

CAN Function[3]	Error Rate (%)[1]
CAN1 = (c_1DIM[4]) + (c_2MPY[5]) + (c_3MFY[6]) + (c_4BW[7]) + (c_5MUN[8]) + (c_6Parity[9]) + (c_7CalvMon[10]) + (c_8NEL[11])	29.92
CAN2 = (c_1DIM[4]) + (c_2MPY[5]) + (c_3MFY[6]) + (c_4BW[7]) + (c_5MUN[8]) + (c_6Parity[9]) + (c_7CalvMon[10])	29.82
CAN3 = (c_1DIM[4]) + (c_2MPY[5]) + (c_3MFY[6]) + (c_4BW[7]) + (c_5MUN[8]) + (c_6Parity[9])	29.93
CAN4 = (c_1DIM[4]) + (c_2MPY[5]) + (c_3MFY[6]) + (c_4BW[7]) + (c_5MUN[8])	29.81
CAN5 = (c_1DIM[4]) + (c_2MPY[5]) + (c_3MFY[6]) + (c_4BW[7])	29.73
CAN6 = (c_1DIM[4]) + (c_2MPY[5]) + (c_3MFY[6])	29.74
CAN7 = (c_1DIM[4]) + (c_2MPY[5])	29.00
CAN8 = (c_1DIM[4]) + (c_3MFY[6])	30.84
CAN9 = (c_2MPY[5]) + (c_3MFY[6])	41.86
CAN10 = (c_3MFY[6])	47.36
CAN11 = (c_2MPY[5])	44.83
CAN12 = (c_1DIM[4])	30.48

[1]Cross-validation error rates were not reported for the test dataset as this method does not apply.
[2]Full model (CAN1) includes the eight variables selected during the SDA.
[3]c^i are the canonical coefficients applied to each term in the CAN function.
[4]DIM = Days in milk.
[5]MPY = Milk protein yield (g/d).
[6]MFY = Milk fat yield (g/d).
[7]BW = Body weight (kg).
[8]MUN = Milk urea N (mg/dL).
[9]Cows were separated into first lactation (parity = 1) or second and beyond lactation (parity = 2).
[10]Calving month ranges from January (1) to December (12).
[11]NE_L = Net Energy of Lactation (Mcal/kg DM).

Figure 5.1. Relationship between observed and predicted DMI (kg/d). Predicted DMI (kg/d) = b_0 + (b_1 x Parity) + (b_2 x MBW) + (b_3 x ADG) + (b_4 x ECM) + RFI (Connor et al., 2013). [DMI (kg/d) = 1.000x + 0.000; intercept P = 1.0000; intercept SE = 0.161; slope P = < 0.0001; slope SE =0.007, R^2 = 0.720; RMSE = 1.743; n = 7,750].

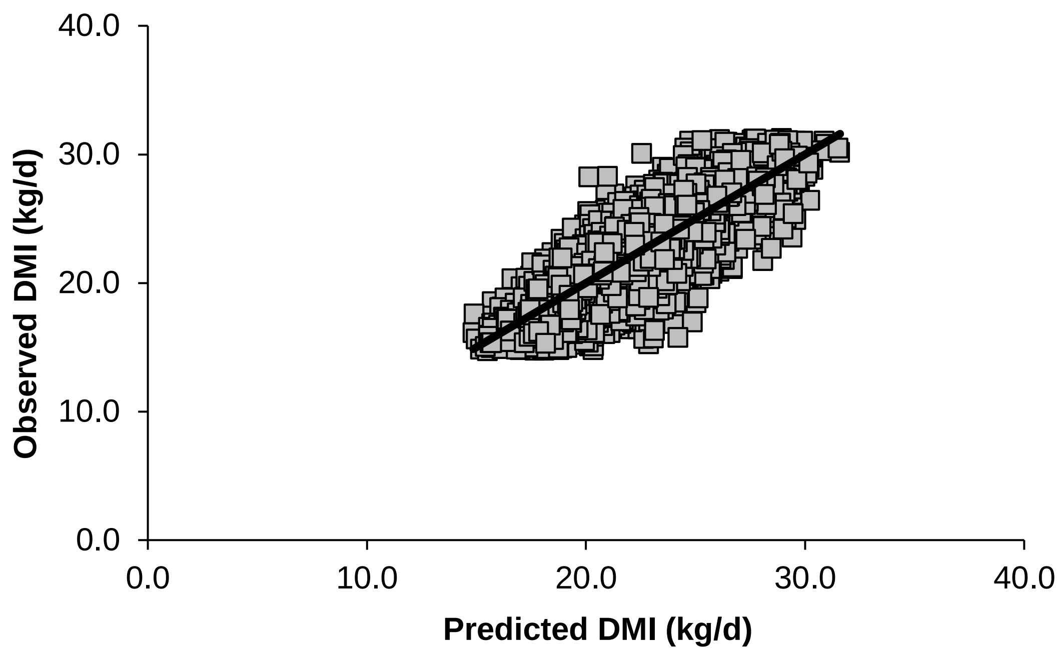


Figure 5.2. Graph of the canonical (CAN) function and canonical frequency distribution for the +RFI (RFI ≥ 1.13) and –RFI (RFI ≤ -1.06) groups. The class means for the +RFI and –RFI groups are 0.590 and -0.585, respectively. Positive and negative positions on the x-axis are dictated by positive and negative canonical coefficients.

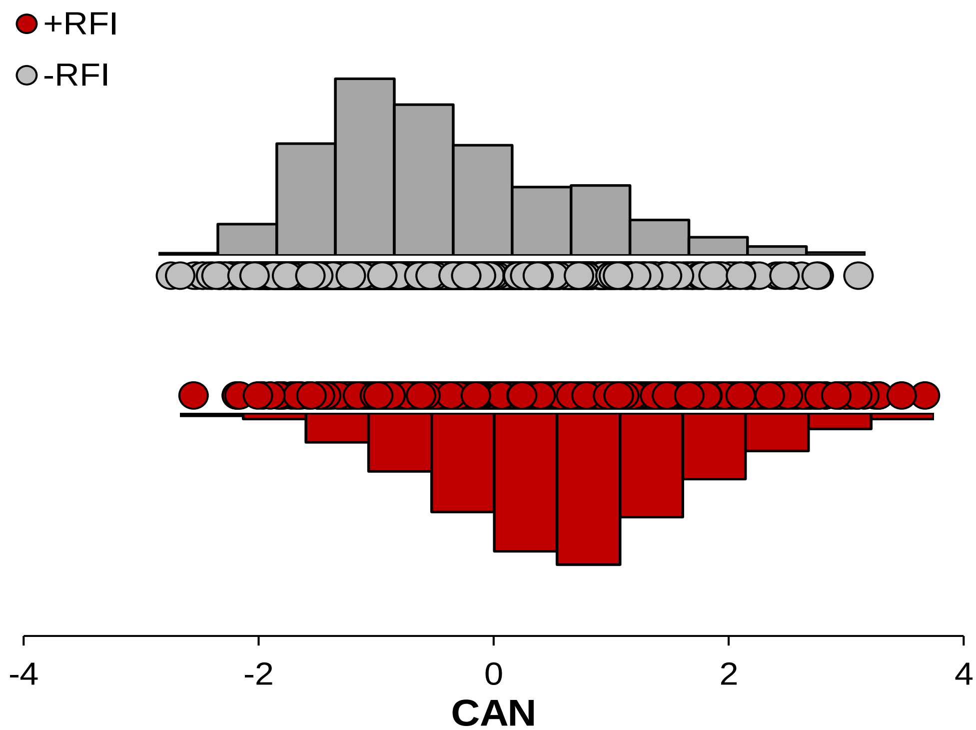


Figure 5.3. Discrimination between +RFI (RFI ≥ 1.13) and –RFI (RFI ≤ -1.06) based on DIM and milk protein yield (g/d). Error rates of misclassification in the training dataset were 27.83% and 27.87% for resubstitution and cross-validation methods, respectively. Resubstitution misclassification error rate in the test dataset was 29.00%.

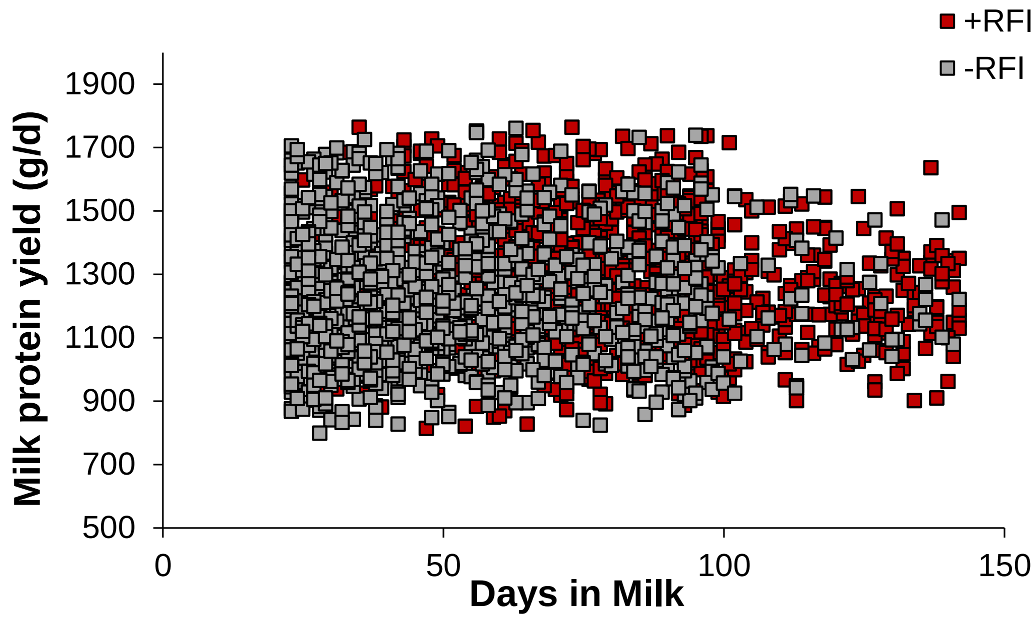


Figure 5.4. Discrimination between +RFI (RFI ≥ 1.13) and –RFI (RFI ≤ -1.06) based on DIM and milk fat yield (g/d). Error rates of misclassification in the training dataset were 28.66% for both resubstitution and cross-validation methods, respectively. Resubstitution misclassification error rate in the test dataset was 30.84%.

