Assessing feed efficiency in early and mid lactation and its associations with performance and health in Holstein cows

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ABSTRACT

Objectives were to evaluate the associations between residual dry matter (DM) intake (RFI) and residual N intake (RNI) in early lactation, from 1 to 5 wk postpartum, and in mid lactation, from 9 to 15 wk postpartum, and assess production performance and risk of diseases in cows according to RFI in mid lactation. Data from 4 experiments including 399 Holsteins cows were used in this study. Intakes of DM and N, yields of milk components, body weight, and body condition were evaluated daily or weekly for the first 105 d postpartum. Milk yield by 305 d postpartum was also measured. Incidence of disease was evaluated for the first 90 d postpartum and survival up to 300 d postpartum. Residual DM intake in mid lactation was associated with RFI (Pearson r = 0.43, and Spearman ρ = 0.32) and RNI (r = 0.44, ρ = 0.36) in early lactation, and with RNI in mid lactation (r = 0.91, ρ = 0.84). Similarly, RNI in mid lactation was associated with RNI in early lactation (r = 0.42, ρ = 0.35). During the first 15 wk postpartum, more efficient cows in mid lactation consumed 3.5 kg/d less DM (Q1 = 19.3 vs. Q4 = 22.8 kg/d) and were more N efficient (Q1 = 31.6 vs. Q4 = 25.8%), at the same time that yields of milk (Q1 = 39.0 vs. Q4 = 39.4 kg/d), energy-corrected milk (Q1 = 38.6 vs. Q4 = 39.3 kg/d), and milk components did not differ compared with the quartile of least efficient cows. Furthermore, RFI in mid lactation was not associated with 305-d milk yield, incidence of diseases in the first 90 d postpartum, or survival by 300 d postpartum. Collectively, rankings of RFI and RNI are associated and repeatable across lactation stages. The most feed-efficient cows were also more N efficient in early and mid lactation. Phenotypic selection of RFI based on measurements in mid lactation is associated with improved efficiency without affecting production or health in dairy cows.

Key words: dairy cow, feed efficiency, health, residual feed intake

INTRODUCTION

The improvement in milk production in dairy cows has historically increased the efficiency of feed utilization by diluting their maintenance nutrient costs (Tyrrell, 1980); however, as a consequence of increased productivity, cows voluntarily consume more feed to support the nutrient demands for milk synthesis (Reynolds, 2004; Baumgard et al., 2017). From 2010 to 2017, feed represented 51% of total costs on a dairy farm (USDA-ERS, 2018); therefore, selecting dairy cows by the efficiency of nutrient utilization is a desirable trait because of its relationship with economic returns and improvement of land utilization (Bach et al., 2020), so long as it does not result in detrimental effects on production or animal health. Ideally, selection would aim to identify superior animals for productive performance, feed efficiency, and resistance to diseases.

Feed conversion ratio is a common measure of feed efficiency in dairy cows and represents the proportion
of feed intake that is recovered as yield of milk or milk corrected for fat or energy. Nevertheless, the feed conversion ratio does not account for the energy used by tissues, and it would result in misleading values of feed efficiency in cows losing weight in early lactation, which usually inflates the feed conversion ratio (VandeHaar et al., 2016). On the other hand, residual DM intake, also called residual feed intake (RFI) is a measure of feed conversion efficiency that is calculated by the difference between observed and predicted DMI, after adjusting for multiple energy sinks, in which the most efficient cows have negative RFI values; that is, they consume less DM to produce the same amount of milk, accounting for body energy changes. The use of RFI has been preferred because it accounts for milk production, BW-related traits, and parity (Bach et al., 2020). Furthermore, RFI has a moderate heritability of 0.36 ± 0.06 (Connor et al., 2013), and it is an economically relevant trait.

