REPEATABILITY OF RESIDUAL FEED INTAKE BY LACTATING DAIRY COWS FED TWO DIETARY FORAGE CONCENTRATIONS

By

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ABSTRACT

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A total of 84 Holstein cows in mid-lactation were studied in three experiments. The study was a crossover design with 2 treatment periods. Treatments were a high forage-low starch diet (**HF**; 36%NDF, 19% starch) and a low forage-high starch diet (**LF**; 26%NDF, 32% starch). Forage comprised 70% of the DM in HF and 47% in LF. Collected data included: dry matter intake (**DMI**), milk yield (**MY**), body weight (**BW**), body condition score (**BCS**), and milk components. Statistical analysis was performed using the MIXED or GLM procedures (SAS 9.4). An RFI value was calculated for each cow when fed each treatment diet then, cows were ranked as high RFI (**HRFI**), medium RFI (**MRFI**) or low RFI (**LRFI**) for each treatment diet.

The HF diet resulted in a decrease of 10% DMI, 5% ECM and 1% BW compared to the LF diet. Rate of BW accretion was 60% lower for cows fed HF compared to for cows fed LF. Milk fat yield, and BCS were not altered by treatments. RFI was relatively repeatable (r=0.54) across the two diets. Of all animals, 56% maintained their group ranking across treatments whereas 38% changed ranking by 1 group. Only 6% moved in ranking from the HRFI to the LRFI group or vice versa. In conclusion, although dietary treatments significantly altered intake, milk production, and energy partitioning, RFI was relatively repeatable for cows fed two diets with different forage concentrations. Thus, genomic breeding values of RFI estimated from cows fed a lower forage (higher starch) diet should still be useful when they are fed a higher forage diet containing less starch.

To my abuelo Anardy Mangual Flores,
responsible for my great passion for animal agriculture;
to my mother, Maribel Mangual Calo, and my wife, Magdanamay Colón Lugo,
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KEY TO ABBREVIATIONS

RFI – Residual Feed Intake

DMI- Dry Matter Intake

LF- Low Forage Diet

HF- High Forage Diet

ECM- Energy Corrected Milk

FCM- Fat Corrected Milk

SCM- Solid Corrected Milk

CP- Crude Protein

BW- Body Weight

Δ BW- Change in Body Weight

BCS- Body Condition Score

ΔBCS- Change in Body Condition Score

NFFS- Non-Forage Fiber Source

DM- Dry Matter

DIM- Days In Milk

SD- Standard Deviation

TMR- Total Mixed Ration

SCC- Somatic Cell Count

MUN- Milk Urea Nitrogen

EDTA- Ethylenediaminetetraacetic acid

NEFA- Non-Esterified Fatty Acid

NE_L- Net Energy of Milk

NE_M- Net Energy of Maintenance

NE_G- Net Energy of Bodyweight Gain

NE_D- Apparent Net Energy Density of Diet

MBW- Metabolic Body Weight

IOFC- Income Over Feed Cost

Chapter 1: Introduction and Review of Literature

The increase in global population presents a challenge for the food production industry. According to a report from the United Nations, the world's population will surpass 10 billion by the year 2100 (U.N., 2015). This increase in population will require an estimated 70% increase in global food production (Godfray et.al., 2010). However, the available arable cropland has decreased in the last 50 years and is projected to continually decline (Bruinsma, 2009). The availability and use of new technologies has increased the efficiency of food production, such as higher yields per acre, which results in similar levels of food production through the usage of significantly less land. Nevertheless, the rate of improvement in yield is projected to decline (Bruinsma, 2009) meaning that current technological efficiency will be even more important. Animal agriculture is vital because livestock convert products otherwise inedible to humans into bioavailable nutrients (Oltjen and Beckett, 1996). Additionally, the FAO (2006) reports that the increase in general wealth will cause an increase in the demand for meat and dairy products. To face these challenges, the dairy industry must improve its efficiency, increasing production with little increase in land usage or environmental impact.

Section 1: Feed efficiency of the dairy industry

Efficiency is defined as output per unit of input. Feed expenses account for over 60% of all variable costs in a dairy operation, as such the use of less feed to achieve the same level of milk production will result in increased feed efficiency and profitability (VandeHaar and St Pierre, 2006). Feed efficiency of the dairy industry and the dairy cow have improved greatly in the last 60 years. In the last 100 years, the industry has quadrupled milk production per cow (VandeHaar and St Pierre, 2006). Although the major contributor to increased milk production per cow has been improvement in the genetic stock, VandeHaar and St Pierre (2006) concluded that increased

knowledge in nutrition allowed cows to reach their genetic potential for milk production. The increase in milk per cow also resulted in an overall increased efficiency (i.e., fewer animals are required to produce the same amount of milk) (VandeHaar, 1998). Reduced numbers of animals require less feed, translating to the same output with reduced input. Therefore, the genetic selection for increased milk production during the past century has indirectly led to improved feed efficiency. Higher producing cows have a greater dry matter intake (**DMI**), and a smaller proportion of available nutrients is used for maintenance, and a greater proportion is partitioned towards production of meat and milk (Holmes and Davey, 1981). This phenomenon is called "dilution of maintenance," and has been the primary reason for increases in feed efficiency over the past 100 years. However, the advantage of greater production efficiency diminishes as intake increases, because the effectiveness of digestion decreases, which negatively affects feed efficiency (Tyrrel and Moe, 1975; NRC, 2001). Consequently, new ways to improve feed efficiency aside from increasing feed intake and diluting maintenance must be sought.

The increase in feed efficiency has also contribute to ameliorate the impact of the dairy industry in the environment. Few studies suggest that animals with increased feed efficiency also have increased digestibility of feed (Richardson and Herd, 2004; Potts et al., 2017); therefore, they excrete less nutrients into the environment and instead, can use more consumed nutrients for productive purposes when compared to inefficient animals. Additionally, another study has proposed that more feed efficient cattle have lower methane emissions compared to least feed efficient cattle (Hegarty et al., 2007). As discussed by VandeHaar et al. (2016), continuous increase in lifetime productivity and efficiency is consistent with good environmental stewardship. In order to continuously improve feed efficiency, it is vital to identify and evaluate the animals that

effectively can capture the greatest portion of the energy provided in the diet and allocate this energy to growth and lactation.

Section 2: Measuring feed efficiency

Multiple measures are used to assess feed efficiency in dairy cattle. One of the most practical approaches to describe feed efficiency is the amount of milk per unit of feed consumed, termed milk-to-feed ratio (Hooven et al., 1972). Currently, the milk-to-feed ratio is often calculated as Energy-Corrected Milk (**ECM**)(kg/d) / DMI (kg/d). However, depending on specific production goals, this measure can be expressed differently. As discussed by Blake and Custodio (1984), variations could include: Fat-Corrected Milk (**FCM**) / DMI, Solids-Corrected Milk (**SCM**)/ DMI, Milk energy output/feed energy or milk protein yield / Crude Protein (**CP**) intake among other variations. This approach examines feed efficiency by encompassing the broader idea of measuring Output / Input. However, efficiency data solely based on milk-to-feed ratio could be misleading because changes in BW or BCS are not considered, which can result in a biased feed efficiency value. As discussed by Varga and Dechow (2013), milk-to-feed ratio does not differentiate if the energy in milk is from intake, tissue mobilization or a combination of the two. As a result, cows that mobilize more tissue (e.g. during early lactation) will appear to be more efficient in the conversion of feed to milk even though they are not. Excessive tissue mobilization can lead to an increased incidence of metabolic diseases, particularly during early lactation (Frigo et al, 2010). DeVries et al. (1999) also discussed the effects of negative energy balance and its impact on health and fertility. Thus, the energetic balance of the animal must be considered when describing feed efficiency.

Gross energetic efficiency is another measure of feed efficiency. VandeHaar (1998), suggested that gross efficiency must include the energy captured in milk and body tissues divided

by feed energy intake. This approach recognizes the importance of tissue mobilization and the energy balance of the animal and may help to avoid the selection of cows based on a false value of efficiency. All previously mentioned methods to establish feed efficiency are not completely independent of other production factors such as body size or level of milk production. Gross efficiency is strongly associated with milk yield because of the dilution of maintenance (Oldenbroeck, 1989). Also, there is a moderate negative correlation between BW and gross efficiency (Vallimont et al., 2011; VandeHaar et al., 2016). Larger cows have a greater maintenance requirement than smaller cows (NRC, 2001), and this has an influence in many measurements of feed efficiency. This dependencies between traits can affect or limit the selection process as some desired traits might be negatively affected by others. To compare feed efficiency of cows with different levels of milk production or body sizes, separated from influences of dilution of maintenance or maintenance requirements another evaluation method is needed and recommended.

Residual feed intake (**RFI**) was first described by Koch et al. (1963) as a measure of feed efficiency in which feed intake was adjusted for BW and production level of the animal. Essentially, this type of evaluation allows researchers to evaluate feed efficiency and compare groups of animals independently of the dilution of maintenance. RFI is the difference between observed intake and predicted intake based on production and cohort. Animals with a negative RFI value eat less than predicted; therefore, they are more efficient when compared to animals with high RFI values. Animals with a positive RFI value eat more than predicted; thus, they are less efficient when compared to animals with lower RFI values. Over many years, the beef industry has conducted multiple studies to establish, evaluate, and validate RFI as an important tool to measure feed efficiency (Koch et al., 1963; Arthur et al., 1996; Herd et al., 2004; Durunna et al.,

2011). However, research on RFI of dairy cattle, has been limited. In recent years, considerable attention has been given to RFI as a possible way to improve feed efficiency of dairy cattle (Pryce et al., 2012; Connor, 2015; VandeHaar et al., 2016).

Section 3: Biological basis of residual feed intake

Bauman et al. (1984) concluded over thirty years ago that understanding the mechanisms that control feed efficiency is key to developing new tools to improve it. Several publications have discussed the sources of RFI variation in beef (Durunna et al., 2012; Herd and Arthur, 2009) and dairy cattle (Connor et al., 2012; Xi et al., 2016). Richardson and Herd (2004) proposed that the variation in RFI could be explained by physiological differences in digestibility (accounting for 10% of RFI), tissue metabolism (accounting for 37% of RFI), heat increment of fermentation (accounting for 9% of RFI), activity (accounting for 10% of RFI), body composition (accounting for 5% of RFI), feeding patterns (accounting for 2% of RFI) and others (accounting for 27% of RFI). In regard to digestibility, Potts et al. (2017) concluded that diet digestibility accounted for up to 31% of the variation observed between high RFI and low RFI cows in their study when cows were fed high fiber diets. However, differences in DMI between high RFI and low RFI cows could explain the increased digestibility. As DMI increases the passage rate of feed through the rumen is increased which decreases time for digestion. So, although digestibility and RFI were negatively correlated in their study, the causal relationship was not clear. In a study by Hegarty et al. (2007) selection of low RFI cows resulted in a reduction in methane emissions; the researchers discussed that a possible explanation for the result was the decreased DMI of low RFI animals. As energy intake decreases, the substrate supply for fermentation is reduced, thus decreasing the supply of hydrogen to the methanogens. But, a study by Basarab et al. (2013) discuss that the relationship between RFI and methane emission can also be the result of different methanogenic profiles

between high and low RFI cattle. This suggest that differences in microbial profiles and rumen metabolism can also influence the variation in RFI. Connor (2015) concluded that understanding the sources of variation in RFI will be important to predict the consequences of selecting for it instead of other traits.