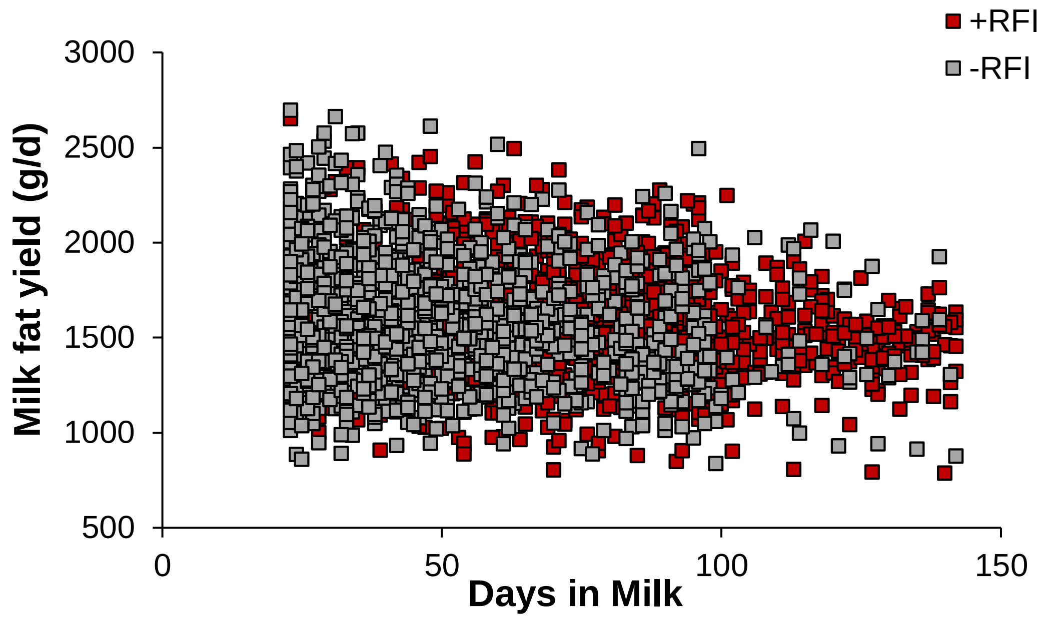
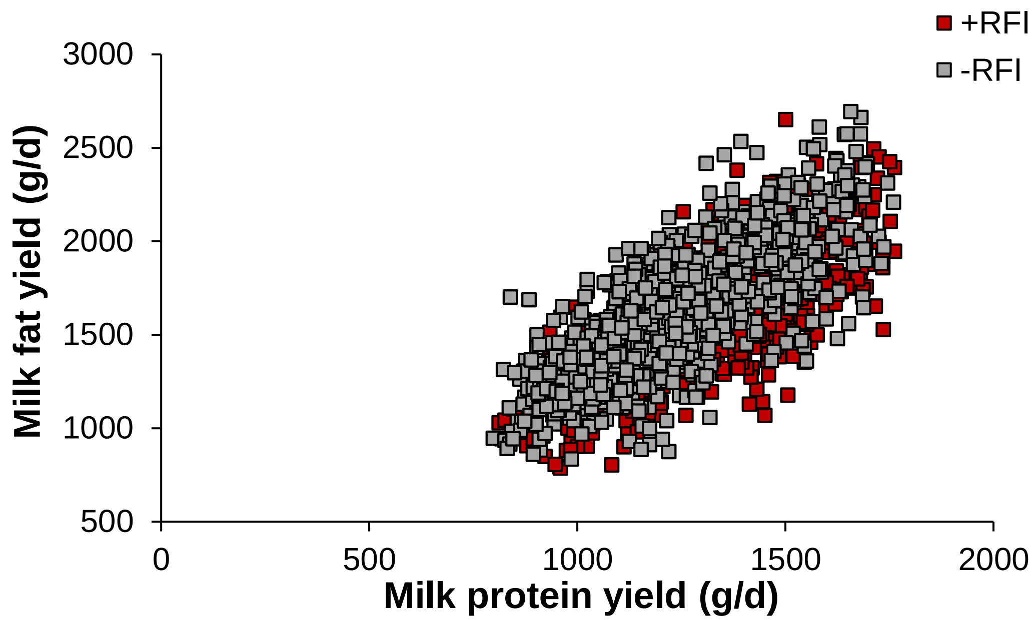


Figure 5.5. Discrimination between +RFI (RFI ≥ 1.13) and –RFI (RFI ≤ -1.06) based on milk protein yield (g/d) and milk fat yield (g/d). Error rates of misclassification in the training dataset were 39.11% and 39.19% for resubstitution and cross-validation methods, respectively. Resubstitution misclassification error rate in the test dataset was 41.86%.



CHAPTER 6: EXPERIMENT 4

Correlation analyses between residual feed intake (RFI) values generated from 3 novel DMI prediction equations and standard RFI[1]

[1]Iwaniuk, M. E., E. E. Connor, and R. A. Erdman. Correlation analyses between residual feed intake (RFI) values generated from 3 novel DMI prediction equations and standard RFI. In preparation for submission to the Journal of Dairy Science.

INTERPRETIVE SUMMARY

Correlation analyses between residual feed intake (RFI) values generated from 3 novel DMI prediction equations and standard RFI. *Iwaniuk et al., page 000.* In Chapter 3 of this dissertation, 8 DMI estimation equations were developed and compared to 2 published equations (Jonker et al., 1998; Kohn et al., 2002). Based on the performance of the novel equations, the top 3 equations were selected, evaluated, and validated. In this study, the 3 validated DMI equations were used to calculate 3 RFI variables (RFI1 – RFI3) for each individual weekly cow record (n = 7,750) and these RFI variables were compared to a standard RFI equation proposed by Connor et al. (2015). Correlation analyses were performed between the standard RFI values and the 3 RFI variables (RFI1 – RFI3) generated using the selected equations developed in Chapter 3. The results of these analyses suggest that RFI values produced from the three novel DMI equations are highly correlated to RFI values generated from a standard DMI prediction equation. Thus, RFI values were not dependent on the equation used to predict DMI and the DMI equations proposed in Chapter 3 are suitable equations to use for RFI calculations.

Correlation analyses between residual feed intake (RFI) values generated from 3 novel DMI prediction equations and standard RFI

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ABSTRACT

Dairy feed costs account for approximately 50% of the total costs of milk production in the U.S. dairy industry. Because feed costs are high, dairy producers are interested in improving the feed efficiency (**FE**) in which cows produce milk by selecting for metabolically superior dairy cows. Research has shown that RFI is an effective tool to genetically select for cows that consume less feed at any given level of milk production. Residual feed intake is phenotypically independent of production traits, relatively heritable ($h^2 = 0.17 – 0.36$), and repeatable within and across lactations in dairy cattle. As a statistical residual, RFI can differ between cows due to true variation in metabolic efficiency as well random variation associated with errors in measurement of dry matter intake (**DMI**; kg/d), DMI prediction, and random noise. Errors associated with DMI prediction depend on the DMI estimation equation utilized to predict DMI and calculate RFI. The hypothesis of this study was that RFI values were independent of the DMI estimation equation used. Three DMI estimation equations developed and validated in Chapter 3 of this dissertation were selected and used in this study to estimate DMI to calculate RFI values (RFI1 – RFI3). These RFI values were compared to RFI values generated by the DMI equation proposed by Connor et al. (2013) which was used to represent "standard" RFI values in the dairy industry. The results of the correlation analyses showed that there were strong correlations (all $r \geq 0.809$; P <0.0001) between RFI values generated by the novel DMI equations and the DMI equation proposed by Connor et al. (2013). Further evaluation of the RFI variables (RFI1 – RFI3) using Model Evaluation Software (**MES**; Tedeschi, 2006) demonstrated that the mean biases (**Mb**) and slope (linear) biases (**Sb**) between the RFI variables and the standard RFI were minimal. In particular, RFI3 had the best model

performance compared to standard RFI values as RFI3 had the least Mb (Mb = 0.01%) and Sb (Sb = 0.082%) compared to RFI1 and RFI2. Additionally, RFI3 had the best accuracy (R^2 = 0.707) and combined accuracy and precision (CCC = 0.839) compared to RFI1 and RFI2. However, it is important to mention that differences in Mb, Sb, R^2, and CCC were minimal among all 3 RFI variables. Thus, it can be concluded that RFI values are not dependent on the DMI estimation equation used to predict DMI. Additionally, these analyses suggest that the 3 novel DMI estimation equations developed and validated in Chapter 3 could be used by dairy producers to effectively estimate DMI and calculate RFI to select for metabolically efficient dairy cows within a herd.