Nutrient digestibility and digestive capacity of the cow are important elements of feed efficiency (Bach et al., 2020). Among the components of a diet, protein sources are the most expensive, and excess excretion of N has been related to environmental challenges; thus, N efficiency can be used as a tool to increase dairy profitability and also contribute to reducing environmental pollution without affecting animal performance (Dado et al., 1994; Phuong et al., 2013; Fadul-Pacheco et al., 2017). A recent study conducted by Liu and VandeHaar (2020) suggested that selecting cows for negative RFI can also improve protein efficiency, given the observed correlations between protein efficiency and RFI in peak (r = −0.42) and late lactation (r = −0.36).

A potential concern in selecting cows for RFI is that measurements are usually taken from cows in mid lactation (Bach et al., 2020), after the period during which cows undergo extensive mobilization of body reserves. Stimulating early-lactation DMI usually is considered beneficial because cows that have less DMI in the first weeks postpartum are more likely to be in negative nutrient balance and develop diseases (Pérez-Báez et al., 2019). In fact, RFI has been reported to be strongly associated with energy balance (r = 0.64); thus more efficient cows exhibited more pronounced negative energy balance (Seymour et al., 2020), reduced blood glucose concentrations in early lactation (Dechow et al., 2017), reduced DMI in the first 90 d postpartum (Connor et al., 2013), and a more frequent change in BCS throughout the first 238 d postpartum (Fischer et al., 2018). A concern with improved feed efficiency in mid lactation is that it might result in increased loss of BW or BCS in early lactation and influence health in dairy cows (Seymour et al., 2020).

Stage of lactation affects feed efficiency, and understanding the repeatability of RFI as cows progress in lactation is important. Studies have demonstrated that RFI is reasonably repeatable during different stages of lactation. Indeed, Connor et al. (2019) observed a strong positive correlation (r = 0.72) between RFI estimated from 23 to 100 d postpartum and RFI estimated from 10 to 305 d postpartum. However, the consequences in early lactation of selection for RFI based on mid-lactation measurements remain unknown. Our first hypothesis was that RFI in mid lactation is correlated with RFI in early lactation; that is, more efficient cows in mid lactation also are more efficient in early lactation. Our second hypothesis was that more efficient cows in mid lactation do not suffer from increased tissue mobilization or diseases in early lactation, although more efficient cows will have reduced energy balance in early lactation. Thus, the objectives were to establish correlations between RFI and residual N intake (RNI) in early and mid lactation and to determine the associations between ranking of RFI in mid lactation with measures of body tissue mobilization, productive performance, incidence of diseases, and survival.

MATERIALS AND METHODS

Cows, Housing, Feeding, and Calculation of Dry Matter Intake

Data from 4 experiments conducted at the University of Florida were used for this study (Greco, 2014; Zenobi et al., 2018; Bollatti et al., 2020a,b; R. Zimpel, University of Florida, unpublished results). The experimental procedures with cows were approved by the Institutional Animal Care and Use committee of the University of Florida.

The experiments were conducted between December 2008 and November 2019, and they all followed randomized block designs and involved dietary manipulations. Data were available from 399 lactating Holstein cows, 154 primiparous and 245 multiparous, with a mean of 2.7 ± 1.1 lactations (1 to 8 lactations). Cows were housed in sand-bedded freestall barns equipped with fans placed above the stalls at intervals of 6 linear meters and water soaker line with nozzles placed above the feedbunk. Cows were randomly assigned to individual feeding gates (Calan Broadbent Feeding System, American Calan Inc.) used to evaluate daily feed intake. Cows had ad libitum access to their respective diets, which were fed as TMR. The TMR were weighed and fed twice daily at approximately 0600 and 1300 h, and refusals were weighed once daily, before the morning feeding, from 1 to 105 d postpartum. The amount
of TMR offered to individual cows was adjusted daily to result in 5 to 10% refusals.