Section 4: Using residual feed intake to enhance feed efficiency of dairy cows

Although the mechanisms for variation are not fully elucidated and warrant further research, considerable attention has been given to RFI as a trait to be included in breeding decisions (Verkamp et al., 1995; Pryce et al., 2014; Connor, 2015; Tempelman et al., 2015; VandeHaar et al., 2016). An advantage of including RFI is that it is independent of the production level of the animal; thus, it allows for the selection of efficiency and milk yield separately. However, if RFI is to be used for selection purposes, two main prerequisites must be met. It must be heritable to ensure progress in future generations, and it must be repeatable to avoid mistakes in the selection process.

Heritability of RFI has been evaluated in multiple studies and at multiple stages of lactation or growth in dairy cattle. Van Arendock et al. (1991) estimated heritability of RFI in 417 growing heifers to be 0.22. Pryce et al. (2012) estimated heritability in 2000 growing heifers to be 0.22 or 0.38 depending on the country (Australia and New Zeeland respectively). For lactating cows, the earliest report was from Van Arendock et al. (1991) who evaluated 360 lactating heifers and estimated heritability to be 0.19. Since that time, more studies have been reported with estimates for heritability of RFI ranging from 0.01 (Vallimont et al., 2011) to 0.32 (Veerkamp et al., 1995). Most recently, Tempelman et al. (2015) published a study with an extensive data base composed of RFI values from 4,900 cows in peak to mid lactation, and estimated the heritability to be between 0.15 to 0.18, depending on the model used in the analysis. Based on this, RFI seems a promising way to improve feed efficiency and is suitable for inclusion in selection indexes.

Traditional breeding programs rely on performance of a sire's daughter which is not available for RFI in commercial herds where individual animal feed intake is not measured. However, through genomics, selection for reduced RFI is possible. Briefly, for genomic evaluation and testing, a reference or test population is evaluated for RFI. Additionally, the test population is genotyped with thousands of single nucleotide polymorphism (SNP) markers. Then, the SNP effects for RFI are jointly estimated for RFI and prediction models are developed. Finally, using the SNP effect estimate models we can predict estimates of genetic merit of RFI for other genotyped animals. However, the successful application of this technique is dependent on the repeatability of RFI as a trait for selection.

In this thesis, repeatability of RFI, refers to the accuracy, and correlation of RFI in cows when it is measured more than once in the same animal. Specifically, we want to know if RFI values (which are always relative to the feed intake of other cows in a cohort group) will be repeated for a cow if measured with different diets, parities, or days in milk. Repeatability is important for genomics as it is assumed that the values obtained from the test population at a specific time, and fed a specific diet, will be representative of that animal's RFI if it were measured for her entire life no matter what diet she was fed. On this matter, Durunna et al. (2012) determined that the repeatability of RFI across periods was moderate ($r = 0.52$) for crossbred beef heifers fed the same diet in both periods. Connor et al. (2013) estimated the repeatability of RFI in early lactation cows to be 0.47 across weeks within lactation and to be 0.56 across lactations when using weekly data during early lactation (0-90 DIM). These studies provide support to the moderate correlation of RFI across time and lactation, which suggest that data obtained at one point in time during mid lactation (either primiparous or multiparous cows in the test population) is an appropriate method for estimating lifetime RFI.

Repeatability across diets is also important if we expect genomic testing to be effective. Currently, most of the test population of the dairy feed efficiency data base used in Tempelman et al. (2015) was evaluated with relatively high starch diets, typical of the northern US. High repeatability across diets will ensure that the values obtained from the test population can be applied to animals when fed different diets fed in different parts of the world or in the future when cattle diets may contain less human consumable feedstuffs. Repeatability of beef steers fed two different diets in the growing and finishing stages was 0.33 (Durunna et al., 2011). However, repeatability of RFI for beef heifers in the growing and finishing phases fed different diets was 0.62 (Kelly et al., 2010a). Finally, repeatability of RFI of 109 lactating dairy cows fed high (30%) and low (14%) starch diets was evaluated during mid-lactation (Potts et al., 2015). They determined RFI repeatability of these cows to be 0.73 when fed these diet, and that weekly repeatability was similar across and within treatment diets. The main difference in dietary fiber supply between the high and low starch diets fed by Potts et al. (2015) was soyhulls, a non-forage fiber, and forage NDF content was similar across treatments. Thus, Potts et al. (2015) showed that RFI was repeatable when starch was replaced with a non-forage fiber. However, whether RFI of lactating cows is repeatable when starch is replaced with forage fiber is not known.

Section 5: Objectives & Hypothesis

In this project, the goal was to further evaluate the repeatability of RFI across diets. The main objective was to determine the repeatability of RFI of cows when fed diets containing different concentrations of forage fiber (and thus starch), one with ~20% forage NDF and ~30% starch and the other with 30% forage NDF and ~20% starch. We hypothesized that RFI for a cow when fed high starch would be repeatable when fed the diet high in forage NDF. If RFI is

repeatable, then the estimated breeding values of RFI determined with current studies like Tempelman et al. (2015) are more likely useful for cows consuming diets with more forage.

Chapter 2: Materials and Methods

Section 1: Animals

All animals and methods were approved by the Michigan State University Institutional Animal Care and Use Committee (**IACUC**). A total of 84 mid-lactation Holstein-Friesian dairy cows (27 multiparous; 57 primiparous) were used in this study. Animals were 97±28 DIM, weighed 634±61 kg and produced 39±10 kg of milk per day at the beginning of their first treatment period. Cows were evaluated in three separate experiments. Specific descriptions of the individual experiments are summarized in Table 1.

Table 1: Description of animals used in the study[1]

Experiment	Animals	Multiparous	Primiparous	DIM (d)	BW (kg)[2]	MY (kg/d)[2]
1	32	14	18	89 ± 27	656 ± 59	40 ± 10
2	32	9	23	98 ± 22	613 ± 62	39 ± 8
3	20	4	16	112 ± 33	631 ± 53	38 ± 10

[1]DIM= Days in milk at the beginning of the study, BW= body weight, MY= Milk yield.
[2]BW and MY values were obtained by calculating the mean ± standard deviation of data collected during the preliminary period.

Section 2: Experimental design and treatments

Experiments followed a crossover design with two treatment periods of either 28 d (Experiments 2 and 3) or 31 d (Experiment 1). Prior to the first treatment period, animals were fed a common diet for either 7 (Experiments 1 and 3) or 5 (Experiment 2) d. Preliminary data were obtained to pair animals based on parity, DIM, BW and MY. Then, within a pair, animals were randomly assigned to a treatment sequence. In one sequence, they were fed a high forage diet (HF) in the first treatment period followed by the low forage diet (LF) in the second treatment period. In the other sequence, the order of diets was reversed. Within each experiment, animals were housed in the same barn in the MSU Dairy Teaching and Research Center (East Lansing, MI). Animals were randomly allocated to tie-stalls. The tie-stall design allowed

measurement of individual cow feed intake. The treatment diet was offered once daily with *ad libitum* access. Cows were blocked from their feed when refusals were collected or when cows left the stalls to the milking parlor. Amount of feed offered was adjusted daily to allow for about 10% orts. Drinking water was available *ad libitum* throughout the day.

The LF diet had 26% NDF and the HF diet had 36% NDF. Both diets were formulated using the Spartan Dairy Ration Evaluator (Michigan State University, East Lansing, MI) to meet NRC (2001) recommendations for protein, minerals and vitamins and fed as totally mixed ration (TMR). A detailed ingredient list is described in Table 2.

Forages were corn silage, alfalfa hay silage, and chopped wheat straw. The HF diet contained 70% forage with 7% corn grain, while the LF had 47% forage with 28% corn grain. Mechanically-extruded soybean meal (Soy Plus®) and solvent-extracted soybean meal were balanced to achieve similar contents of predicted metabolizable protein in the two diets. The nutrient composition in Table 2 is reported on a DM basis and was calculated using the feed analysis of the corn and alfalfa hay silages. The nutritional value of the rest of ingredients was calculated from book values of NRC (2001). As shown in Table 2, over 70% of the fiber in the HF and LF diets was provided by forage sources.

Table 2: Composition of treatment diets[1,2]

	Experiment 1		Experiment 2		Experiment 3	
Diet	HF	LF	HF	LF	HF	LF
Ingredients %Diet						
Corn Silage	38.1	27.4	38.1	27.4	38.1	27.4
Alfalfa Hay Silage	26.2	17.5	26.2	17.5	26.2	17.5
Wheat Straw	5.2	2.7	5.2	2.7	5.2	2.7
Cotton Seeds	5.2	5.2	5.2	5.2	5.2	5.2
Soy Bean Meal	7.1	15.6	7.1	15.6	7.1	15.6
Soy Plus®	8.2	----	8.2	----	8.2	----
Ground Dry Corn	7.0	15.5	7.0	15.5	7.0	15.5
High Moisture Corn	----	12.6	----	12.6	----	12.6
Vitamin-Mineral Mix[3]	2.0	2.0	2.0	2.0	2.0	2.0
Limestone	0.2	0.7	0.2	0.7	0.2	0.7
Sodium Bicarb	0.8	0.8	0.8	0.8	0.8	0.8
Nutritients % of DM						
NDF	0.2	27.0	36.0	27.0	34.0	26.0
Forage NDF	31.0	21.0	31.0	21.0	29.0	20.0
Cude Protein	18.0	18.0	17.0	17.0	18.0	18.0
Metabolizable protein	12.0	11.5	11.6	11.4	11.9	11.6
Starch	20.0	32.0	20.0	32.0	20.0	32.0
Fatty Acid	3.7	3.2	3.7	3.2	3.7	3.2

[1]High (HF) and Low (LF) forage diets fed to animals during experiments, 1 (n=32), 2 (n=32) and 3 (n=20).

[2]Nutrient composition estimated with the Spartan Dairy 3 software® using updated feed ingredient analysis.

[3]Vitamin and mineral mix composed of: Ground corn (34.1%), Salt(25.6%), Ca Carbonate (21.8%), Biofos (9.1), Mg oxide (3.9%), Soybean oil (2%) and <1% of the following Vitamin A, Vitamin E, Vitamin D3, Mn sulfate, Zn sulfate, Fe sulfate, Cu sulfate, Selenium, Iodine and Co Carbonate.