Key Words: feed efficiency, residual feed intake, dry matter intake, correlation

INTRODUCTION

Feed costs account for approximately 50% of the total production costs associated with dairy milk production in the United States (Beck and Ishler, 2016; Hardie et al., 2017; Valvekar et al., 2010). Because feed costs affect profitability, dairy producers are interested in estimating the feed efficiency (**FE**) of which individual cows utilize feed nutrients for milk production in order to select for highly efficient cows within their herds (Connor, 2015). The improvement of dairy FE through management and genetic selection of highly efficient cows results in the following 3 positive outcomes: 1) increased profitability for dairy producers, 2) increased milk production which will help meet the nutritional demands of the growing global population, and 3) reduced impact of the dairy industry on the environment (Capper et al., 2009; VandeHaar et al., 2016).

There are several methods utilized within the U.S. dairy industry to estimate FE on an individual cow basis. One method that has increased in popularity during the past 2 decades is the use of residual feed intake (**RFI**) analysis (Connor, 2015; VandeHaar et al., 2016). Essentially, RFI is calculated using the following 3 steps: 1) actual dry matter intake (**DMI**; kg/d) is measured on individual cows in a cohort of cows in a herd, 2) DMI is predicted for an individual cow basis using a DMI prediction equation generated from production and BW data for the cohort of cows, and 3) RFI is calculated as the difference between a cow's actual and predicted DMI (Berry and Crowley, 2013; Connor, 2015; Koch et al., 1963). If an individual cow consumes more feed than predicted to produce a specified quantity of milk, she will have a positive RFI value and she is considered to have low metabolic FE. Conversely, if an individual cow consumes more feed than predicted to produce a specified quantity of milk, she will have a negative RFI value and she is considered to have a high metabolic FE (Connor, 2015; Potts et al., 2015).

There are several advantages to using RFI to calculate metabolic efficiency of individual cows. First, RFI values have been shown to be repeatable for individual cows within and across lactations such that stage of lactation and parity (when included in the DMI prediction equation) do not affect RFI values as they do affect other measures of FE such as the milk feed ratio (energy-corrected milk (kg/d) per unit of DMI (kg/d)) (Connor et al., 2013; Connor, 2015; Tempelman et al., 2014). In addition, RFI has been shown to have a relatively moderate heritability (h^2 =0.17–0.36) compared to other traits utilized for genetic selection (Connor et al., 2013; Tempelman et al., 2014; Cassell, 2009; Holstein Association USA, 2018. Lastly, high correlations have been shown to exist between RFI measured in growing heifers and subsequent RFI calculated during lactation (r = 0.58; Nieuwhof et al., 1992). This suggests that RFI can be assessed prior to first lactation in dairy cows and may allow for selection of more efficient cows prior to breeding and calving (Macdonald et al., 2014; Nieuwhof et al., 1992).

Although there are several advantages to using RFI to as a tool for evaluating FE, there are also several major disadvantages to using RFI. In particular, RFI is a residual value calculated as the difference between actual and estimated DMI. Due to the statistical nature of residuals, RFI contains both true variation in metabolic efficiency between cows due to genetic and the environmental conditions as well as random variation due to errors in DMI measurement, DMI prediction, and random error (VandeHaar et al., 2016). If DMI is measured inaccurately on-farm or DMI is poorly predicted for the cohort of cows using a DMI prediction equation, RFI values may be inflated as the variation due to these random errors falls into the residual (VandeHaar, et al., 2016). Thus, in some cases, RFI may not truly reflect differences in metabolic efficiency between cows; it may reflect errors associated with various stages of the analysis (VandeHaar et al., 2016).

In regard to errors associated with poor DMI predictions, it is possible that RFI values may differ even for the same individual cow depending on the DMI estimation equation used in the analysis. Because DMI is a labor-intensive and costly parameter to measure, numerous DMI estimation equations have been developed to predict DMI using on-farm measurements (Connor, 2015; Faverdin et al., 2017; Halachmi et al., 2004). Typically, DMI estimation equations contain the following 4 parameters: energy-corrected milk (**ECM**; kg/d), metabolic body weight (**MBW**; $BW^{0.75}$), and average daily gain (**ADG**; g/d) (Connor, 2015). However, a standard DMI equation does not exist; thus, it is at the researcher's discretion to select a DMI estimation equation to predict DMI to be used in the calculation of RFI.

The hypothesis of this study was that RFI values differ significantly between within-cow observations due to differences in predicted DMI values from varying DMI estimation equations. Therefore, the objective of this study was to determine if the 3 selected DMI equations developed and validated in Chapter 3 of this dissertation generate significantly different RFI values (RFI1 – RFI3) as compared to a standard DMI estimation used to estimate RFI in the U.S. dairy industry. The DMI estimation equation developed by Connor et al. (2013) was selected to represent a standard-industry DMI equation and this model included the following parameters: ECM, MBW, ADG, and parity. The results of this study will determine whether or not RFI is repeatable across the proposed DMI estimation equations and this knowledge can be utilized by individuals within the dairy industry to improve dairy FE through management and genetic selection.

MATERIALS AND METHODS

Dataset and DMI Estimation Equations

The initial data used for this study were obtained from the laboratory of Dr. Erin Connor at the United States Department of Agriculture (**USDA**), Beltsville Agricultural Research Center, Beltsville, MD. All data collection involving animals was approved by the Northeast Area Animal Care and Use Committee. The detailed methodology for development, assessment, selection, and validation of the 3 novel DMI estimation equations presented in this study is presented in Chapter 3 of this dissertation. The top 3 DMI estimation equations from Chapter 3 were used in this study to generate predicted RFI values (RFI1 – RFI3) and those equations are presented in Table 6.1. In addition, the DMI estimation equation proposed by Connor et al. (2013) was used in this study to represent a standard DMI equation to estimate RFI in the U.S. dairy industry. This equation is also presented in Table 6.1.

The detailed methodology for the data manipulation and production parameter estimates for the final dataset used in this study to estimate DMI and calculate RFI for the 4 equations is presented in Chapter 4 of this dissertation. The final dataset consisted of 7,750 weekly cow mean observations. The descriptive statistics for the continuous variables that were used to estimate DMI are presented in Table 6.2.

DMI Estimations and RFI Calculations

For the 3 selected DMI estimation equations developed in Chapter 3 of this dissertation, DMI was estimated by 2-wk intervals of week of lactation (**WOL**) using PROC NLIN (SAS 9.4., SAS Institute, Inc., Cary, NC). The standard predicted DMI and RFI were estimated for each weekly cow record by 2-wk intervals of WOL using PROC REG (SAS 9.4.) and the following equation proposed by Connor et al. (2013):

$$\text{Predicted DMI (kg/d)} = b_0 + (b_1 \times \text{Parity}) + (b_2 \times \text{MBW}) + (b_3 \times \text{ADG}) + (b_4 \times \text{ECM}) \quad (1)$$

Where:

b_0 = Intercept
b_1 = Partial regression coefficient of intake on parity (primiparous vs multiparous)
b_2 = Partial regression coefficient of intake on metabolic BW (**MBW**, $BW^{0.75}$; kg)
b_3 = Partial regression coefficient of intake on average daily gain (**ADG**; g/d)
b_4 = Partial regression coefficient of intake on energy-corrected milk (**ECM**; kg/d)

Once DMI was estimated for each weekly cow record, the "standard" RFI was calculated as:

$$\text{RFI} = \text{Observed DMI} - \text{Predicted DMI} \quad (2)$$

Equations for estimating RFI1, RFI2, and RFI3 based on DMI Equations 1, 2, and 3 from Chapter 3 of this dissertation are described in Table 6.1.

Correlation Analyses of RFI Variables

Pearson's correlation coefficient (**PCC**) is a measure of linear association between 2, continuous variables and the range of PCC is -1.0 to +1.0 in which negative values close to -1.0 indicate a strong, negative correlation and positive values close to +1 indicate a strong, positive correlation (Rodgers and Nicewander, 1988). To calculate the PCC between the standard RFI value and the 3 RFI variables (RFI1 – RFI3) generated from the novel DMI equations, PROC CORR (SAS 9.4.) was utilized. Each Pearson correlation analysis produced an associated *P*-value which represented the probability that the observed PCC (or one more extreme) was due to random chance, assuming the null hypothesis was true (Norman and Streiner, 1994). Statistical significance was declared at $P < 0.05$.

Detection of Mean and Linear Biases

Mean and linear biases between the standard RFI and the RFI variables (RFI1 – RFI3) generated from the 3 proposed DMI equations were evaluated using the Model Evaluation System (**MES**, College Station, TX; http://nutritionmodels.com/mes.html) described by Tedeschi (2006). The methodology used to detect and interpret mean and linear biases are discussed in detail in the "Materials and Methods" section of Chapter 3 in this dissertation.

RESULTS

The DMI prediction equation proposed by Connor et al. (2013) as well as the 3 novel DMI equations developed in Chapter 3 of this dissertation are presented in Table 6.1. In addition, the descriptive statistics for the response variables used to calculate DMI in each equation are presented in Table 6.2. Lastly, the averaged coefficients for each term in the 4 DMI estimation equations are presented in Table 6.3.

The results of the regression analyses between observed DMI and prediction DMI for each DMI equation are presented in Table 6.4 and Figures 6.1 – 6.4. The equation proposed by Connor et al. (2013) explained the most variation out of all 4 DMI estimation models (R^2 = 0.720; Root Mean Square Error (**RMSE**) = 1.743; $P < 0.0001$; Figure 6.1). In terms of consistency of variation of DMI explained, the R^2 for the DMI prediction equation used to calculate RFI in this current study mirrored the R^2 value for the same model as reported by Connor et al. (2013) using a subset of the current dataset (R^2 = 0.72; Standard deviation (**SD**) = 4.64). As observed in Chapter 3, the equation used to calculate RFI3 explained more variation associated with DMI (R^2 = 0.690; RMSE = 1.834; $P <$ 0.0001; Figure 6.4)) compared to the DMI equations used in RFI1 (R^2 = 0.660; RMSE = 1.920; $P < 0.0001$; Figure 6.2) and RFI2 (R^2 = 0.647; RMSE = 1.957; $P < 0.0001$; Figure 6.3); however, differences between the amount of variation explained in each equation were minimal.