Dietary ingredients were sampled twice weekly and dried at 55°C for 48 h in a forced-air oven, and dry weights were recorded. Dried feed samples were ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific), composited at each 4- to 8-wk period, depending on the experiment, and analyzed for N (method 990.03), ADF (method 973.18), NDF (method 2002-04), fat (method 2003.05), minerals (method 985.01), and DM at 105°C for 24 h (method 930.15; all methods from AOAC International, 2012). The DM contents of diets were calculated by multiplying the DM obtained at 55°C by the DM obtained at 105°C. It was assumed that refusals had the same DM content as the diet offered. Intake of DM for individual cows each day that refusals had the same DM content as the diet offered minus the amount refused multiplied by the DM content of the TMR in a particular week.

**Body Condition Score and Body Weight**

In each experiment, cows were scored for body condition on the day of calving and again once weekly by trained evaluators using a 1-to-5 scale with increments of 0.25 units as depicted in the Elanco body condition score chart (Elanco Animal Health, 2009). Cows were weighed twice daily, immediately after each milking, on a walk-through scale (AfiWeigh, SAE AfiKim) as they left the parlor, and the mean daily BW was calculated. Daily means were used to generate weekly means for BW. Weekly changes in BW were calculated by subtracting the mean BW in a given week minus the mean BW in the preceding week. For the first week postpartum, the value obtained was the difference between the mean value from 1 to 7 d postpartum minus the value of the day of calving. The obtained value was divided by the number of days between measurements, to generate a daily change in BW in kilograms per day.

**Milk Yield and Composition**

Milk yield was recorded using electronic milk flow meters (AfiFlo, SAE AfiKim) at each milking, and the daily yield was calculated by adding the individual milk weights per cow. In experiments 1, 2, and 3, cows were milked twice daily, between 1000 and 1100 h and again between 2200 and 2300 h. In experiment 4, cows were milked 4 times daily at 0600, 1100, 1800, and 2300 h. In experiments 1, 2, and 3, milk samples were obtained from 2 consecutive milkings, morning and night, once weekly for the first 105 d postpartum. In experiment 4, milk was sampled from the first and third milkings of the day, and samples were taken twice weekly, every Monday and Wednesday, until 105 d postpartum. Samples of milk were analyzed for fat, protein, and lactose at the Southeast Milk Inc. DHI laboratory (Belleview, FL). Milk yield and concentrations of fat, protein, and lactose from each milking were used to calculate the final concentrations of milk components for each week, which were then applied to calculate the yields of milk components for the respective weeks.

**Calculations**

All calculations were performed using the mean values for each week postpartum. Cow was the unit of analyses. Energy-corrected milk yield and the NE\textsubscript{L} content in milk were calculated according to NRC (2001) as follows: ECM (kg/d) = [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)]; NE\textsubscript{L} in milk (Mcal/kg) = [(0.0929 × fat %)] + [(0.0563 × protein %)] + [(0.0395 × lactose %)]. The NE\textsubscript{L} content in milk was multiplied by milk yield to calculate the amount of NE\textsubscript{L} secreted in milk daily. The 305-d milk yield was obtained for all cows that completed 305 d in lactation, whereas for those that left the herd or were dried off before 305 d postpartum, the value was estimated as the sum of daily milk yield observed until the cow left the herd or was dried off plus the projected subsequent production until 305 d using Wood’s lactation curve equation (Wood, 1967).

Body energy that was retained or mobilized as body reserves was calculated from changes in BW (kg/d), loss or gain, and considering BCS (1 to 5) measurements to adjust the NE\textsubscript{L} content in tissue gained or mobilized using an equation from NRC (2001) as follows: body energy change (Mcal/d) = (1.88 + 1.036 × BCS) × change in BW. Values for body energy change are expressed as megacalories per day of NE\textsubscript{L}.