Section 3: Sample collection & analysis

Methods for the assessment of treatment effect over animal production

Cows were fed once daily at 1000 h (Experiment 3) or 1200 h (Experiments 1 and 2). Feed intake was recorded daily and calculated as the difference between the amount of feed offered and the amount refused the next day. All feed amounts were weighed using scales (Fairbanks FB1100-2 Series, Kansas City, MO). That were calibrated every three months. Corn silage and alfalfa silage were sampled twice weekly (Monday and Thursday) for analysis of dry matter (**DM**) content on site using the Koster Moisture Tester (Model C, Brunswick, OH). Values were used to adjust the amounts of forages mixed into the diets to keep nutrient and energy concentrations constant through time. Cows were milked twice daily in a double-seven herringbone automatic milking parlor, at 0430 and 1530 h (Experiments 1 and 2) or 0340 and 1440 h (Experiment 3). Milk yield was recorded electronically for each milking throughout the project. Milk samples were collected on 4 consecutive milkings each week throughout the experiment and at each milking during the last 5 d of each treatment period. All individual samples collected from each cow in each period were analyzed for fat, true protein, lactose, total solids, somatic cell count (**SCC**) and milk urea nitrogen (**MUN**) using infrared spectroscopy (AOAC, 1990) by Universal Lab Services (East Lansing, MI). BW was recorded three times weekly (Monday, Wednesday and Friday) immediately after morning milking approximately at 0520h (Experiments 1 and 2) or 0430 (Experiment 3), on a calibrated livestock scale (XR3000 Tru-Test Corp., Mineral Wells, TX). Body condition score (**BCS**) was assessed on animals at the beginning and end of each treatment period by three different scorers using a 1 to 5point scale with 0.25 increments (1= very thin and 5= obese) at any time during a 24h period. Scorers were trained following scoring guidelines described by Edmonson et al (1989) but were not blinded to treatments.

Blood Metabolites

During the last 5 d of each experimental period, blood samples were collected every 15 h to evaluate the effect of treatment on key metabolites for experiments 1 and 2. Blood samples were collected via venipuncture of the medial caudal vein. Blood was collected using two 5-ml vacutainer tubes containing EDTA (BD, Franklin Lakes, NJ, USA) for the analysis of insulin and non-esterified fatty acids (**NEFA**); and one 5-ml vacutainer tube containing sodium fluoride (BD Franklin Lakes NJ, USA) for the analysis of glucose. Following collection, samples were kept on ice for 20 to 30 min and then processed to separate plasma. Separation was performed using a centrifuge for 15 min at 2,000 x *g* at 4°C. After separation, the plasma portion was transferred to 1-ml tubes and stored at -20°C keeping EDTA samples and sodium fluoride samples separated. After the collections were finished, samples were thawed. Individual time point samples within period from each cow were composited to obtain one sample per cow per period and then frozen at -20°C until analyses were performed. One composite was done for EDTA tubes and a separate composite was prepared for sodium fluoride tubes. Plasma samples were analyzed for insulin, glucose, and NEFA concentration. Insulin was quantified using the radioimmunoassay kit, PI-12K Porcine Insulin RIA (EMD Millipore, Billerica, MA, USA) performed following all manufacturer's protocols and specifications. Glucose concentration was quantified using the Glucose kit #510; (Sigma Chemical Co., St. Louis, MO, USA) following all manufacturer's protocols and recommendations. Concentration of NEFA was quantified using the NEFA HR-2 Kit (Wako Chemicals, Richmond, VA, USA). The kit was used following all manufacturer's protocols and specifications. All tests were analyzed in duplicate, and any sample with a CV greater than 8% for the duplicates was re-analyzed.

Section 4: Calculations & statistical analysis

Milk yield was calculated as the mean milk yield of all days within each treatment period for a cow using electronic parlor records. Fat yield, protein yield and lactose yield were calculated for each milking in which samples were analyzed. The components from the 4 consecutive milkings each week were summed to obtain the yield of a 48-h period and divided by 2 to determine daily yield of components. Data was used to determine weekly concentration (%) of fat, protein, and lactose. Energy corrected milk (**ECM**) was calculated using the equation of Tyrrell and Reid (1965):

$$\text{ECM (kg)} = 0.327 * \text{milk (kg)} + 12.95 * \text{fat (kg)} + 7.65 * \text{protein (kg)}$$

The daily change in body weight (Δ**BW**) for each cow in each period was calculated by linear regression of BW over time with the REG procedure of SAS v.9.4 (SAS Institute, Inc. Cary, NC). After the first regression of BW by day, data points outside of ± 3.5 SD were established as outliers and removed before performing a second regression. The slope of the second regression was established as the mean daily ΔBW for an individual cow in each experimental period. Change in BCS (Δ**BCS**) was calculated as the difference between the mean BCS at the end and the beginning of a treatment period.

Energy partitioning

Energy partitioning calculations were performed considering the three possible major energy sinks of a mid-lactation cow, energy for maintenance, energy for milk production and energy for BW gain. Net energy of milk (NE_L) was calculated using the equation from the NRC (2001):

$$NE_L = 9.29 * \text{fat (kg)} + 5.63 * \text{protein (kg)} + 3.95 * \text{lactose (kg)};$$

each coefficient describes the heat of combustion of individual components in Mcal/kg according to the NRC, (2001).

The energy allocated for maintenance (NE_M) was calculated according to the NRC (2001) as:

$$NE_M = 0.073 * MBW$$

Metabolic body weight **(MBW)** was calculated as $BW^{0.75}$ where BW is the mean BW of an individual cow during an individual treatment period. The coefficient of 0.073 is the estimated fasting heat production of cows housed in tie-stalls, provided by NRC (2001). Net energy of gain (NE_G)refers to the energy transferred to or from body tissue when BW is gained or lost, and was calculated as:

$$NE_G = (2.88+1.036 * BCS) * \Delta BW$$

where BCS is the mean BCS of a cow in one treatment period and ΔBW refers to the BW change of a cow in kg/d during that period. This equation was derived using values in Table 2-5 of the NRC (2001). Individual percent of total NE allocated for a particular variable was calculated by:

$$\%NE_L, \%NE_M \text{ or } \%NE_G = \left(\frac{NE_L, NE_M \text{ or } NE_G}{NE_L + NE_M + NE_G}\right) x\ 100$$

where the numerator of the equation was the variable of interest in Mcal/d, with the final value expressed as the percent of mega calories per day allocated to a particular variable. Multiples of maintenance were calculated to obtain an indicator of intake using 2 different methods. Multiple of maintenance using a cow's energy requirements ($\mathbf{MM_R}$)was calculated as:

$$MM_R = \frac{(NE_L + NE_M + NE_G)}{NE_M,}$$

Multiples of maintenance using measured intake (**MM_I**)was calculated as:

$$MM_I = \frac{(DMI*NE_{D,})}{NE_M}$$

where DMI is the observed mean DMI of a 28-day period.

NE_D refers to the apparent net energy density of the diet, calculated as:

$$NE_D = \frac{NE_L + NE_M + NE_G}{DMI}$$

where each term represents the mean energy of all animals partitioned towards lactation, maintenance, and BW gain, respectively, averaged over the treatment period.

Statistical analysis

Statistical analysis of each dependent variable was analyzed using the MIXED procedure of SAS v.9.4 (SAS Institute, Inc. Cary, NC) by the following initial model:

$$Y_i = m + experiment + diet + parity + period\ (experiment) + diet*experiment + diet*parity + diet*period(experiment) + experiment*parity + experiment*period(experiment) + parity*period\ (experiment) + cow(experiment) + \varepsilon_i,$$

where Y is the dependent variable of interest, with the fixed effects of experiment (1, 2 or 3), diet (HF or LF), parity class (primiparous or multiparous) and period nested within experiment; followed by all interactions of fixed effects with ε as the error term of the model. Cow nested within experiment and parity, (Cow (experiment)) was included as a random effect.

Efficiency calculations

The milk-to-feed ratio was calculated by dividing weekly ECM (kg/d) by DMI (kg/d) and then averaged to one value per cow per period.

Income over feed cost (**IOFC)** was calculated as:

IOFC = milk income - feed cost

Feed cost was calculated using the economic value for commodities in the midwestern United States in the fall of 2013, summarized in Table 3.

Table 3: Price of used commodities

Feed ingredient	$/kg of DM[1]
Corn Silage	$0.22
Alfalfa Hay Silage	$0.11
High Moisture Corn	$0.33
Ground Dry Corn	$0.34
Soybean Meal	$0.56
Soy Plus[2]	$0.51
Cotton Seed	$0.41
Wheat Straw	$0.10
Vitamin-Mineral Mix[3]	$0.45
Limestone	$0.22
Sodium Bicarb	$0.22

[1]Economic values for commodities in the Midwestern United States in the fall of 2013.

[2]Source: Dairy Nutrition Plus, Soy Plus distributors

[3]Price paid by MSU dairy for the specific mineral blend used in the study

Milk income was determined based on individual production of milk components following the values of fat ($5.04/kg), protein($3.29/kg), and other solids yields ($0.12/kg) as determined by the USDA under federal order No. 33 for May 2016. Based on average production of all cows and the economic values of components milk price was determined to be $13.08 per 45.4kg. RFI was calculated using the following statistical model developed by Potts et al. (2015), and analyzed using the GLM procedure of SAS v.9.4 (SAS Institute, Inc. Cary, NC):

$DMI_i = \beta_0 + \beta_1 \times NE_{Li} + \beta_2 \times MBW_i + \beta_3 \times NE_{Gi}$ + parity + experiment + sequence (experiment) + diet (sequence*experiment) + ε_i,

where DMI is the mean DMI of a treatment period, NE_L is the energy of milk for a treatment period, MBW is the mean MBW of a treatment period and NE_G is the change in the energy of BW for the treatment period, Parity refers to primiparous or multiparous, Experiment refers to individual experiment (1, 2, or 3), Sequence refers to treatment sequence for a cow within an experiment and was used as the cohort of animals that received treatment diets in the same sequence nested within experiment and diet nested within the sequence and experiment interaction. Finally, the ε term refers to the residual in the model, or RFI value for the *i* cow under each diet. Ranking of animals by RFI was done using the SD of the mean RFI of each diet individually and used to categorize animals into 3 levels for RFI: Low (LRFI), Medium (MRFI), and High (HRFI) where LRFI= RFI < -0.5 SD, MRFI= -0.5 SD < RFI < 0.5 SD, and HRFI= RFI> 0.5 SD. An RFI group was established for each animal fed each diet. Weekly RFI was also evaluated and calculated using weekly data analyzed by the previously described model, with one exception, the term diet(sequence*experiment) was substituted with week(sequence*experiment).