Descriptive statistics for the 4 RFI variables generated from the DMI estimation equations are presented in Table 6.5. Overall, the means of the 4 RFI were close to zero which is consistent with the nature of residuals (Cox and Snell, 1968). In addition, the RFI values from -8.482 to +8.179, depending on the equation utilized.

The results of the correlation analyses between the standard RFI values and the 3 RFI variables (RFI1 – RFI3) are presented in Table 6.6. The strongest positive, linear correlation occurred between standard RFI and RFI3 (r = 0.841) as shown in Figure 6.7. The second strongest correlation occurred between standard RFI and RFI2 (r = 0. 815) which is shown in Figure 6.6. Lastly, the weakest correlation occurred between standard

RFI and RFI1 ($r = 0.809$) as shown in Figure 6.5. All correlation analyses were statistically significant ($P < 0.0001$).

The results of the evaluation conducted using the MES (Tedeschi, 2006) between RFI and RFI variables (RFI1 – RFI3) are presented in Table 6.7. Accuracy between standard RFI values and the RFI variables (RFI1 - RFI3) was determined using R^2, while the concordance correlation coefficient (**CCC**) assessed both accuracy and precision simultaneously (Tedeschi, 2006). The CCC is a measure of the agreement between measured (RFI) and predicted (RFI1 – RFI3) variables and a value of 1.0 is indicative of perfect agreement between 2 variables (Tedeschi, 2006). On average across all of the RFI variables, RFI3 had the highest accuracy ($R^2 = 0.707$) and highest measure of both accuracy and precision (CCC = 0.839) compared to RFI1 ($R^2 = 0.654$; CCC = 0.805) and RFI2 ($R^2 = 0.663$; CCC = 0.809).

To determine mean and linear biases, several evaluations were conducted. The Mb in RFI (kg/d) was calculated and this statistic represents, on average, the tendency of a prediction (RFI1 – RFI3) to over or underestimate a parameter as compared to the observed (RFI) value (Tedeschi, 2006). The RFI3 estimate had the lowest Mb (-0.0087 kg/d) compared to RFI1 (-0.0184 kg/d) and RFI2 (-0.0223 kg/d). The Mb for RFI3 suggests that on average RFI3 underestimated RFI values by 0.0087 kg/d. Additionally, root mean square error of prediction (**RMSEP**) was determined and this value represents the reliability and predictability of the model (RFI1 – RFI3) on observed (RFI) values (Tedeschi, 2006). To calculate RMSEP, all squared prediction errors associated with the model are summed together and then the square root of the sum is taken (Tedeschi, 2006). Similar to Mb, RFI3 had the lowest RMSEP (RMSEP = 1.014 kg/d) compared to RFI1 and

RFI2 which both had an RMSEP equal to 1.148 kg/d. Lastly, the mean square errors of prediction (**MSEP**) were decomposed into the percentage mean bias, slope (linear) bias, and random error to determine the source of error in the model (RFI1 – RFI3) prediction (Tedeschi, 2006).

On average, the Mb and Sb for all 3 RFI variables (RFI1 – RFI3) were relatively small (Mb $\leq$ 0.04%; Sb $\leq$ 3.66%) compared to random error (error $\geq$ 96.30%). Again, RFI3 had the smallest Mb (Mb = 0.01%) and Sb (Sb = 0.82%) compared to RFI1 (Mb = 0.03%; Sb = 2.50%) and RFI2 (Mb = 0.04%; Sb = 3.66%). Additionally, RFI3 had the highest random error (error = 99.17%) compared to RFI1 (error = 97.48%) and RFI2 (96.30%). Overall, the DMI equation used to generated RFI3 values proved produce RFI values most similar to standard RFI values compared to the DMI equations used to generate RFI1 and RFI2 values.

DISCUSSION

In recent years, utilizing RFI to estimate dairy FE has become the focus of a substantial amount of research in the U.S. dairy industry (Connor, 2015; VandeHaar et al., 2016). Due to how it is calculated, RFI is phenotypically independent of production traits such as body size, ADG, and milk yield (Connor et al., 2013; Van Arendonk et al., 1991). Thus, research suggests that RFI represents metabolic differences in feed utilization between cows independent of production differences (Connor et al., 2013). Because RFI values can be utilized to estimate metabolic FE, research has been conducted to examine RFI heritability and repeatability to determine if RFI is a suitable metric to use for management and genetic selection of highly feed efficient dairy cows.

In regard to heritability (h^2), research has shown that RFI is relatively heritable ($h^2 = 0.17$- 0.36) compared to other production traits ($h^2 < 0.10$) which suggests that it can be used for genetic selection to improve dairy FE over time (Connor et al., 2013; Holstein Association USA, 2018; Tempelman et al., 2014). In addition, RFI has been shown to be repeatable for individual cows both within and across lactations (Connor et al., 2013; Tempelman et al., 2014). Thus, this research suggests that RFI can be measured at any stage of lactation or any parity (when included in the DMI prediction equation) and still reflect an accurate prediction of metabolic efficiency for an individual cow (Connor, 2015; Tempelman et al., 2014). Lastly, RFI values measured on heifers have been shown to be strongly correlated to subsequent RFI values calculated on the same animals during lactation (Macdonald et al., 2014; Nieuwhof et al., 1992). Therefore, theoretically, a dairy producer could estimate the RFI of a heifer cow to predict her metabolic efficiency for future lactations without having to wait until she calves and enters the milking herd. In summary, research has shown that RFI is indicative of metabolic FE and RFI values are both heritable and repeatable which makes RFI a good candidate for genetic selection to improve dairy FE.

Although research has examined the heritability and repeatability of RFI within and across lactations, research has yet to be conducted that examines the repeatability of RFI when different DMI estimation equations are used to predict DMI. This question deserves substantial consideration as numerous DMI estimation equations exist and various equations are currently being utilized to predict DMI to calculate RFI (Connor, 2015). Therefore, it is important to determine if RFI is dependent on the DMI equation utilized to estimate DMI.

As discussed previously, RFI is a residual that is calculated as the difference between actual and predicted DMI (Berry and Crowley, 2013; Connor, 2015; Koch et al., 1963). Thus, RFI contains true variation due to differences in metabolic FE between cows, but it also contains random variation due to errors associated with actual DMI measurements, prediction equation errors, and random noise (VandeHaar et al., 2016). Because prediction equation errors are associated with the equation used, the hypothesis of this study was that different DMI estimation equations would generate significantly different RFI values as prediction error is inherently engrained in RFI (VandeHaar et al., 2016). However, the results of this study indicated that RFI values generated from different DMI estimation equations show good agreement suggesting that RFI may be robust in terms of differences in DMI predictions used for its calculation.

As shown in Table 6.4 and Figures 6.1 - 6.4, the regression relationships between actual DMI and estimated DMI were similar among the 4 DMI estimation equations. The equation used to predict DMI to calculate standard RFI values accounted for 72.0% of the total variation associated with DMI which mirrored the percentage reported by Connor et al. (2013) using the same equation in their study with lactating dairy cattle. The DMI equations used to calculate RFI1, RFI2, and RFI3 accounted for 66.0, 64.7, and 69.0% of the total variation associated with DMI, respectively (Figures 6.2 – 6.4). Manafiazar et al. (2013) reported that 68% of total variation associated with DMI in dairy cattle was accounted for when MBW, empty body weight, and milk production energy requirements were included in the DMI equation. Because the results of the regression analyses in this study are similar to previously published results, it can be concluded that the equations used to predict DMI for RFI1, RFI2, and RFI3 adequately predicted the DMI of lactating dairy cows.

Although we hypothesized that RFI values would be dependent on the DMI estimation equation, the results of the correlation analyses suggest that there is a strong correlation between the standard RFI and the estimated RFI values, regardless of the DMI estimation equation used. Correlations between the standard RFI values and the RFI variables (RFI1 – RFI3) generated from the proposed DMI equations developed in Chapter 3 of this dissertation were 0.809, 0.815, and 0.841, respectively ($P < 0.0001$). These correlations suggest that there is good agreement between RFI values regardless of the DMI estimation equation used in the analysis. To the knowledge of the authors, this is the first study that has examined the relationship between RFI values generated using different DMI prediction equations and it could be concluded that RFI may be robust and repeatable across different DMI estimation equations.

While RFI values from the 3 proposed DMI equations showed good agreement with standard RFI values, it is important to note that the magnitude of RFI values differed such that standard RFI had larger values as compared to RFI1, RFI2, and RFI3. This observation can be seen in Figures 6.5 – 6.7 in which the slope of the line of correlation between each proposed RFI (RFI1 – RFI3) and standard RFI is between 0.72 and 0.80, which departs from unity (1.0). It may be possible that there is inherent error associated with standard RFI values that is not present in RFI1, RFI2, and RFI3 such that standard RFI values are larger in magnitude. The intercepts of the line of correlation between each proposed RFI (RFI1 – RFI3) and standard RFI range from -0.01 to -0.02 which suggests that these relationships lack large mean biases as each intercept is approximately zero (Figures 6.5 – 6.7).