Net energy balance was calculated using daily caloric intake from DMI and energy content of the diets according to NRC (2001) and the NE\textsubscript{L} system. The contents of NE\textsubscript{L} of diets fed were calculated for each experiment via NRC (2001) software using the analyzed nutrient content of ingredients and adjusting for the mean DMI observed in the experiment. Net energy for lactation required for maintenance was calculated based on metabolic BW (BW\textsuperscript{0.75}) as follows: NE\textsubscript{L} for maintenance (Mcal/d) = BW\textsuperscript{0.75} × 0.08. The NE\textsubscript{L} required for milk synthesis was calculated according to the yields of fat, protein, and lactose in milk as previously described. The balance of NE\textsubscript{L} was calculated as follows: net energy balance (Mcal/d) = intake of NE\textsubscript{L} – (NE\textsubscript{L} required for maintenance + NE\textsubscript{L} required for milk synthesis).

Nitrogen intake was calculated after determining the daily CP intake from DMI and the CP content of the
Incidence of Diseases and Survival

Diseases were recorded for each cow for the first 90 d postpartum and included milk fever, retained placenta, metritis, mastitis, displaced abomasum, lameness, and pneumonia. Milk fever was diagnosed based on clinical signs that included recumbency that responded to an i.v. administration of a solution containing Ca borogluconate. All cows were examined on the day after calving, and retained placenta was defined as a cow that failed to expel the fetal membranes within 24 h after parturition. All cows underwent a complete physical examination on d 4, 7, 10, and 12 postpartum, which included evaluation of rectal temperature, hydration status, heart and respiration rates, ruminal contractions, auscultation and percussion just cranial to the left and right paralumbar fossae, auscultation of the lungs and heart, transrectal palpation of the uterus and evaluation of uterine discharge, evaluation of lameness score, and collection of urine for assessment of presence of ketones. In addition, any cow with altered behavior that might indicate sickness, such as inappetence or a milk yield deviation by more than 15% relative to production on the previous day, were also examined. Rectal temperature was measured, and cows were considered to have fever if greater than 39.5°C. Cows were palpated transrectally, and presence of an enlarged flaccid uterus with fetid watery reddish-brownish discharge was defined as metritis. Metritis concurrent with fever was considered puerperal metritis. Cows with puerperal metritis received antimicrobial therapy. Diagnosis of displaced abomasum was performed by percussion and auscultation of the left and right flanks and confirmed during surgical correction by omentopexy. Immediately before every milking, all cows were examined by herd personnel for signs of clinical mastitis, and those with mastitis received intramammary antimicrobial therapy. Cows with fever, increased respiratory frequency, and detection of wheeze, rhonchus, or crackle sounds at auscultation were considered to have respiratory disease, which was treated with antimicrobial therapy. Cows were considered to have multiple diseases if diagnosed with more than 1 type of disease in the first 90 d postpartum (e.g., retained placenta and mastitis). Morbidity was defined as a cow having at least 1 disease that included milk fever, retained placenta, metritis, mastitis, displaced abomasum, lameness, or pneumonia in the first 90 d postpartum. Culling or death was recorded up to 300 d postpartum.

Statistical Analyses

Data were analyzed using the MIXED and GLIMMIX procedures of SAS 9.4 (SAS/STAT version 9.4; SAS Institute Inc.) for continuous and categorical data, respectively. The associations calculated in the current study were all based on phenotypic measurements. Analyses were performed using combined data from the 4 experiments.

Predicted DM intakes were calculated for each cow in early lactation, from 1 to 5 wk postpartum, and in mid lactation, from 9 to 15 wk postpartum. The linear models to predict DMI for an individual cow regressed the early-lactation or the mid-lactation mean DMI with major energy sinks during the respective periods. The statistical models included fixed effects of NE\textsubscript{L} secreted in milk, metabolic BW, body energy change, parity (primiparous vs. multiparous), season of calving, treatment within experiment, and the residual error term. Then, RFI (kg/d) was calculated as the difference between observed and predicted DMI in early and mid lactation.

Similar to DMI, the linear model for predicted N intake in early and mid lactation regressed the early-lactation or mid-lactation mean N intake with major N sinks during the respective periods. The statistical models included the fixed effects of milk N, metabolic BW, body N change, parity, season of calving, treatment within experiment, and the residual error term. Then, RNI (kg/d) was calculated as the observed minus the predicted N intake for each cow.
RESULTS

A total of 399 cows were included in the study and contributed data for statistical analysis in early lactation; however, 6 cows were excluded from the analyses using mid-lactation measurements because they were removed from their respective experiments before 13 wk postpartum, and, therefore, RFI in mid lactation could not be calculated.