Analysis of animal performance comparing two efficiency groups

To evaluate which variables can significantly contribute to RFI variation, we evaluated the different responses to treatment diets by the cows with the highest and lowest RFI in each treatment sequence. Selected animals were in the LRFI or HRFI group, respectively, in both treatment periods. Efficiency level was established as a group. Dependent variables were analyzed using the following model:

$$Y_i = m + RFIg + \varepsilon_i,$$

Where m is the overall mean, RFIg represent the efficiency level, with 1= Lowest RFI animals and 2 = highest RFI animals and ε refers to the error term of the model. This model was run for all dependent variables including: DMI, ECM, BW, ΔBW, BCS, ΔBCS MMR, MMI, Milk components (Fat, Protein, MUN and SCC), RFI, Milk-to-feed, IOFC and plasma metabolites (glucose, insulin, and NEFA) using the GLM procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC, USA).

Chapter 3: Results

Section 1: Treatment effect on animal production

Effect of diet in production variables are shown in Table 4. Cows fed the HF diet had 10% lower DMI (2.4 kg/d; $P < 0.01$), and 5% less ECM (2.1 kg/d; $P < 0.01$) compared with those fed the LF diet. This negative effect on production by the HF diet also was evident for milk yield (8% or -3.1 kg/d, $P < 0.01$) and 4% FCM (3% or -1.2 kg/d, $P < 0.01$). Compared with the LF diet, cows fed the HF diet had increased milk fat percentage (0.3% greater, $P < 0.01$) but no difference was observed in fat yield. Protein concentration (-0.2%) and yield (-0.20 kg/d) decreased ($P < 0.01$) when cows were fed the HF diet compared to the LF diet. MUN increased by 11% (1.8 mg/dl; $P < 0.01$) for cows fed the HF diet compared with those fed the LF diet. Milk quality evaluated using SCC was not different for cows fed the two diets. Furthermore, cows fed the HF diet gained less BW ($P < 0.01$ for ΔBW), which was reflected in the means of BW per dietary period. However, although BW gain was different for cows fed the two different diets, there was no difference in BCS or ΔBCS.

Energy partitioning results are reported in Table 5. The fraction of energy partitioned towards BW gain more than doubled in the LF diet compared to the HF diet ($P < 0.01$). However, the fraction of energy partitioned towards maintenance ($P < 0.01$) was greater for cows fed the HF than the LF diet. Cows fed the HF diet tended ($P < 0.07$) to partition a greater fraction of energy towards milk than the cows fed the LF diet.

The LF diet increased the concentration of insulin in plasma by 26% ($P < 0.01$) and the concentration of glucose by 3.3% ($P < 0.01$) compared to HF diet. The LF diet also decreased the concentration of NEFA by 20% ($P < 0.01$) compared to the HF diet (Table 6).

Weekly responses to dietary treatments are divided by treatment sequences and reported in Figures 1, 2 and 3. The weekly diet effects reported for DMI, ECM yield, and BW were similar to the overall treatment effects.

Table 4: Treatment effect on production variables[1]

	Diets[2]			
Variable[3]	HF	LF	SEM	*P*-value[4]
DMI (kg/d)	22.0	24.4	0.15	<0.01
Milk Yield (kg/d)	35.1	38.2	0.25	<0.01
ECM Yield (kg/d)	36.5	38.6	0.27	<0.01
4%FCM Yield (kg/d)	33.9	35.1	0.28	<0.01
% Fat	3.8	3.5	0.03	<0.01
Fat Yield (kg/d)	1.3	1.3	0.02	0.79
% Protein	2.98	3.13	0.01	<0.01
Protein Yield (kg/d)	1.0	1.2	0.01	<0.01
MUN (mg/dl)	15.9	14.1	0.13	<0.01
SCC (1000/mL)	122	98	33	0.47
BW (kg)	643	647	1.1	<0.01
ΔBW (kg/d)	0.3	0.5	0.07	<0.01
BCS (1-5 Scale)	3.1	3.1	0.01	0.78

[1]All values represent the LSMeans for a 28 day period.

[2]HF= High forage diet and LF= Low forage diet.

[3]ECM= 0.327*Milk Yield + 12.95*Fat Yield + 7.65* Protein Yield, 4% FCM= 0.4*Milk Yield + 15*Fat Yield, BW= Body weight, ΔBW= Change in body weight, BCS= Body condition score and ΔBCS = Change in body condition score.

[4]*P*-value associated with the main effect of diets.

Table 5: Diet effect on energy partitioning[1]

	Diet[2]			
Variable	HF	LF	SEM	*P*-value[3]
NE lactation (Mcal/d)	25.0	26.4	0.19	<0.01
% to milk	69.4	67.9	0.82	0.07
NE maintenance (Mcal/d)	9.3	9.4	0.01	<0.01
% to maintenance	26.4	24.5	0.35	<0.01
NE body tissue (Mcal/d)	1.6	3.0	0.43	<0.01
% to body tissue	4.2	7.6	1.10	<0.01

[1]Values represent LS Means over a 28 day period.

[2]HF= High forage diet and LF= Low forage diet.

[3]*P*-value associated with the main effect of diets.

Table 6: Plasma concentration of blood metabolites[1]

	Diet[2]			
Variable[3]	HF	LF	SEM	*P*-value[4]
Glucose	58.6	60.6	0.43	<0.01
Insulin	0.50	0.68	0.03	<0.01
NEFA	124	99	3.2	<0.01

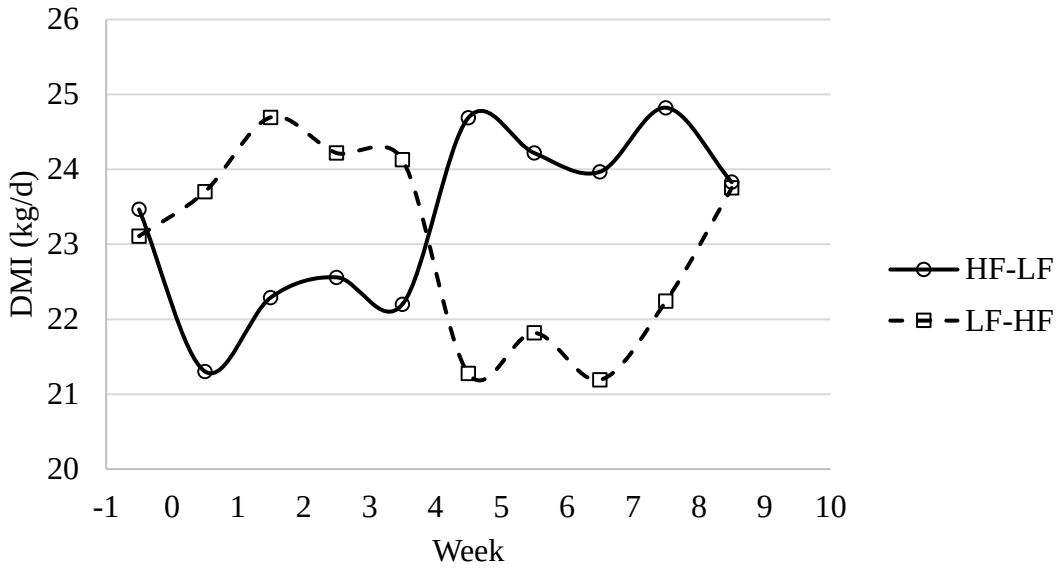
[1]All values represent the LSMean of a 28 days Period (n=64 cows).

[2]HF= High forage diet and LF= Low forage diet.

[3]Units: Glucose (mg/dl), Insulin (mg/L) and NEFA (µeq/L).

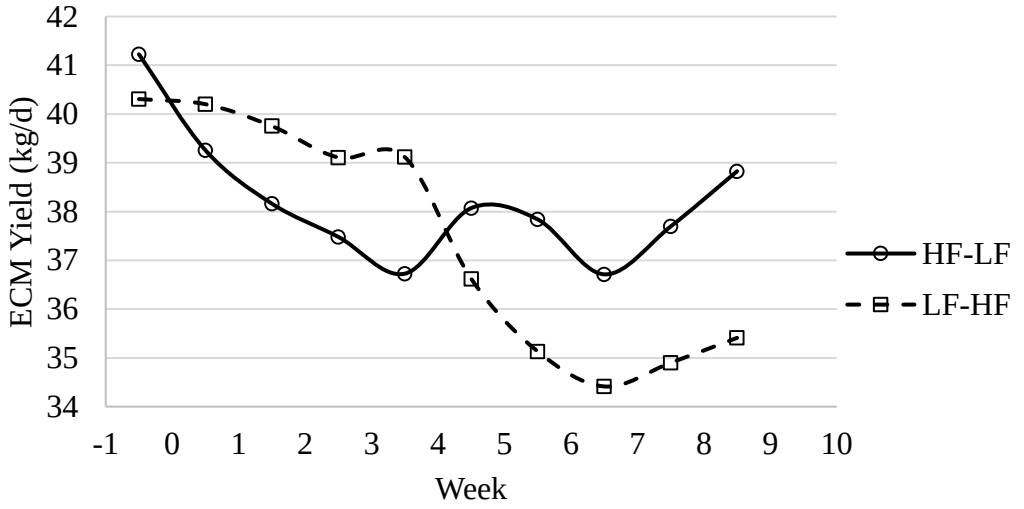
[4]P-value associated with the main effect of diets.

Figure 1: Weekly response in dry matter intake [1, 2]



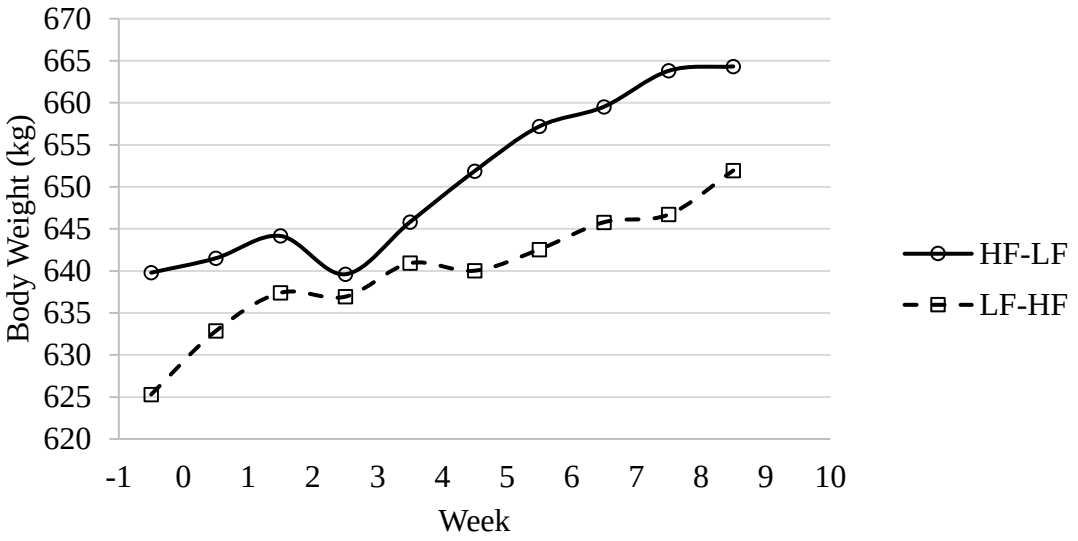
[1]HF-LF sequence= animals fed HF diet in period 1 (wk 1-4) and LF diet on period 2 (wk 5-8), LF-HF sequence= animals fed LF diet in period 1 (wk 1-4) and HF diet in period 2 (wk 5-8).
[2]Overall diet effect on DMI was significant (P <0.01), with SEM of ± 0.15 kg.