To further explore the relationships between the standard RFI values and the RFI variables (RFI1 – RFI3), the following evaluations were conducted: accuracy (R^2), combined accuracy and precision (CCC), overall mean bias (Mb), errors associated with predictions (RFI1 – RFI3) (RMSEP), and the decomposition of prediction errors (MSEP) into Mb, Sb, and random errors presented as percentages of MSEP. Overall, the R^2 and CCC values for all 3 RFI variables were greater than 0.654 and 0.805, respectively, which indicates that these RFI variables had good agreement with the standard RFI values. In addition, the Mb and RMSEP for all 3 RFI variables were less than -0.0223 and 1.148, respectively, which is relatively low as RFI values ranged from ranged from -8.482 to +8.179 in these datasets. The Mb for all RFI variables was negative which indicated that RFI was under-predicted in the 3 proposed DMI equations as compared to the standard DMI equation used to generate RFI values (Connor et al., 2013). However, it is important to note that the Mb and Sb for the RFI variables were relatively low (Mb ≤ 0.04%; Sb ≤ 3.66%) compared to random error (error ≥ 96.30%), suggesting that most of the errors of the prediction can be attributed to natural variation and not biases associated with the RFI predictions (RFI1 – RFI3).

In regard to performance, RFI3 showed the best agreement with standard RFI values as it had the lowest overall Mb, RMSEP, Mb (% of MSEP), and Sb (% of MSEP) compared to RFI1 and RFI2. Conversely, RFI3 had the highest R^2, CCC, and random error (% of MSEP) compared to RFI1 and RFI3 which suggests that it had the strongest correlation to the standard RFI values compared to the other RFI variables. It is important to note that differences between the 3 RFI variables in regard to the evaluation parameters are relatively small; therefore, all 3 equations used to predict DMI proposed in Chapter 3 of this dissertation may be suitable to use for RFI calculations.

In summary, it can be concluded that RFI values generated from different DMI equations showed good agreement such that RFI values were not dependent on the DMI prediction equation. In addition, the results of this experiment demonstrated that the 3 novel DMI estimation equations can be used as alternative equations to predict DMI to calculate RFI. To estimate DMI using the equations developed in Chapter 3, the following variables must be measured on-farm or estimated: milk yield, milk protein concentration, BW, milk urea nitrogen, and dietary crude protein concentration. Conversely, the DMI equation proposed by Connor et al. (2013) requires milk yield (kg/d), milk fat concentration (%), milk protein concentration (%), BW (kg), ADG (g/d), and parity to be recorded on-farm and most importantly direct measurement of DMI in order to calculate RFI. The results of this study provide dairy producers with 3 new DMI estimation methods that may be utilized to estimate DMI to calculate RFI using more readily available on-farm measurements.

CONCLUSIONS

Previous research has shown that RFI is a heritable and repeatable trait that can be used to genetically select for metabolically efficient dairy cattle. Calculating RFI requires the prediction of DMI. Several DMI estimation equations have been developed and used to calculate RFI. The results of this study indicate that RFI values generated from different DMI estimation equations (RFI vs. RFI1 – RFI3) are strongly correlated such that RFI values appear to be relatively independent of the DMI equation used during their calculation. In addition, the results of this study suggest that the 3 selected DMI estimation equations developed and validated in Chapter 3 of this dissertation can be used by the dairy industry to successfully estimate DMI and calculate RFI to select for metabolically efficient dairy cows; thus, eliminating the need to directly measure DMI of individual cows.

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Table 6.1. Equations used to estimate individual cow DMI to calculate RFI.

Eq.	DMI[1] Estimation Equations[2]
RFI[3]	DMI (kg/d) = b_0[4] + (b_1[5] x Parity[6]) + (b_2[7] x MBW[8]) + (b_3[9] x ADG[10]) + (b_4[11] x ECM[12]) + RFI[13]
RFI1[14]	DMI (kg/d) = (MilkN[15] + (B[16] × BW[17]) + (C[18] × MUN[19]))/(0.83[20] × DietN[21] - 3[22]) + RFI1
RFI2[23]	DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) + RFI2
RFI3[24]	DMI (kg/d) = (MilkN + (B × BW))/(I[25] × DietN – MFN[26]) + RFI3

[1]DMI = Dry matter intake (kg/d).
[2]Equations used to estimate DMI for RFI1 – RFI3 were the top 3 estimation equations developed, evaluated, and validated in Chapter 3 of this dissertation.
[3]Equation by Connor et al. (2013) used to predict DMI to estimate RFI. This equation represents a standard DMI prediction equation used to calculate RFI in the U.S. dairy industry.
[4]Intercept.
[5]Partial regression coefficient of intake on parity.
[6]Parity = Primiparous (first lactation; 1) or multiparous (second lactation and beyond; 2).
[7]Partial regression coefficient of intake on MBW.
[8]MBW = Metabolic body weight ($BW^{0.75}$).
[9]Partial regression coefficient of intake on ADG.
[10]ADG = Average daily gain (g/d).
[11]Partial regression coefficient of intake on ECM.
[12]ECM = Energy-corrected milk (kg/d) = ((12.95 x lbs milk fat) + (7.65 x lbs milk protein) + (0.327 x lbs milk)/2.2) (DRMS, 2014).
[13]RFI is calculated as actual DMI minus predicted DMI which represents statistical error in each equation.
[14]Equation used to predict DMI to calculate RFI1 corresponds to Equation 2 in Chapter 3 of this dissertation.
[15]MilkN = (Milk protein yield (g/d)/6.25)/(0.93).
[16]B = coefficient used to estimate N output based on ΔBW.
[17]BW = Body weight (kg).
[18]C = Coefficient used to estimate N output based on ΔMUN.
[19]MUN = Milk urea nitrogen (mg/dL).
[20]0.83 = Constant used to estimate the digestibility of dietary N (NRC, 2001).
[21]DietN = (Dietary crude protein (%)/6.25) × 10.
[22]3 = Constant used to estimate metabolic fecal N (MFN).
[23]Equation used to predict DMI to calculate RFI2 corresponds to Equation 3 in Chapter 3 of this dissertation.
[24]Equation used to predict DMI to calculate RFI3 corresponds to Equation 6 in Chapter 3 of this dissertation.
[25]I = Coefficient used to estimate digestibility of dietary N.
[26]MFN = Coefficient used to estimate metabolic fecal N (g/d).

Table 6.2. Descriptive statistics for the continuous variables used to estimate the individual DMI of lactating dairy cows.

Item[1]	Mean	SD[2]	Minimum	Maximum
DMI[3], kg/d	22.5	3.3	14.7	31.2
Milk Yield, kg/d	44.0	7.2	27.6	64.3
Milk Fat, %	3.54	0.45	2.17	4.74
Milk Protein, %	2.82	0.23	1.80	3.87
ECM[4], kg/d	44.0	7.1	26.0	68.1
MilkN[5], g/d	212	33	137	303
BW[6], kg	583	61	456	764
MBW[7], kg	119	9	99	145
MUN[8], mg/dL	11.8	2.6	4.7	18.3
Dietary CP[9], %	16.6	0.7	14.7	18.5
DietN[10], g/d	26.5	1.2	23.5	29.6
ADG[11], g/d	0.34	0.69	-6.66	6.15
Parity[12]	1.44	0.50	1	2

[1]Sample size for each variable (n) = 7,750 means averaged weekly on an individual cow basis.
[2]SD = Standard deviation.
[3]DMI = Dry matter intake.
[4]ECM = Energy-corrected milk = ((12.95 x kg milk fat) + (7.65 x kg milk protein) + (0.327 x kg milk)/2.2) (DRMS, 2014).
[5]MilkN = (Protein yield (g/d)/6.25)/(0.93).
[6]BW = Body weight
[7]MBW = Metabolic body weight ($BW^{0.75}$).
[8]MUN = Milk urea nitrogen.
[9]CP = Crude protein (% DM basis).
[10]DietN = (CP/6.25) × 10.
[11]ADG = Average daily gain (g/d).
[12]Parity = Primiparous (first lactation; 1) or multiparous (second lactation and beyond; 2).

Table 6.3. Coefficients (**coeff**) and standard error (**SE**) for each term in the 4 DMI equations used to generate RFI values.

Eq.	DMI Prediction Equations[1]	Model Terms	Estimate Coeff.	SE
RFI[2]	DMI[3] (kg/d) = b_0[4] + (b_1[5] x Parity[6]) + (b_2[7] x MBW[8]) + (b_3[9] x ADG[10]) + (b_4[11] x ECM[12]) + RFI[13]	b0	-1.45	0.542
		b1	0.718	0.111
		b2	0.116	0.005
		b3	0.515	0.074
		b4	0.203	0.007
RFI1[14]	DMI (kg/d) = (MilkN[15] + (B[16] × BW[17]) + (C[18] × MUN[19]))/(0.83[20] × DietN[21] – 3[22]) + RFI	B	0.353	0.001
		C	0.676	0.041
RFI2[23]	DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) + RFI	B	0.367	0.000
RFI3[24]	DMI (kg/d) = (MilkN + (B × BW))/(I[25] × DietN – MFN[26]) + RFI	B	0.378	0.002
		I	-0.013	0.012
		MFN	-19.55	0.337

[1]Equations used to predict DMI for RFI1 – RFI3 were the top 3 estimation equations developed, evaluated, and validated in Chapter 3 of this dissertation.
[2]Equation by Connor et al. (2013) used to predict DMI to estimate the standard RFI. This equation represents a standard DMI prediction equation used to calculate RFI in the U.S. dairy industry.
[3]DMI = Dry matter intake.
[4]Intercept.
[5]Partial regression coefficient of intake on parity.
[6]Parity = Primiparous (first lactation; 1) or multiparous (second lactation and beyond; 2).
[7]Partial regression coefficient of intake on MBW.
[8]MBW = Metabolic body weight ($BW^{0.75}$).
[9]Partial regression coefficient of intake on ADG.
[10]ADG = Average daily gain (g/d).
[11]Partial regression coefficient of intake on ECM.
[12]ECM = Energy-corrected milk (kg/d) = ((12.95 x lbs milk fat) + (7.65 x lbs milk protein) + (0.327 x lbs milk)/2.2) (DRMS, 2014).