Residual DMI, Residual N Intake, and Correlations

The mean RFI ranged from \(-2.2\) to \(+2.3\) kg/d in Q1 to Q4 when RFI was calculated in early lactation and from \(-2.1\) to \(+2.3\) kg/d in Q1 to Q4 when RFI was calculated in mid lactation (Supplemental Tables S1 and S2). Grouping cows into quartiles of RFI in mid lactation resulted in RNI with means ranging from \(-56\) to \(51\) g of N per day in early lactation, and \(-58\) to \(60\) g of N per day in mid lactation (Supplemental Tables S1 and S2). Distribution of RFI and RNI are depicted in Figures 1 and 2. Cows with residuals below the lines in each figure were those more feed or N efficient, whereas those above the lines were less efficient.

Rankings of RFI in early and mid lactation and of RNI in early and mid lactation were repeatable \((P < 0.001)\), and correlations between the 2 stages of lactation were at least 0.32 for RFI and at least 0.35 for RNI (Figures 3A and 3B). Strong correlations \((P < 0.001)\) between RFI and RNI were observed in both early and mid lactation (Figures 3C and 3D). Although reranking was observed, approximately 45% of the cows in Q1 and Q4 in mid lactation also were in Q1 and Q4, respectively, in early lactation (Supplemental Table S3, https://figshare.com/s/5a14509269596dcf133c).

Associations Between RFI in Mid Lactation and Performance in Early Lactation

Quartile of RFI in mid lactation was associated \((P < 0.001)\) with RFI and RNI in early lactation, and the relationships were linear and positive (Table 1). Similarly, quartile of RFI in mid lactation was associated \((P < 0.001)\) with linear decreases in DM and NE\(_{\text{L}}\) intakes in early lactation (Table 1), which resulted in a linear decrease \((P < 0.001)\) in NE\(_{\text{L}}\) balance in early lactation as efficiency improved. By contrast, quartile of RFI in mid lactation was not associated with body energy or N changes, daily BW change, or yields of milk, ECM, fat, protein, and total NE\(_{\text{L}}\) secreted in milk (Table 1). More efficient cows in mid lactation had greater \((P = 0.001)\) BCS in early lactation, a relationship that was linear with quartile of RFI (Table 1). The proportion...
of dietary N partitioned into milk N increased ($P < 0.001$) as RFI quartile decreased, and the association was quadratic with quartile of RFI in mid lactation. When both milk N and body N changes were considered, the efficiency of N use by dairy cows in early lactation followed a cubic ($P = 0.02$) association with quartile of RFI in mid lactation. Cows in Q3 had the greatest efficiency, whereas those in Q4 had the least efficiency.

**Associations Between RFI in Mid Lactation and 305-Day Milk Yield, Incidence of Diseases, and Survival**

Quartile of RFI in mid lactation was not associated with 305-d milk yield (Table 2). Similarly, quartile of RFI in mid lactation was not associated with SCS in the first 15 wk postpartum. Morbidity and multiple diseases by 90 DIM affected 39.3% and 15.0% of the cows in the study, respectively, and 9.5% of the cows left the study by 300 d postpartum because of either death or culling (Table 2). Quartile of RFI in mid lactation was not associated with any of the diseases evaluated in the first 90 DIM. Similarly, quartile of RFI in mid lactation was not associated with morbidity, risk of multiple diseases, or survival of cows to 300 DIM. Milk fever was not reported in Table 2 because the low frequency of cases resulted in poor performance of the statistical model.