Figure 2: Weekly response in ECM Yield[1, 2]



[1]HF-LF sequence= animals fed HF diet in period 1 (wk 1-4) and LF diet on period 2 (wk 5-8), LF-HF sequence= animals fed LF diet in period 1 (wk 1-4) and HF diet in period 2 (wk 5-8).
[2]Overall diet effect on ECM was significant (P <0.01), with SEM of ± 0.27 kg.

Figure 3: Weekly response in body weight[1, 2]



[1]HF-LF sequence= animals fed HF diet in period 1 (wk 1-4) and LF diet on period 2 (wk 5-8), LF-HF sequence= animals fed LF diet in period 1 (wk 1-4) and HF diet in period 2 (wk 5-8).
[2]Overall diet effect on BW was significant (P <0.01), with SEM of ± 1.10 kg.

Section 2: Analysis of repeatability of RFI

The model used to estimate DMI explained 90% of the variation in DMI (r^2= 0.90); the remaining 10% was RFI. Values for individual RFI of each cow within each diet plotted against each other, are shown in Figure 4. Repeatability across the two dietary treatments was 0.54 and was determined by the Pearson correlation coefficient using the CORR procedure of SAS v.9.4 (SAS Institute, Inc. Cary, NC).

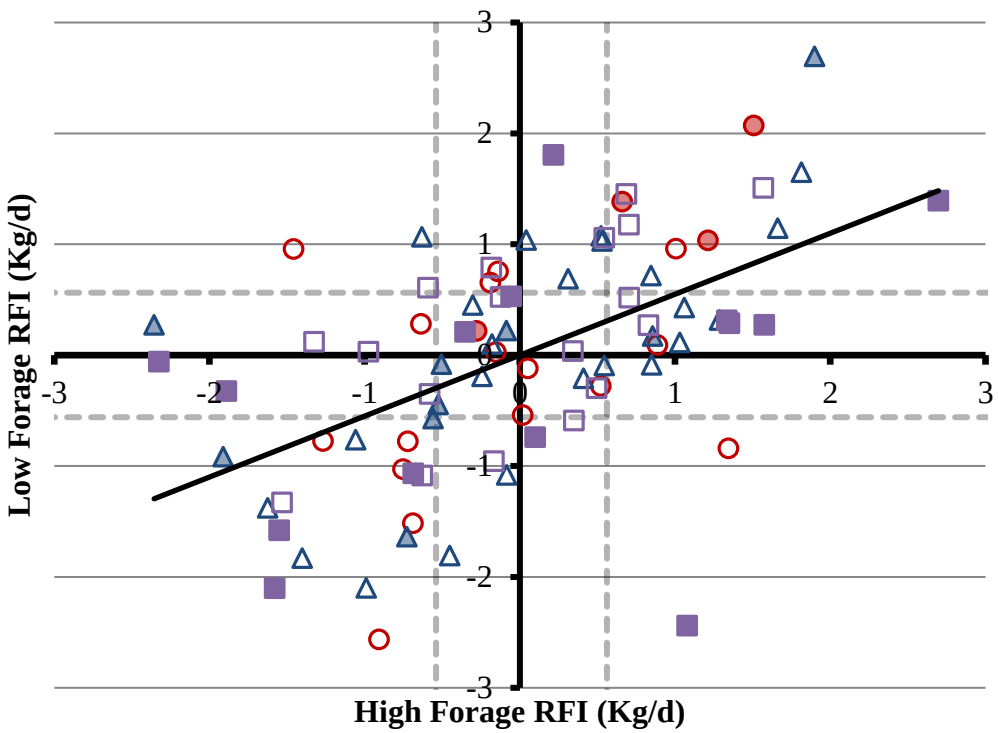
Weekly RFI values for each cow were determined and averaged to compare repeatability within and across diets. As seen in Figures 1, 2, and 3, the effect of overall dietary treatment effects was reflected in weekly means by treatment for DMI, ECM, and BW. To avoid confounding effects of dietary adjustments in BW for the weekly correlation analysis, the RFI values for wk 1 and wk 5 were removed before averaging the weekly correlations (repeatability). Values for within diet

repeatability in Table 7 represent the mean of weekly correlations between weeks 2, 3 and 4 for the diet fed in period 1 (Diet 1); and the mean of weekly correlations between weeks 6, 7 and 8 for the diet fed in period 2 (Diet 2). Across diet correlation represent the mean of weekly correlations of weeks 2, 3, and 4 with weeks 6, 7, and 8. Repeatability of RFI of cows fed same diet (within: Period 1 or Period 2) and across diets are shown by Pearson correlation coefficients in Table 7, and established for multiple subgroups.

As described in the Materials and Methods, an RFI group was defined using SD, and cows were assigned to an efficiency group for each diet. The efficiency group re-ranking is summarized in Figure 6. “No change” refers to the cows that were in the same efficiency group when fed either dietary treatment. The “1 group change” refers to cows that changed by one efficiency group (for example, from MRFI to LRFI or vice versa) as diets changed. Finally, “Drastic change” refers to animals that changed from the most efficient group (LRFI) to the least efficient group (HRFI) or vice-versa when diets were switched. Diet effect did not affect the RFI classification of 56% of the cows (n = 47) across all three experiments. Of the remaining 44% of cows, 38% (n = 32) had a 1 group change and only 6% of the cows (n = 5) changed RFI group drastically.

Table 8 presents Pearson (above diagonal) and Spearman (under diagonal) correlation coefficients of RFI and milk-to-feed ratio, along with other production variables. There was no correlation of RFI with BW, ΔBW or ECM as expected because these variables were included in the DMI ANOVA model used to compute RFI. Milk-to-feed ratio was correlated to ECM yield (r = 0.71) but not correlated to BW (Table 8). RFI was correlated negatively to milk-to-feed ratio (r = -0.38) and IOFC (r = -0.28); whereas there was a positive correlation (r = 0.79) between IOFC and milk-to-feed ratio (Table 9).

Figure 4: Repeatability of residual feed intake across diets[1]



[1]Repeatability of residual feed intake across high forage and low forage diets (y=0.55x; R^2=0.3). Each data point represents 1 cow's RFI value on each diet (n=84), evaluated in Experiment 1 (n=32), Experiment 2 (n=32) or Experiment 3 (n=20). Open squares indicate primiparous cows in Experiment 1 (n=18), closed squares indicate multiparous cows in Experiment 1 (n=14), open triangles indicate primiparous cows in Experiment 2 (n=23), closed triangles indicate multiparous cows in Experiment 2 (n=9), open circles indicate primiparous cows in Experiment 3 (n=16), and closed circles indicate primiparous cows in Experiment 3 (n=16).

Table 7: Within and across diet repeatability of residual feed intake

Category	n	Period 1[1]	Period 2[2]	Across[3]
All				
Pearson coefficient	84	0.62	0.66	0.47
Parity				
Primiparous	57	0.57	0.72	0.54
Multiparous	27	0.67	0.53	0.39
Sequence[4]				
High-Low	42	0.68	0.62	0.53
Low-High	42	0.55	0.73	0.39
Experiment				
1	32	0.72	0.84	0.43
2	32	0.62	0.58	0.49
3	20	0.46	0.58	0.50

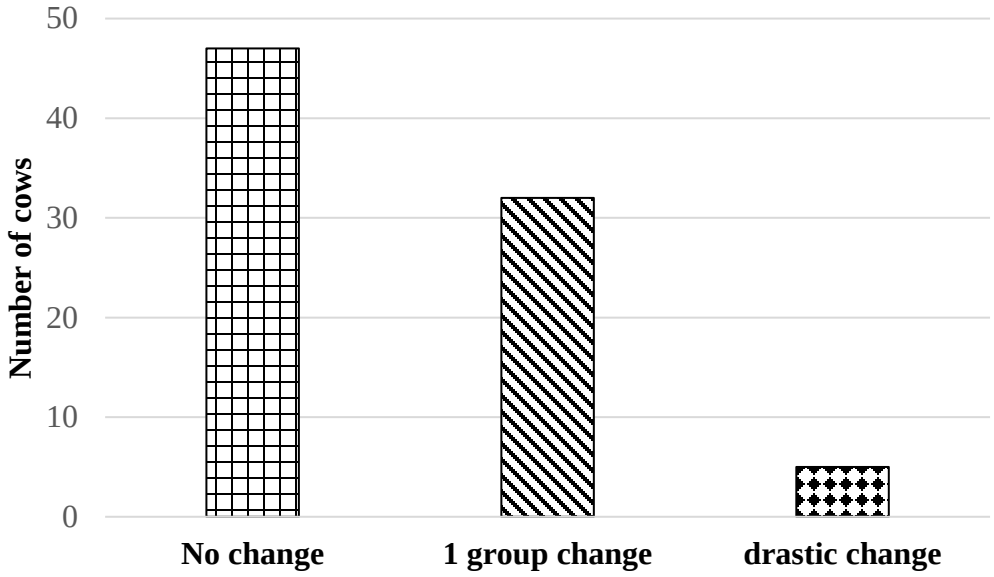
[1]Represents the average correlation between weeks 2, 3, and 4.

[2]Represents the average correlation between weeks 6, 7, and 8.

[3]Represents the average correlation between weeks 2 ,3, and 4, with weeks 6, 7, and 8.

[4]High-Low (HF diet in period 1 and LF diet in period 2), Low-High (LF diet in period 1 and HF diet in period 2).

Figure 5: Re-ranking of animals across diets[1,2]



[1] Efficiency groups were establish using SD from the mean of the individual RFI value of the cow High RFI group refers to the cows who's RFI is over 0.5 SD, Med-RFI group refers to cows who's RFI is between -0.5 and 0.5 SD, Low RFI group refers to the cows who's RFI is under -0.5 SD An RFI group was established for each cow on each dietary treatment

[2] No change (n=47) refers to the 56% of cows that maintain efficiency ranking when diets were switched, 1 group change (n=32) refers to the 38% of cows that changed efficiency ranking by one group when diets were switched, drastic change (n=5) refers to the 6% of cows that changed efficiency ranking drastically from efficient to inefficient or vice versa

Table 8: Correlation of feed efficiency values and production variables[1, 2, 3]

	RFI	**DMI**	**ECM**	**BW**	**ΔBW**	**Milk:Feed**
RFI	1	**0.32**	0.01	0.003	0.01	**-0.38**
DMI	**0.28**	1	**0.83**	**0.66**	0.08	**0.20**
ECM	-0.03	**0.80**	1	**0.49**	**-0.16**	**0.71**
BW	0.01	**0.65**	**0.50**	1	-0.03	0.02
ΔBW	0.01	**0.16**	-0.05	-0.002	1	**-0.37**
Milk:Feed	**-0.36**	**0.17**	**0.67**	0.06	**-0.24**	1

[1]Bold values represent a *P*-value <0.05.