[13]RFI is calculated as actual DMI minus predicted DMI which represents statistical error in each equation.
[14]Equation used to predict DMI to calculate RFI1 corresponds to Equation 2 in Chapter 3 of this dissertation.
[15]MilkN = (Milk protein yield (g/d)/6.25)/(0.93).
[16]B = coefficient used to estimate N output based on ΔBW.
[17]BW = body weight (kg).
[18]C = coefficient used to estimate N output based on ΔMUN.
[19]MUN = milk urea nitrogen (mg/dL).
[20]0.83 = constant used to estimate the digestibility of dietary N (NRC, 2001).
[21]DietN = (Dietary crude protein (%)/6.25) × 10.
[22]3 = constant used to estimate metabolic fecal N (MFN).
[23]Equation used to predict DMI to calculate RFI2 corresponds to Equation 3 in Chapter 3 of this dissertation.
[24]Equation used to predict DMI to calculate RFI3 corresponds to Equation 6 in Chapter 3 of this dissertation.
[25]I = coefficient used to estimate digestibility of dietary N.
[26]MFN = coefficient used to estimate MFN.

Table 6.4. Regression relationships between estimated and actual DMI for the proposed DMI estimation equations.

Eq.	Slope	SE	*P*-value	Int.	SE	*P*-value	R^2	RMSE[1]	*P*-value
RFI[2]	1.000	0.007	< 0.0001	0.000	0.161	1.0000	0.720	1.743	< 0.0001
RFI1[3]	0.953	0.008	< 0.0001	1.085	0.176	< 0.0001	0.660	1.920	< 0.0001
RFI2[4]	0.944	0.008	< 0.0001	1.281	0.180	< 0.0001	0.647	1.957	< 0.0001
RFI3[5]	0.975	0.007	< 0.0001	0.568	0.168	0.0007	0.690	1.834	< 0.0001

[1]RMSE = root mean square error.
[2]DMI (kg/d) = b_0 + (b_1 x Parity) + (b_2 x MBW) + (b_3 x ADG) + (b_4 x ECM) + RFI (Connor et al., 2013).
[3]DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3) + RFI1 (Chapter 3; Equation 2).
[4]DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) + RFI2 (Chapter 3; Equation 3).
[5]DMI (kg/d) = (MilkN + (B × BW))/(I × DietN – MFN) + RFI3 (Chapter 3; Equation 6).

Table 6.5. Descriptive statistics for the 4 RFI variables[1,2] generated from the DMI estimation equations.

RFI	Mean	SD[3]	Minimum	Maximum
RFI[4]	0.000	1.743	-8.449	8.083
RFI1[5]	0.018	1.925	-7.849	8.057
RFI2[6]	0.022	1.963	-8.482	8.179
RFI3[7]	0.009	1.835	-8.192	7.423

[1]RFI = Residual feed intake measured as actual DMI minus predicted DMI.
[2]Sample size (n) for each RFI variable was 7,750 weekly cow RFI values.
[3]SD = Standard deviation.
[4]DMI (kg/d) = b_0 + (b_1 x Parity) + (b_2 x MBW) + (b_3 x ADG) + (b_4 x ECM) + RFI (Connor et al., 2013).
[5]DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3) + RFI (Chapter 3; Equation 2).
[6]DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) + RFI (Chapter 3; Equation 3).
[7]DMI (kg/d) = (MilkN + (B × BW))/(I × DietN – MFN) + RFI (Chapter 3; Equation 6).

Table 6.6. Pearson correlation coefficients[1,2,3] between the standard RFI values[4] and the RFI variables[5,6,7] generated from the 3 selected DMI estimation equations.

RFI	RFI[4]
RFI1[5]	0.809
RFI2[6]	0.815
RFI3[7]	0.841

[1]Pearson correlation coefficients (r) are a measure of association between 2 continuous variables.
[2]Sample size (n) for each correlation was 7,750 weekly cow RFI values.
[3]All correlation analyses had $P < 0.0001$.
[4]DMI (kg/d) = $b_0 + (b_1 \times \text{Parity}) + (b_2 \times \text{MBW}) + (b_3 \times \text{ADG}) + (b_4 \times \text{ECM}) + \text{RFI}$ (Connor et al., 2013).
[5]DMI (kg/d) = $(\text{MilkN} + (B \times \text{BW}) + (C \times \text{MUN}))/(0.83 \times \text{DietN} - 3) + \text{RFI}$ (Chapter 3; Equation 2).
[6]DMI (kg/d) = $(\text{MilkN} + (B \times \text{BW}))/(0.83 \times \text{DietN} - 3) + \text{RFI}$ (Chapter 3; Equation 3).
[7]DMI (kg/d) = $(\text{MilkN} + (B \times \text{BW}))/(I \times \text{DietN} – \text{MFN}) + \text{RFI}$ (Chapter 3; Equation 6).

Table 6.7. Evaluation of the RFI variables (RFI1[1], RFI2[2], and RFI3[3]) generated from the DMI equations developed in Chapter 3 as compared to standard RFI values[4] (Connor et al., 2013).

					MSEP decomposition[7] (%)		
RFI	R^2	Mean bias	CCC[5]	RMSEP[6]	Mean bias[8]	Slope bias[9]	Random error[10]
RFI1	0.654	-0.0184	0.805	1.148	0.03	2.50	97.48
RFI2	0.663	-0.0223	0.809	1.148	0.04	3.66	96.30
RFI3	0.707	-0.0087	0.839	1.014	0.01	0.82	99.17

[1]DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3) + RFI (Chapter 3; Equation 2).
[2]DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) + RFI (Chapter 3; Equation 3).
[3]DMI (kg/d) = (MilkN + (B × BW))/(I × DietN – MFN) + RFI (Chapter 3; Equation 6).
[4]DMI (kg/d) = b_0 + (b_1 x Parity) + (b_2 x MBW) + (b_3 x ADG) + (b_4 x ECM) + RFI (Connor et al., 2013).
[5]CCC = Concordance correlation coefficient.
[6]RMSEP = Root mean squared errors of prediction.
[7]MSEP = Mean squared errors of prediction.
[8]Mean bias is the difference between standard RFI and RFI variable (RFI1 – RFI3) values.
[9]Slope bias is the linear bias associated with the correlation.
[10]Random error represent natural (unaccounted for) errors between standard RFI and each RFI variable (RFI1 – RFI3).

Figure 6.1. Relationship between observed and predicted DMI (kg/d) for RFI. Predicted DMI (kg/d) = b_0 + (b_1 x Parity) + (b_2 x MBW) + (b_3 x ADG) + (b_4 x ECM) + RFI (Connor et al., 2013). [DMI (kg/d) = 1.000x + 0.000; intercept *P* = 1.0000; intercept SE = 0.161; slope *P* = < 0.0001; slope SE =0.007, R^2 = 0.720; RMSE = 1.743; n = 7,750].

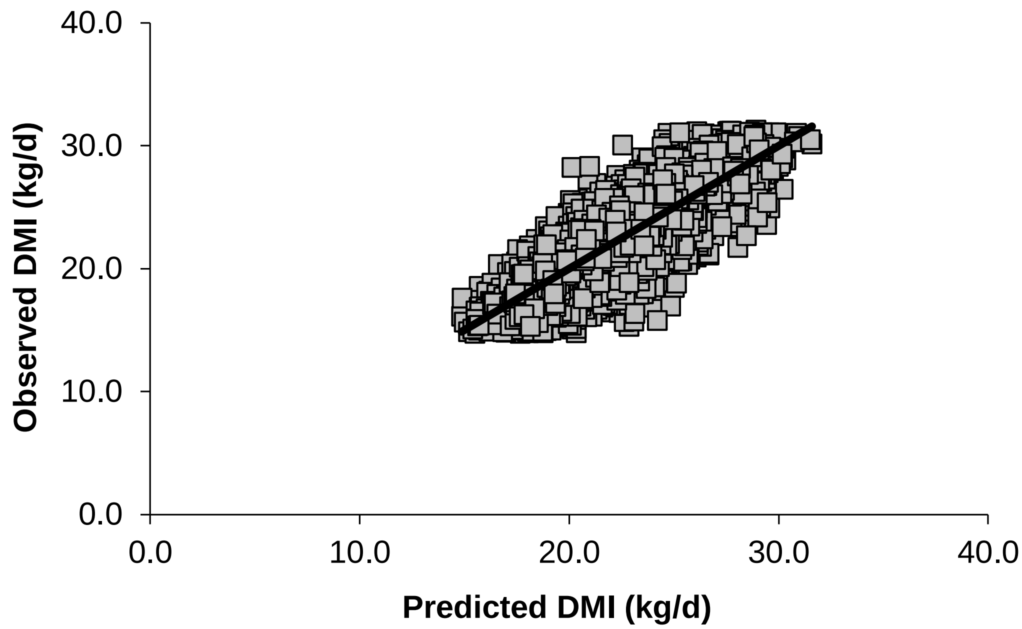


Figure 6.2. Relationship between observed and estimated DMI (kg/d) for RFI1. Estimated DMI (kg/d) = (MilkN + (B × BW) + (C × MUN))/(0.83 × DietN - 3) + RFI. [DMI (kg/d) = 0.953x + 1.085; intercept $P < 0.0001$; intercept SE = 0.176; slope $P = < 0.0001$; slope SE =0.008, $R^2 = 0.660$; RMSE = 1.920; n = 7,750].