**Associations Between RFI in Mid Lactation and Performance in the First 105 Days Postpartum**

Unsurprisingly, as RFI in mid lactation decreased (i.e., more efficient cows) so did DMI, and this response was observed in the first 15 wk postpartum (Q1–Q4; 19.3 vs. 20.5 vs. 21.6 vs. 22.9 ± 0.3 kg/d, LSM ± SEM), although the magnitude of difference among quartiles changed as lactation progressed based on the interaction ($P < 0.001$) between RFI quartile and week postpartum (Figure 4A). Similarly, a linear increase ($P < 0.001$) in the amount of ECM produced per kilogram of DMI was observed as RFI quartile in mid lactation decreased (Figure 4B). In the first 15 wk postpartum, cows in Q1 produced 0.27 kg more ECM for each kg

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**Figure 1.** Observed and predicted DMI in early (EL, A) and mid lactation (ML, B), and distribution of residual DMI (RFI) in EL (C) or ML (D) in lactating dairy cows. Early lactation represents measurements taken from 1 to 5 wk postpartum, whereas ML represents measurements taken from 9 to 15 wk postpartum. The most feed-efficient cows are those represented by the symbols below the lines, and the least feed-efficient cows are those represented by the symbols above the lines.
of DM consumed than cows in Q4 (Q1–Q4; 2.051 vs. 1.972 vs. 1.953 vs. 1.778 ± 0.026 kg/kg). The improvement in yield of ECM per kilogram of DMI associated with quartile of RFI originated from less intake because yields of milk (Q1–Q4; 39.0 vs. 39.5 vs. 40.5 vs. 39.4 ± 0.7 kg/d), ECM (38.6 vs. 39.4 vs. 40.6 vs. 39.3 ± 0.7 kg/d), fat (1.42 vs. 1.46 vs. 1.50 vs. 1.44 ± 0.03 kg/d), or protein (1.09 vs. 1.12 vs. 1.15 vs. 1.13 ± 0.02 kg/d) in the first 15 wk postpartum were not associated with RFI (Figures 4C to 4F). The efficiency of dietary N intake partitioned into milk N and body N change in the first 15 wk of lactation increased linearly (P < 0.001) as RFI in mid lactation decreased (Q1–Q4; 31.6 vs. 30.7 vs. 31.1 vs. 28.4%), although differences among quartiles were mostly after 6 wk postpartum (data not shown).

Quartile of RFI in mid lactation was associated (P = 0.003) with BCS postpartum, and the latter increased in a linear fashion (P < 0.001) as efficiency increased (Q1–Q4; 3.23 vs. 3.17 vs. 3.14 vs. 3.10 ± 0.03; Figure 5A). Residual DMI was not associated with BW of cows (Figure 5B), but more efficient cows in mid lactation had greater (P = 0.03) daily loss of BW (Q1–Q4; −0.41 vs. −0.34 vs. −0.23 vs. −0.24 ± 0.05 kg/d), which was reflected in greater loss of body energy in the first 15 wk of lactation (−2.65 vs. −2.30 vs. −1.58 vs. −1.65 ± 0.31 Mcal/d; Figures 5C and 5D).

**Net Energy Balance and Body Energy Change in Early Lactation as a Function of RFI in Early Lactation**

Figure 6 depicts the linear regression plots between RFI in early lactation and NEB balance (Figure 6A) or body energy change (Figure 6B) both in early lactation and segregated according to parity group. A positive association (P < 0.0001) was observed between RFI in early lactation and NEB balance such that, as RFI increased, NEB balance also increased, and the response was observed in both primiparous and multiparous cows, with R² ranging from 0.33 to 0.40. However, no association was observed between RFI in early lactation and body energy changes in early lactation in primiparous or multiparous cows.
DISCUSSION