[2]Pearson correlation coefficients are above the diagonal, and Spearman coefficients are under the diagonal.

[3]RFI= Residual feed intake, ECM= 0.327*Milk Yield + 12.95*Fat Yield + 7.65*Protein Yield, BW= Body weight, ΔBW= Change in body weight.

Table 9: Correlation between feed efficiency values[1, 2, 3]

	IOFC	Milk-to-feed	RFI
IOFC	1	0.85	-0.26
Milk-to-feed	0.79	1	-0.38
RFI	-0.22	-0.36	1

[1]All coefficients had a *P*-value <0.01.

[2] Pearson correlation coefficients are above the diagonal, and Spearman coefficients are under the diagonal.

[3] IOFC= Income over feed cost and RFI= Residual feed intake.

Section 3: Evaluation of RFI Variability

As expected, RFI differed between the most efficient (LRFI) and the least efficient (HRFI) cows ($P < 0.01$, Table 10). Although the two efficiency groups did not differ statistically in ECM production, BW or ΔBW, the LRFI group had a 13% reduction in DMI ($P < 0.01$, Table 10). This was the expected result and provides further evidence of how feed efficiency can reduce intake without a major effect over production. No significant difference was observed in milk components (Table 10). LRFI cows had greater milk-to-feed ratio ($P = 0.02$) than HRFI cows, while no difference was observed in IOFC (Table 10).

Concentrations of measured metabolites in plasma for the two RFI groups are shown in Table 10. No significant difference was observed in the concentration of glucose or insulin in plasma between the LRFI and HRFI groups. However, cows in the LRFI group had a 27% increase in concentration of NEFA (35 μeq/L greater, $P < 0.01$) compared with cows in the HRFI group. Finally, multiples of maintenance based on intake were greater for cows in the HRFI ($P < 0.01$). As expected, no difference was observed between groups in the multiples of maintenance calculated based on production requirement values ($P = 0.23$) as its calculation is based on NE_M, BW and ΔBW.

Table 10: Comparison of production on two efficiency groups[1]

	Efficiency Group[2]			
Variable[3]	HRFI	LRFI	SEM	*P*-value[4]
n	10	10		
DMI (kg/d)	24.4	21.2	0.69	<0.01
Milk Yield (kg/d)	36.7	33.8	2.06	0.32
ECM Yield (kg/d)	37.2	36.0	1.9	0.67
BW (kg)	644	644	14	0.97
ΔBW (kg/d)	0.53	0.22	0.13	0.11
BCS (1-5 Scale)	3.08	3.16	0.07	0.45
ΔBCS (28d period)	-0.02	-0.06	0.04	0.51
MM_R	3.98	3.77	0.124	0.23
MM_I	4.35	3.67	0.101	<0.01
Milk Components				
% Fat	3.59	3.88	0.13	0.11
% Protein	3.09	3.14	0.07	0.64
MUN (mg/dl)	14.8	14.4	0.36	0.37
SCC (1000/ml)	65	68	16	0.87
Feed Efficiency[3]				
RFI	1.3	-1.5	0.11	<0.01
Milk-to-feed	3.5	4.3	0.40	0.17
IOFC (US $/d)	2.90	3.08	0.12	0.33
Plasma Metabolites				
Insulin (mg/L)	0.71	0.55	0.08	0.17
Glucose (mg/dl)	59.7	59.5	1.2	0.9
NEFA (ueq/L)	96	131	7.8	<0.01

[1]All values represent overall LS Mean for both dietary treatments.

[2]LRFI= Low RFI or efficient cows, HRFI= High RFI or inefficient cows.

[3]ECM= 0.327*Milk Yield + 12.95*Fat Yield + 7.65*Protein Yield, BW= Body weight, ΔBW= Change in body weight, BCS= Body condition score, ΔBCS = Change in body condition score, RFI= Residual feed intake, IOFC= Income over feed cost, and NEFA= Non-esterified fatty acids

[4]P-value associated with the main effect of efficiency group.

Chapter 4: Discussion

Section 1: Treatment effect on production variables

As expected, DMI decreased for cows fed the HF compared with LF diet (Table 4). High starch diets can affect DMI through metabolic regulation explained by the hepatic oxidation theory (Allen et al., 2009), and high NDF diets can decrease DMI due to physical fill (Allen, 2000). Fiber particles have a greater retention time in the rumen when compared to more digestible compounds such as starch or proteins. Continuous intake coupled with longer retention time can cause distention of the reticulo-rumen. This distention causes pressure on the mechanoreceptors present in the rumen wall (Harding and Leek, 1972). The signal of these mechanoreceptors stimulates the satiety centers of the brain to terminate a meal (Forbes et al., 1996) thus, suppressing intake. The effect of high NDF is greater when the source of fiber is forage compared with non-forage fiber sources. Particle size of forages are typically larger and less uniform than non-forage fiber sources (NFFS), which results in additional rumination time needed per unit of NDF and longer retention time for forages when compared to NFFS. Our data showed a 10% decrease in DMI when cows were fed the HF diet compared to the LF diet. Over 75% of total fiber was provided by a forage source in the LF diet, and this increased to 80% in the HF diet. We speculate that physical fill was the major limitation to greater DMI for cows fed the HF diet. Due to the strong positive correlation between DMI and ECM ($r = 0.8$, Table 8), we also presume that reduced DMI partly limited ECM of cows fed the HF diet compared to cows fed the LF diet. These observations are similar to the results reported by Ipharraguerre et al. (2002) and Oba and Allen (2000), where increased NDF diets decreased DMI and milk yield of cows. The decrease in milk production of cows fed a high NDF (low starch) diet is also consistent with the results of Potts et al. (2015), who reported a decrease of 5% (2.1 kg/d) milk yield per day, when dietary starch decreased 16% and was replaced

by fiber. Our data shows a decrease of 8% (3.1 kg/d) milk yield per day, when dietary starch was decreased by 12% and was replaced by fiber. Although our difference in starch was smaller between diets, the drop in milk production was bigger. The reason is likely that our fiber was from forage and was coupled with a drop in DMI, whereas Potts et al. (2015) replaced starch with non-forage fiber.

Dietary treatments also altered milk components in our study. Protein concentration and yield were increased for cows fed the LF diet, which is consistent with Potts et al. (2015). This increase in protein concentration and yield likely was due to the greater concentration of starch in the LF diet. Increasing availability of highly digestible carbohydrates provides additional energy to the rumen microbes which increases the synthesis and absorption of microbial protein (Grum et al., 1996). Additionally, the increased concentration of starch increases the synthesis of propionic acid, an important glucogenic precursor for ruminants; which could spare amino acids from being used as glucogenic precursors and save them for used in protein synthesis. Finally, high starch diet can up regulate the mTOR pathway, which is activate in high-energy metabolic states and results in an increase in milk protein synthesis (Morita et al., 2015). The study conducted by Burgos et al. (2010) suggest that nutrients can regulate mammary protein synthesis through the mTOR pathway in dairy cows. This helps explain the observed increase of milk protein yield by the LF diet. Although MUN values for both diets were within the recommended range, the LF diet caused a lower MUN concentration in milk compared to the HF diet. As discussed by Khezri et al. (2006), this is likely the result of the LF diet providing sufficient, rapidly available carbohydrates to stimulate the capture of ammonia by the rumen microbes.

The HF diet increased milk fat percentage of cows when compared to the LF diet, which is consistent with published research, as exemplified in Oba and Allen (2000) and Potts et al. (2015).

Increased concentration of dietary fiber results in a higher acetate:propionate ratio in the rumen (Oba and Allen, 2000), but the increase in milk fat concentration from high fiber is often simply the result of less milk volume without a drop in milk fat synthesis. The milk dilution factor likely explains the differences observed in our data as there was a change in fluid milk yield but no difference in fat yield. Additionally, high starch diets can have a negative effect on fiber digestion due to depressed rumen pH (Russell and Wilson, 1996); however, this likely does not explain our results as the concentration of total NDF and forage NDF exceeded the recommended minimums (NRC, 2001). Finally, another possible explanation for changes in milk fat concentration is dietary effects on energy partitioning. Cows fed the LF diet partitioned twice as much energy towards body tissue accretion than those fed the HF diet. This is consistent with their increased concentrations of glucose and insulin in plasma, as insulin is known to promote body fat accretion (Bauman, 2000), and our changes in partitioning are similar to those of Boerman et al. (2015). Thus, although cows fed LF diets consumed more energy, much of the increased energy was partitioned to body tissue accretion rather than milk fat yield.

Although we observed significant differences in BW, ΔBW, and partitioning of energy towards BW, no significant differences were observed in BCS or ΔBCS. This contrasts with the results reported by Potts el al. (2015) who found that diets significantly altered ΔBCS. A possible explanation for this difference is that, 70% of the cows in our study were primiparous cows whereas only 40% of the cows were primiparous in Potts et al. (2015). As animals mature, fat accretion patterns change across adipose tissue depots. Therefore, primiparous and multiparous cows may respond to diets differently in how gains in BCS and BW are related. A significant correlation ($P < 0.01$) between ΔBW and ΔBCS is reported for multiparous cows but no correlation for primiparous cows (Table A1-Apendix). Although the amount of maintenance energy was

greater for cow fed the LF diet compared to cows fed the HF diet, the fraction of energy partitioning toward maintenance was less. The dilution of maintenance likely explains this result. As energy intake increases, the portion required for maintenance is reduced. Thus, the increase in DMI for cows when fed the LF diet resulted in a smaller portion used for maintenance than when they were fed the HF diet. This decreased proportion for maintenance assumes that it is proportional to MBW and that is accurate and it does not change with diet changes.

In summary, our data shows that our diets altered several production traits. This confirms that the two diets were different from each other, giving validity to their use for testing the repeatability of RFI across diets differing in forage content.