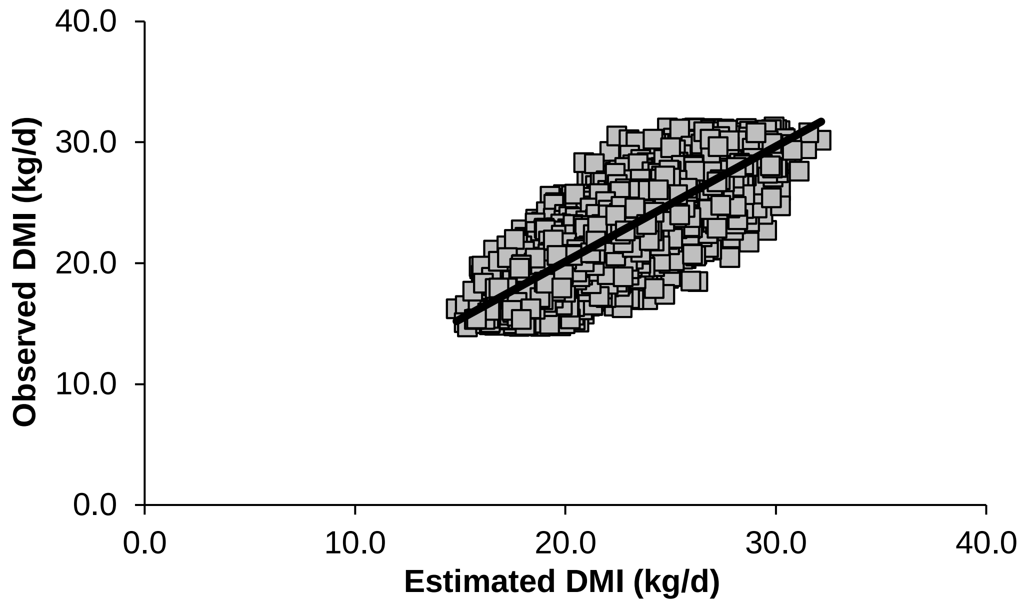


Figure 6.3. Relationship between observed and estimated DMI (kg/d) for RFI2. Estimated DMI (kg/d) = (MilkN + (B × BW))/(0.83 × DietN - 3) + RFI. [DMI (kg/d) = 0.944x + 1.281; intercept P < 0.0001; intercept SE = 0.180; slope P = < 0.0001; slope SE =0.008, R^2 = 0.647; RMSE = 1.957; n = 7,750].

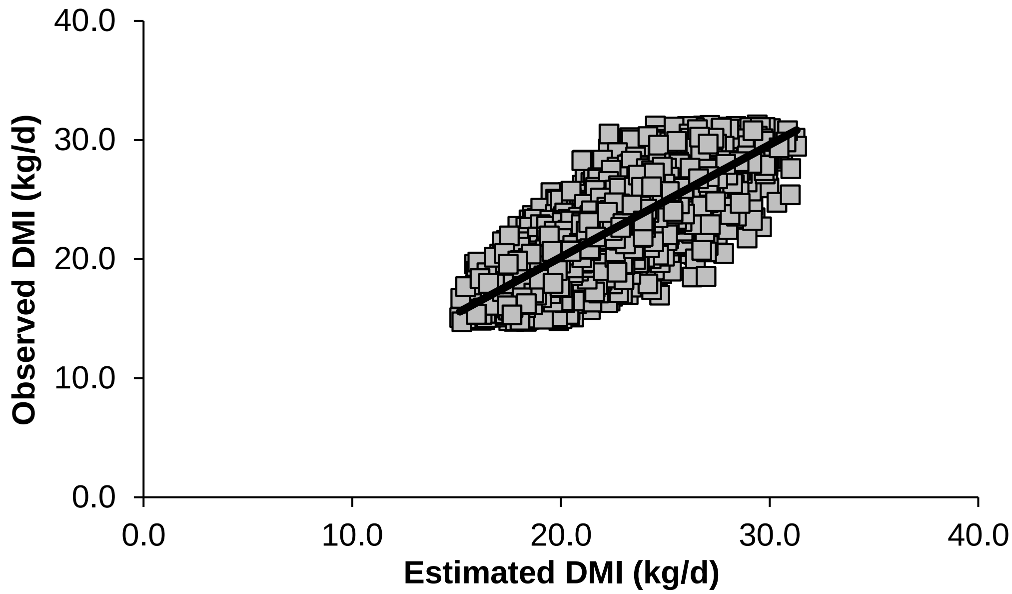


Figure 6.4. Relationship between observed and estimated DMI (kg/d) for RFI3. Estimated DMI (kg/d) = (MilkN + (B × BW))/(I × DietN – MFN) + RFI. [DMI (kg/d) = 0.975x + 0.568; intercept P = 0.0007; intercept SE = 0.168; slope P = < 0.0001; slope SE =0.007, R^2 = 0.690; RMSE = 1.834; n = 7,750].

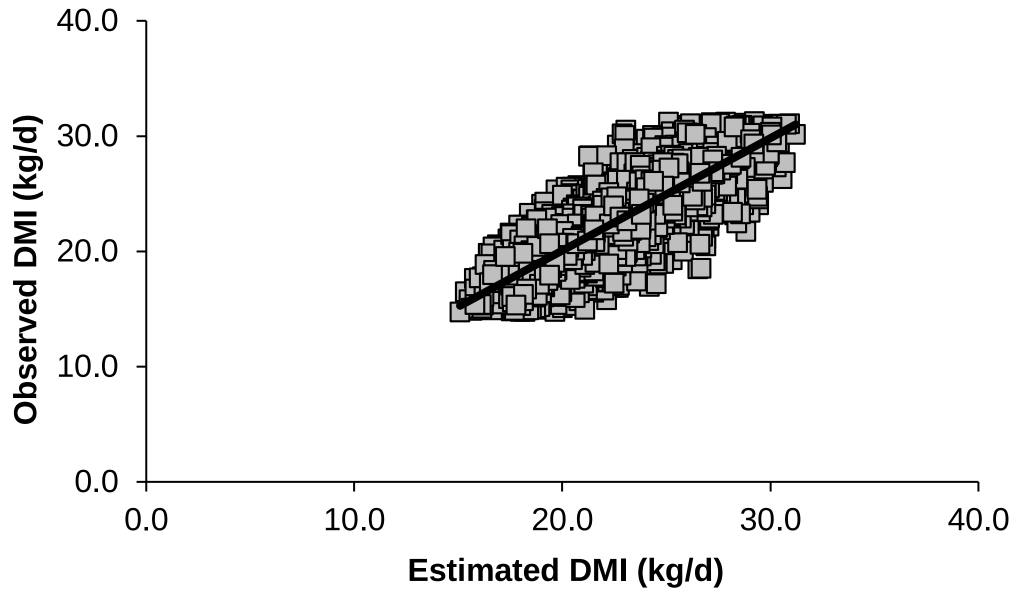


Figure 6.5. Correlation between RFI and RFI1 (r = 0.809; *P* < 0.0001).

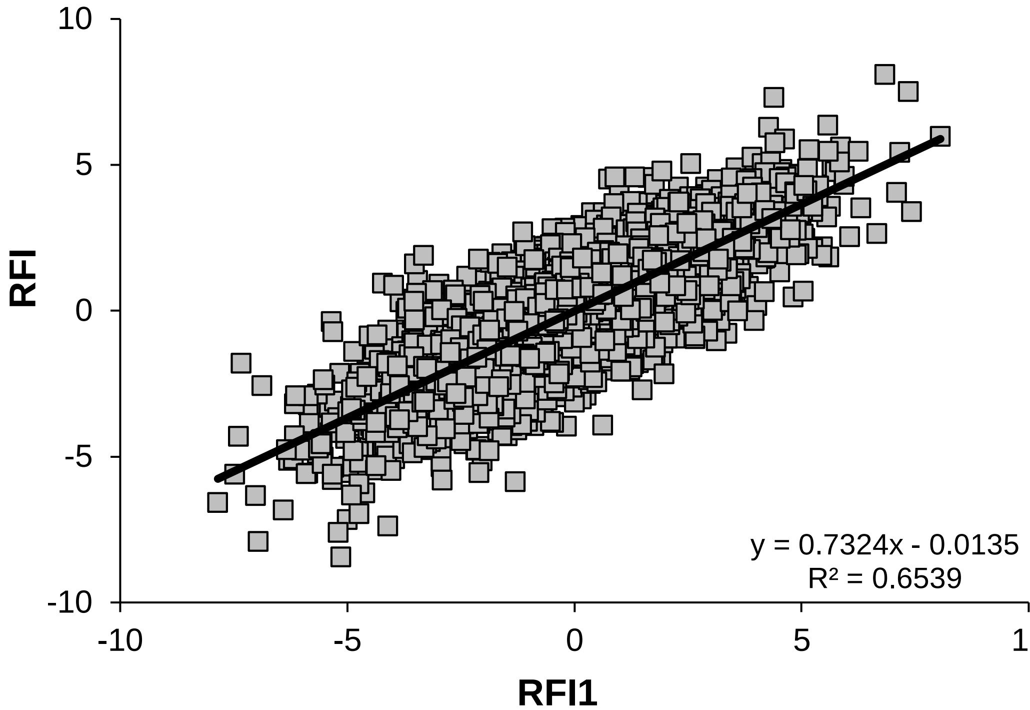


Figure 6.6. Correlation between RFI and RFI2 (r = 0.815; *P* < 0.0001).