Residual DM intake or RFI has been a potential target trait for genomic selection in dairy cattle mainly because of its relationship with farm profitability and a potential reduction of the impacts of dairy farming on the environment. In many instances, DMI is measured to calculate RFI after the first weeks of lactation to avoid the period in which cows undergo extensive BW loss, which often tends to inflate measures of feed efficiency. Although RFI seems to be repeatable (Connor et al., 2019) and to have a moderate heritability (Connor et al., 2013), limited knowledge exists relative to the consequences of selecting cows for RFI based on measurements in mid lactation and potential effects on early lactation. The present study showed that RFI in mid lactation is correlated with RFI or RNI in early lactation and strongly correlated with RNI in mid lactation; thus, selecting cows for improved feed efficiency based on RFI is expected to result in improved feed and N efficiency in the first weeks of lactation, which results in reduced DMI. Nevertheless, despite the reduced DMI in early lactation observed for cows with negative RFI in mid lactation, we detected no associations with risk of disease, survival, productive performance in the first 5 or 15 wk postpartum, or milk yield in the first 305 d of lactation.

Determinants of RFI have been widely reported typically past early lactation (Bach et al., 2020), probably because of increased RFI heritability after 53 d postpartum (Connor et al., 2013), less fluctuation in DMI measurements, return to a neutral or positive NE\textsubscript{L} balance (Bauman and Currie, 1980), or less influence from disorders that are mainly observed in the first weeks postpartum (Ribeiro et al., 2016). Such influences would favor collection of RFI past the early lactation period and, therefore, result in measurements of feed efficiency that might have implications for cows in early lactation. Nevertheless, the relationship between RFI estimated at different stages of lactation is not well reported in the literature, likely because of the challenges of measuring DMI and the physiological heterogeneity of lactational cycles (Li et al., 2017; Hurley et al., 2018). To include RFI in selection programs, the

![Figure 3. Relationship between residual DMI and residual N intake in early (EL, 1 to 5 wk postpartum) and mid lactation (ML, 9 to 15 wk postpartum) in dairy cows and respective Pearson (r) and Spearman (\(\rho\)) correlations. (A) Residual DMI in EL and ML, \(r = 0.43\), \(\rho = 0.32\); (B) residual N intake in EL and ML, \(r = 0.43\), \(\rho = 0.35\); (C) residual N intake in EL and residual DMI in EL, \(r = 0.87\), \(\rho = 0.82\); (D) residual N intake in ML and residual DMI in ML, \(r = 0.91\), \(\rho = 0.84\).]
### Table 1. Associations between quartiles of residual DM intake (RFI) in mid lactation and performance of cows in early lactation

<table>
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<th>Item, early lactation</th>
<th>RFI in mid lactation, quartiles</th>
<th>SEM</th>
<th>P-value</th>
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<tr>
<td></td>
<td>Q1 (−2.13 kg/d)</td>
<td>Q2 (−0.55 kg/d)</td>
<td>Q3 (+0.42 kg/d)</td>
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<tr>
<td>Cows, n</td>
<td>98</td>
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<td>RFI,* kg/d</td>
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<td>RNI,§ g/d</td>
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<td>BCS,* 1 to 5</td>
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</table>

¹Measurements taken from 1 to 5 wk postpartum. RNI = residual N intake.
²Residual DM intake was calculated in wk 9 to 15 postpartum based on the difference between observed and predicted DMI, and cows grouped into quartiles (Q1–Q4). Values in parentheses are the means of RFI for each quartile. Predicted DMI in mid lactation was calculated following a statistical model that considered NE₆ secreted as milk, metabolic BW, change in BW energy, parity, season of calving, and treatment within experiment.
³Body energy change was calculated as the NE₆ either accreted or lost as body tissue based on the daily change in BW adjusted for the BCS.
⁴Body N change was calculated assuming that each 1 kg of BW contains 21.6 g of N that was either accreted (BW gain) or released (BW loss).
⁵N efficiency was calculated based on the amount of dietary N intake recovered as N in milk and in body tissue.