Section 2: Repeatability of RFI

Our results showing that RFI is moderately repeatable (r=0.54) across diets are similar to several published studies in beef and dairy cattle. Repeatability of RFI across diets varies from 0.33 (Durunna et al., 2001) to 0.73 (Potts et al., 2015). Potts et al. (2015), found the repeatability of RFI across high and low starch diets for lactating dairy cows to be relatively high (r = 0.73). Although our study used the same animal model and our dietary treatment also differed in fiber and starch content, our reported repeatability was lower. The most likely reason for this difference is that Potts et al. (2015) replaced starch with fiber from soyhulls to achieve the difference in fiber concentrations, whereas forage was the primary source of fiber in the current study. There is ample data that shows how the variation in fiber sources (forage or non-forage) can impact DMI and other variables in lactating dairy cows (Clark and Armentano, 1993; Cuningham et.al.,1993; Clark and Armentano, 1997; among others). More recently a study by Halachmi et al. (2004) reported significant differences in *in vitro* NDF, OM digestibility and feeding behavior for two diets differing in NDF source (soy hulls vs. corn silage); which directly compare the two fibers sources

used by Potts et al. (2015) and the present study. Their results showed that forage NDF decreases digestibility and intake of dairy cows when compared to non-forage NDF. Additionally, Ipharraguerre et. al. (2002) reported that up to 30% corn grain can be substituted by soyhulls with no effect on DMI. Thus, the fact that we replaced corn grain with forage, instead of soyhulls, likely explains why we observed differences in DMI but Potts et al. (2015) did not. RFI is a measurement strongly related to DMI. In contrast to Potts et al. (2015), our dietary treatments differed considerably in forage NDF concentration, negatively impacting DMI. Consequently, we propose that the variation of forage NDF between diets is the main reason for a reduction in RFI repeatability across diets when compared to Potts et al. (2015). As previously discussed digestion and passage rate can be more variable for forage sources.

Repeatability of RFI across diets differing in forage content has been reported for beef cattle (Durunna et al., 2011 and Cassady et al., 2016). Durunna et al (2011) observed the repeatability of RFI across the growing and finishing periods of beef steers to be 0.33 with the growing steers fed a forage-based diet and the finishing steers fed a concentrate-based diet. Compared to those results, our value was higher, but this was partly expected considering that differences in stage of development could potentially influence repeatability of RFI. For heifers fed the same diet in both the growing and mature period, the repeatability of RFI was 0.54 (Durunna et al., 2012). Heifers fed the same diet during the growing and finishing period had an RFI repeatability of 0.62 (Kelly et al., 2010b); a value that is similar to the value reported by Cassady et al. (2016). Steers fed a grain-based diet during the growing and finishing periods had an RFI repeatability of 0.63. However, the same study by Cassady et al. (2016) fed two diets differing in forage and grain concentration during the growing period and reported a RFI

repeatability of 0.40. This data shows that both diet and physiological state of the animal likely influence repeatability of RFI.

Another method to evaluate RFI repeatability is to examine the repeatability of feed efficiency rankings across diets. Using this method, we obtained results comparable to the values reported by Potts et al. (2015) and others (Figure 6). In our study, 56% of the animals maintained the same efficiency group across diets, a result identical to the 56% reported by Potts et al. (2015). Additionally, in our study 38% of animals changed efficiency ranking by one efficiency group e.g. from the High RFI group to the Medium RFI, which compares with 40% in Potts et al. (2015). Finally, drastic changes in efficiency group e.g. from the High RFI group (least efficient) to the Low RFI group (most efficient) or vice versa were recorded for 6% of the animals in our study and for 4% of the animals in Potts et al. (2015). Therefore, although our overall repeatability for RFI was lower than that of Potts et al (2015), re-ranking of the feed efficiency groups for animals was similar. This also parallels the results of Durunna et al. (2011), who reported that feeding finishing compared to growing diets to steers resulted in no change in RFI group for 45% of the animals, a 1-group change for 46%, and a drastic change in RFI group for 8%. Durunna et al. (2012) observed similar changes in re-ranking of RFI groups when crossbred heifers were fed the same diet across two periods.

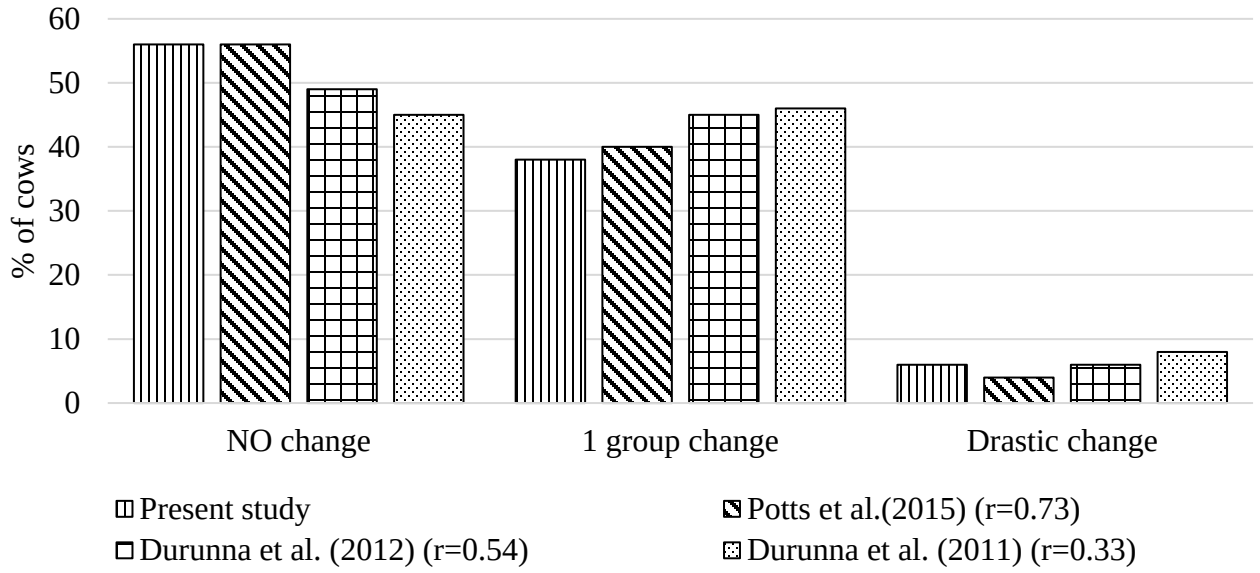
Weekly correlations within and across diets are reported in Table 7. We found the repeatability across diets using weekly RFI calculations was 0.47, which was lower than the overall repeatability of 0.54. This is expected because the weekly measurement of RFI will have a greater error associated to it. This correlation of weekly RFI of cows fed two different diets was relatively similar when evaluating different parity groups, sequence cohorts, or experiments (Table 7). Based on weekly data from our study, RFI repeatability was reasonably high within diet (r = ~0.64). This

provides evidence that even within a diet, correlation is not perfect. Therefore, a correlation of r=0.54 across two diets does not mean that the change in RFI ranking or correlation was mostly caused by differences in diets. Alternatively, our correlation value of 0.54 is 84% of the within diet correlation value, and thus it represents a moderate repeatability of RFI across dietary forage concentration.

Finally, our data shows a negative correlation between IOFC and RFI. As such, efforts to reduce RFI in dairy cows (selecting for increased feed efficiency) will result in an increase of IOFC. The same is true for the correlation between RFI and milk-to-feed ratio. Our data also supports that selection for RFI can be coupled to other desired traits because is independent from variables such as milk production or BW. There was no correlation between RFI, ECM, BW, or ΔBW (Table 8); thus, an improvement in RFI can be pursued without major consequences in milk yield or BW values; this is also discussed by VandeHaar et.al. (2016). Our recommendation is to use selection indexes for decreased RFI coupled with increased milk production to maximize profitability and environmental stewardship.

In summary, RFI was moderately repeatable across dietary forage concentration. Therefore, analysis of RFI under high starch diets can be used to obtain a fair estimation of RFI rankings in cows fed low starch diets with a high concentration of forage.

Figure 6: Re-ranking summary of four experiments[1]



[1]Bars with upright lines represent efficiency re-ranking of dairy cows across high and low forage fiber diets (r=0.54) in the present study, bars with diagonal lines represent the re-ranking of dairy cows across high and low starch diets reported by Potts et al. (2015) (r=0.73), plat bars represent the re-ranking of beef heifers fed the same diet across the growing and finishing periods reported by Durunna et al., (2012) (r=0.54), dotted bars represent re-ranking of beef steers across the growing period (fed growing diet) and the finishing (fed finishing diet) periods reported by Durunna et al., (2011) (r=0.33).

Section 3: Variation between two efficiency groups differing in RFI

Multiple studies have evaluated differences in production between cattle that rank low in RFI (efficient) compared with cattle that rank high in RFI (inefficient) dairy cows (Williams et al., 2011; Connor et al., 2013; Potts et al., 2015; and Xi et al., 2016). Our data showed that DMI was 13% lower (3.2 kg/d) for the low RFI cows compared to the high RFI cows with no statistical differences in ECM production ($P = 0.67$) or ΔBW ($P = 0.11$). Our results are consistent with Xi et al. (2016), who observed that low RFI cows ate 8% less (1.6 kg/d) than high RFI cows, Connor et al. (2013), who found the low RFI cows ate 15% less (3.7 kg/d) than high RFI cows, and Potts et al. (2015), who found that compared with high RFI low RFI cows had a reduction in DMI under

high and low starch diets of 14% (4.2 kg/d) and 20% (5.8 kg/d) respectively. As expected in all three studies, the reduction in DMI by low compared to high RFI cows was not associated with a change in milk production or BW change. Williams et al. (2011) studied growing dairy heifers and observed 18% less (1.7 kg/d) DMI for low compared to high RFI animals, and the decreased DMI of the low RFI group was not associated with a change in growth rate. These results support the hypothesis that selection for low RFI could reduce feed costs without altering production; thus, increasing the profitability of dairy cows.

The mechanisms to explain the physiological source of RFI (the DMI not attributable to differences in milk production, BW, or change in BW) are inconclusive. Richardson and Herd (2004) estimated the contribution of different physiological processes to variation in RFI. In their study, they estimated that protein turnover and metabolism could contribute to 37% of the variation in RFI, which is supported by McDonagh et al. (2001) who studied efficiency in steers. Xi et al. (2016), reports an increase of 0.2% in the concentration of milk protein in low RFI cows compared to high RFI cows. Conversely, our data shows no significant difference on the concentration of milk protein between the two efficiency groups, which parallels results reported by Connor et al. (2013) and Macdonald et al. (2014). The concentration of urea in milk is an indicator of protein metabolism in dairy cows. Xi et al. (2016) found that high RFI cows had greater MUN concentration than low RFI cows (P <0.01). Thus, we expected that cows that produced more milk per unit of feed might have a lower concentration of MUN. However, we observed no difference in MUN between low RFI and high RFI cows. Additional research is needed to determine if protein metabolism is different in low RFI cows vs high RFI cows.

We found a weak positive correlation between SCC and RFI (r = 0.20), although no difference was observed in mean SCC of low RFI and high RFI cows. In accordance with our data,

Xi et al. (2016) found no statistical difference in SCC between RFI groups; but, reported a positive correlation between SCC and RFI (r = 0.55). Interestingly two cows with second and third highest RFI ranking had the highest SCC (1.3 million and 526,000 respectively); because these cows where outliers, they were removed from the dataset before making comparisons across RFI groups. If they had remained in the data, the mean SCC of high RFI cows would have been much higher. Perhaps these cows ate more than expected (had high RFI) due to energy losses associated with the inflammatory process. Hou et al. (2012) suggests that genetic differences between cows with different efficiency (RFI) could be related to differences in immune responses which could relate SCC and RFI. Present data is not conclusive but, the observations of the two case animals and the positive correlation observed by us and others is evidence that further research is needed determine what is the relationship between RFI and some immune responses that impact milk quality.