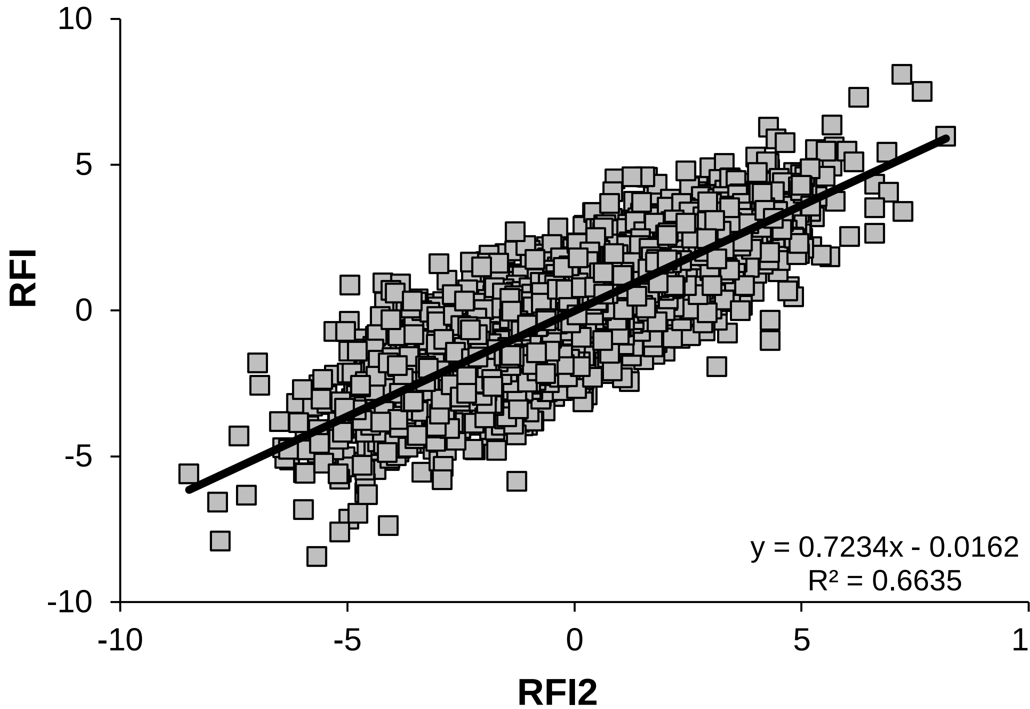
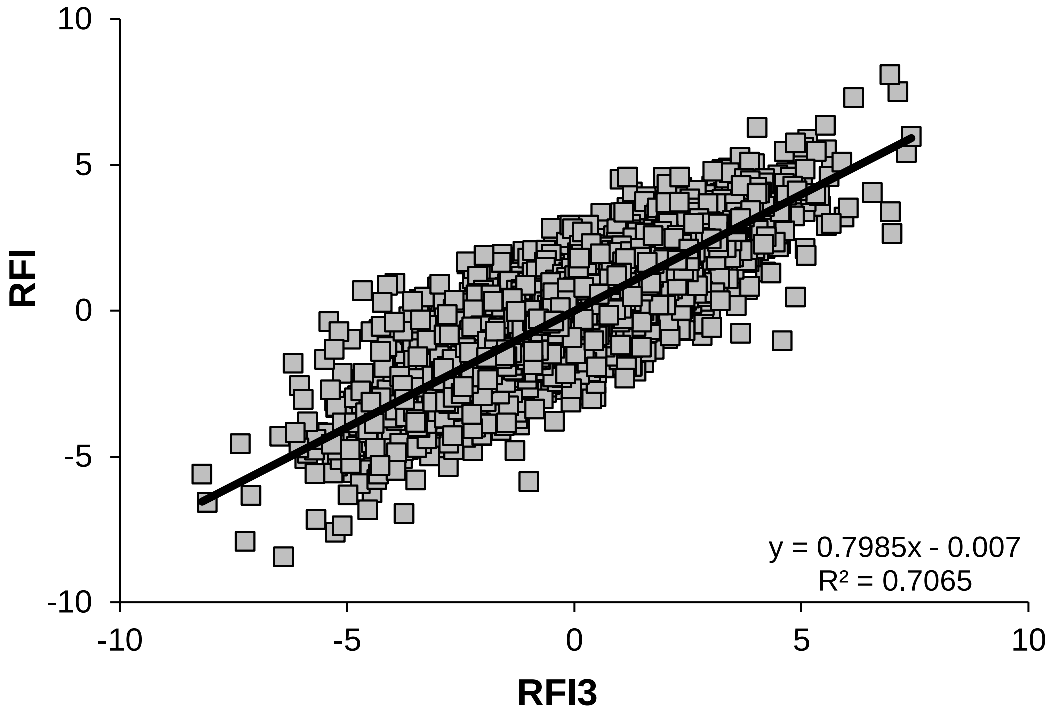


Figure 6.7. Correlation between RFI and RFI3 (r = 0.841; *P* < 0.0001).



CHAPTER 7: Summary

Summary and Future Directions

Summary

Due to high feed costs, dairy producers are interested calculating dairy feed efficiency (**FE**) on individual cows to select for the most efficient cows within their herds. There are 3 common approaches to estimating individual cow FE in the U.S. dairy industry and all 3 methods require an estimate of dry matter intake (**DMI**; kg/d). Because DMI is costly and labor-intensive to measure on individual cows, DMI must be estimated using equations. The first objective of this dissertation was to develop and validate equations that estimate DMI on an individual cow basis using the concept of nitrogen (**N**) balance derived from commonly available on-farm parameters. Results from the first experiment (Chapter 3) indicated that DMI could be successfully estimated on an individual cow basis using commonly measured on-farm parameters. The most successful equation (Equation 6) requires the following parameters to be measured in order to estimate FE: 1) milk N based on milk yield and milk protein concentration on the individual cow, 2) BW of the individual cow, and 3) dietary N from the herd ration composition. Because these inputs are relatively straight-forward to measure, this equation may be used in the dairy industry as a simple, practical method to estimate individual cow DMI even when cows are fed in a group setting.

As previously mentioned, all 3 commonly used approaches to estimate individual cow FE require a measurement of individual cow DMI which may be difficult to obtain on a standard dairy operation. The second objective of this dissertation was to determine if commonly measured biological, production or dietary variables could be used to successfully discriminate between high and low FE dairy cows without requiring DMI. In this experiment, FE was defined as the ratio of energy-corrected milk (ECM; kg/d) per unit

of DMI. The results of the second experiment (Chapter 4) suggested that days in milk (**DIM**), milk fat yield (g/d), and BW had the most discriminatory power to discriminate among cows based on their FE status. Using these 3 variables, cows were correctly assigned to their respective FE group (high vs. low) at a success rate of 89%. Because FE was negatively correlated with DIM and BW, but positively correlated with milk fat yield, smaller cows with high milk fat yields in early lactation tended to be the most feed efficient animals in the herd. Dairy producers can use the results of this study to select for cows with high FE without requiring the measurement of DMI.

Similarly, the third objective of this dissertation was to determine if commonly measured biological, production, or dietary variables could be used to successfully discriminate between dairy cows based on their residual feed intake (**RFI**) status. Residual feed intake is calculated as the difference between actual and predicted DMI. Because DMI is predicted from a model including several biological, production, and dietary variables, RFI is considered to be phenotypically independent of the variables used to estimate DMI in the calculation of RFI. The results of the third experiment (Chapter 5) suggested that RFI was independent of all of the parameters investigated in this study, except for DIM. Thus, the results of this experiment are congruent with previously published results suggesting that RFI is indicative of differences in metabolic efficiency between cows independent of most biological, production, and dietary variables.

Lastly, RFI is calculated as the difference between actual and predicted DMI such that RFI is the residual term in the statistical model for DMI. As a residual, RFI contains true variation due to metabolic difference among cows as well as random variation caused by errors in actual DMI measurements or DMI estimations. Therefore, different equations used to estimate DMI may generate different RFI values due to differences in errors of predictions. The final objective of this dissertation was to determine if RFI values were dependent on the equation used to estimate DMI. The results of this experiment (Chapter 6) suggested that RFI values generated from different DMI equations are strongly correlated such that RFI values are independent of the DMI equation utilized in the calculation. Thus, dairy producers can select the equation to estimate DMI that is most suitable for their operation without causing an "equation bias" on the RFI calculation used to determine individual cow FE status.

Future Directions

The DMI equations developed in Chapter 3 were designed to estimate DMI until approximately 140 DIM of lactation. Future work should be conducted to develop equations to estimate DMI throughout mid and late lactation using the N balance approach. In addition, updated equations that estimate urinary N from MUN (mg/dL) should be developed and evaluated using data from N balance experimental studies. Results from the second (Chapter 4) and third (Chapter 5) experiments suggest that dietary factors did not possess much discriminatory power to differentiate between high and low FE cows. The dataset used for these projects contained records for cows receiving similar dietary treatments; therefore, it is not surprising that dietary factors were not highly influential in discerning between cows based on FE and RFI status. The discriminant analyses approach used in these projects should be repeated on a dataset in which there is high variation in dietary parameters to assess the impact of diet composition on dairy FE and RFI. Lastly, results from the fourth experiment (Chapter 6) suggest that RFI was independent of the equation used to estimate DMI. Future studies should be conducted to compare RFI values generated from other published DMI equations commonly used in the dairy industry to further explore the robustness and repeatability of RFI across different DMI equations.

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