Our data shows that NEFA concentration was 27% (+35µeq/L; $P< 0.01$) higher in the plasma of low RFI cows than high RFI cows. Our findings are opposed to the results of Xi et al. (2016) where low RFI cows had a 19% lower NEFA concentration that high RFI cows. The authors speculate that the observed difference might have been influenced by a reduction in carcass fat of low RFI cows, but no difference was observed in BW or chest girth measurements between groups, and BCS data was not reported. Our data shows a negative correlation (r= -0.31) between RFI and NEFA concentration which was also reported by Kelly et al. (2010a) (r= -0.21; 86 growing beef heifers), and Wood et al. (2014) (r= -0.17; 324 pregnant beef cows evaluated across 9 studies). The negative correlation between RFI and NEFA may indicate that low RFI cows are mobilizing fat to support a lower DMI but, we did not detect it. It could also indicate that efficient cows metabolize fat differently than inefficient cows. However, a different study by Kelly et al. (2010b) found no significant correlation between NEFA and RFI in growing heifers. Thus, with the

inconsistencies of reported correlations, and the lack of studies that relate RFI and NEFA in lactating cows, is not clear if RFI and NEFA are related. Further studies are needed to clearly establish the relationship between key plasma metabolites and RFI in lactating dairy cows.

Chapter 5: Conclusions and closing remarks

Cows produced less milk and partitioned less energy toward body tissue when fed diets with 30% forage NDF and 20% starch compared to 20% forage NDF and 30% starch. This production responses confirmed that the two dietary treatments were nutritionally different and were valid diets for testing the repeatability of RFI across diets. Repeatability was moderate at r = 0.54 across these dietary treatments, and only 6% of 84 cows had a drastic change in RFI ranking when dietary treatments were switched. This supports our hypothesis that RFI is a repeatable trait across diets differing in dietary forage concentration. Therefore, we suggest that genomic breeding values obtained for RFI when cows of a reference population are fed high starch diets can be applied through a selection index for animals on lower starch, high forage diets. Finally, further research is needed to elucidate the mechanism that influences the variation of RFI so we can design management and nutritional strategies to maximize feed efficiency.

APPENDIX

Table A1: Correlation of BW and BCS of primiparous and multiparous cows[1, 2, 3]

	BW	ΔBW	BCS	ΔBCS
BW	1	0.03	**0.63**	0.14
ΔBW	0.12	1	-0.06	**0.25***
BCS	**0.45**	0.04	1	0.03
ΔBCS	0.02	0.12	0.06	1

[1]Bold values represent a *P*-value of <0.05 and * represent a *P*-value of <0.1

[2] Correlation coefficient are Multiparous above diagonal and Primiparous below diagonal

[3] BW=Body weight, ΔBW= Change in body weight, BCS=Body condition score and ΔBCS= Change in body condition score

Table A-2: Raw RFI weekly correlation of all animals[1]

Week[2,3]	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
Wk1	1	**0.61**	**0.54**	**0.50**	**0.30**	**0.32**	**0.35**	0.21
Wk2	**0.62**	1	**0.70**	**0.54**	**0.28**	**0.39**	**0.56**	**0.38**
Wk3	**0.51**	**0.65**	1	**0.61**	**0.41**	**0.43**	**0.56**	**0.33**
Wk4	**0.52**	**0.56**	**0.60**	1	**0.62**	**0.52**	**0.56**	**0.55**
Wk5	**0.31**	**0.31**	**0.43**	**0.64**	1	**0.58**	**0.49**	**0.47**
Wk6	**0.35**	**0.40**	**0.42**	**0.50**	**0.59**	1	**0.59**	**0.70**
Wk7	**0.34**	**0.51**	**0.51**	**0.55**	**0.49**	**0.59**	1	**0.59**
Wk8	**0.27**	**0.45**	**0.37**	**0.58**	**0.47**	**0.65**	**0.66**	1

[1] Pearson correlation coefficient are above the diagonal (n=84); Spearman's coefficients are below the diagonal (n=84). All significant values (P <0.05) are expresed in bold.

[2]Week of experiment. Diet change occurred after compleation of wk 4, thus cows received same diet either High or Low forage) in weeks 1 throught 4 and the other diet in wk 5 throught 8.

[3]Weekly residual feed intake was estimated from the performance of each cow for the specific wk, with change in body energy being calculated using change in BW defined as the diference between average BW for the wk minus the average BW for the previos wk.

Table A-3: Raw RFI weekly correlation by treatment sequence [1,2]

Week[3,4]	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
Wk1	1	**0.65**	**0.73**	**0.53**	**0.38**	**0.32**	**0.39**	0.15
Wk2	**0.53**	1	**0.78**	**0.60**	**0.39**	**0.48**	**0.63**	**0.37**
Wk3	0.22	**0.57**	1	**0.59**	**0.46**	**0.45**	**0.65**	0.28
Wk4	**0.44**	**0.45**	**0.66**	1	**0.58**	**0.51**	**0.52**	**0.56**
Wk5	0.13	0.11	**0.31**	**0.70**	1	**0.58**	**0.46**	**0.47**
Wk6	**0.32**	0.27	**0.38**	**0.53**	**0.57**	1	**0.57**	**0.70**
Wk7	0.29	**0.45**	**0.40**	**0.63**	**0.54**	**0.62**	1	**0.51**
Wk8	**0.33**	**0.39**	**0.44**	**0.56**	**0.48**	**0.71**	**0.76**	1

[1] Values are the Pearson correlation coefficient. Sequence High-Low are above the diagonal (n=42); Sequence Low-High are below the diagonal. All significant values ($P < 0.05$) are depicted in bold.

[2] Sequence High -Low refers to cows that recived the high forage diet in period 1 and the low forage diet in period two; the sequence Low-High refers to the cows that received the low forage diet in period 1 and the high forage diet in period 2.

[3]Week of experiment. Diet change occurred after compleation of wk 4, thus cows received same diet either High or Low forage) in weeks 1 throught 4 and the other diet in wk 5 throught 8.

[4]Weekly residual feed intake was estimated from the performance of each cow for the specific wk, with change in body energy being calculated using change in BW defined as the diference between average BW for the wk minus the average BW for the previos wk.

Table A-4: Raw RFI weekly correlation by parity group[1]

Week[2,3]	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
Wk1	**1**	**0.75**	**0.61**	**0.64**	**0.34**	**0.46**	**0.59**	**0.34**
Wk2	**0.53**	**1**	**0.75**	**0.51**	0.20	0.36	**0.66**	0.23
Wk3	**0.50**	**0.67**	**1**	**0.57**	**0.38**	**0.44**	**0.65**	0.28
Wk4	**0.40**	**0.54**	**0.66**	**1**	**0.69**	**0.43**	**0.49**	**0.47**
Wk5	**0.28**	**0.29**	**0.44**	**0.57**	**1**	**0.63**	**0.41**	**0.56**
Wk6	0.26	**0.37**	**0.40**	**0.55**	**0.52**	**1**	**0.53**	**0.77**
Wk7	0.21	**0.45**	**0.46**	**0.59**	**0.50**	**0.59**	**1**	**0.41**
Wk8	0.12	**0.45**	**0.36**	**0.59**	**0.37**	**0.59**	**0.68**	**1**

[1] Values refer to the Pearson correlation coefficient. Primiparous cows are above the diagonal (n=57); multiparous cows are below the diagonal (n=27). All significant values (P <0.05) are depicted in bold.

[2]Week of experiment. Diet change occurred after compleation of wk 4, thus cows received same diet either High or Low forage) in weeks 1 throught 4 and the other diet in wk 5 throught 8.

[3]Weekly residual feed intake was estimated from the performance of each cow for the specific wk, with change in body energy being calculated using change in BW defined as the diference between average BW for the wk minus the average BW for the previos wk.

Table A-5: Raw RFI weekly correlation by Experiment[1]

Week[2,3]	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
Wk1	1	**0.77**	**0.51**	**0.64**	**0.40**	**0.41**	**0.44**	0.36
Wk2	**0.65**	1	**0.69**	**0.78**	**0.41**	0.28	**0.41**	0.33
Wk3	**0.71**	**0.79**	1	**0.71**	**0.52**	**0.42**	**0.47**	**0.44**
Wk4	**0.52**	**0.48**	**0.58**	1	**0.72**	**0.46**	**0.57**	**0.49**
Wk5	**0.37**	**0.36**	**0.55**	**0.67**	1	**0.7**	**0.7**	**0.69**
Wk6	**0.34**	**0.44**	**0.46**	**0.52**	**0.66**	1	**0.81**	**0.83**
Wk7	**0.55**	**0.74**	**0.67**	**0.57**	**0.51**	**0.62**	1	**0.89**
Wk8	0.22	**0.38**	0.27	**0.53**	**0.54**	**0.73**	**0.41**	1

[1] Values refer to the Pearson correlation coefficient between weekly RFI values. Cows in experi ment 1 are above the diagonal (n=32); Cows in experiment 2 are below the diagonal (n=32). All significant values (P <0.05) are depicted in bold.

[2]Week of experiment. Diet change occurred after compleation of wk 4, thus cows received same diet either High or Low forage) in weeks 1 throught 4 and the other diet in wk 5 throught 8.

[3]Weekly residual feed intake was estimated from the performance of each cow for the specific wk, with change in body energy being calculated using change in BW defined as the diference between average BW for the wk minus the average BW for the previos wk.

Table A-6: Raw RFI weekly correlation in Experiment 3[1]

Week[2,3]	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
Wk1	**1**							
Wk2	**0.56**	**1**						
Wk3	**0.57**	0.38	**1**					
Wk4	0.22	**0.46**	**0.53**	**1**				
Wk5	**0.56**	0.29	**0.65**	**0.53**	**1**			
Wk6	0.30	0.37	**0.47**	**0.66**	**0.68**	**1**		
Wk7	0.28	**0.52**	**0.48**	**0.61**	0.27	0.40	**1**	
Wk8	0.08	0.28	0.32	**0.76**	**0.58**	**0.77**	**0.57**	**1**

[1] Values refer to the Pearson correlation coefficient between weekly RFI values of cows in Experiment 3 (n=20). All significant values (P <0.05) are depicted in bold.

[2]Week of experiment. Diet change occurred after compleation of wk 4, thus cows received same diet either High or Low forage) in weeks 1 throught 4 and the other diet in wk 5 throught 8.

[3]weekly residual feed intake was estimated from the performance of each cow for the specific wk, with change in body energy being calculated using change in BW defined as the diference between average BW for the wk minus the average BW for the previos wk.

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