### Table 2. Associations between quartiles of residual feed intake (RFI) in mid lactation and 305-d milk yield, incidence of disease, and survival of cows

<table>
<thead>
<tr>
<th>Item</th>
<th>RFI in mid lactation, quartiles</th>
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<td>Q1 (−2.13 kg/d)</td>
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<td>Cows, n</td>
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<td>305-d milk,² kg</td>
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<td>Diseases by 90 d postpartum, %</td>
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<td>Metritis</td>
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<td>Mastitis</td>
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<tr>
<td>Displaced abomasum</td>
<td>1.0</td>
</tr>
<tr>
<td>Lameness</td>
<td>10.2</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>2.0</td>
</tr>
<tr>
<td>Multiple diseases⁴</td>
<td>13.3</td>
</tr>
<tr>
<td>Morbidity⁵</td>
<td>37.8</td>
</tr>
<tr>
<td>Left herd by 300 d, %</td>
<td>10.2</td>
</tr>
</tbody>
</table>

¹Residual feed intake was calculated in wk 9 to 15 postpartum based on the difference between observed and predicted DMI, and cows grouped into quartiles (Q1–Q4). Values in parentheses are the means of RFI for each quartile. Predicted DMI in mid lactation was calculated following a statistical model that considered NE₆ secreted as milk, metabolic BW, change in BW energy, parity, season of calving, and treatment within experiment.
²Milk measured daily for the first 305 d in lactation. Cows that left the study before 305 d postpartum (n = 37) had the remainder of the lactation (from culling or death to 305 d) estimated using Wood’s lactation curve (Wood, 1967).
³Evaluated weekly in the first 15 wk postpartum.
⁴Multiple diseases: diagnosis of more than 1 type of disease in the first 90 d postpartum.
⁵Morbidity = diagnosis of at least 1 disease (milk fever, retained placenta, metritis, mastitis, displaced abomasum, lameness, or pneumonia) in the first 90 d postpartum.
animal’s rank of RFI ideally must be repeatable within and across lactations, and across different diets and production systems (Ginguina et al., 2020). The Pearson correlation between RFI in early and mid lactation was 0.43, and the Spearman’s correlation between quartiles in early and mid lactation was 0.32, suggesting that RFI in mid lactation is correlated with RFI in early lactation, although re-ranking of cows exists. In fact, studies have demonstrated phenotypic and genetic repeatability of RFI (Nieuwhof et al., 1992; Connor et al., 2013), and correlations have been observed across different stages of lactation and across different parity groups within the same individual cows (Hurley et al., 2018), across diets (Potts et al., 2015), and across production systems (Ginguina et al., 2020). A recent study conducted by Connor et al. (2019) reported a strong correlation ($r = 0.72$) between RFI estimated during the first 100 d with that estimated during 305 d postpartum, and a correlation of 0.37 between RFI when heifers around 10 to 14 mo of age and RFI of the
same animals in the first 100 d of the first lactation. Hurley et al. (2018) studied correlations of residual energy intake from 2,505 lactations originating from 1,290 grazing dairy cows across lactation stages and parity groups. They observed moderate correlations between residual energy intake estimated from 8 to 90 DIM with residual energy intake estimated from 91 to 180 (r = 0.19) or after 180 DIM (r = 0.23). When correlations of residual energy intake between lactations within the same cows were evaluated, they ranged from 0.13 to 0.23 (Hurley et al., 2018). Mäntysaari et al. (2012) reported correlations in 145 primiparous Nordic Red dairy cows between residual energy intake measured from 2 to 10 wk postpartum with residual energy intake estimated from 91 to 180 (r = 0.19) or after 180 DIM (r = 0.23). When correlations of residual energy intake between lactations within the same cows were evaluated, they ranged from 0.13 to 0.23 (Hurley et al., 2018). Mäntysaari et al. (2012) reported correlations in 145 primiparous Nordic Red dairy cows between residual energy intake measured from 2 to 10 wk postpartum with residual energy intake measured 11 to 20 wk postpartum (r = 0.47) or 21 to 30 wk postpartum (r = 0.30).

Selection for low DMI and high milk yield is desirable to improve feed efficiency (Tetens et al., 2014), so long as body tissue reserves, health, and reproduction are not compromised. The present results showed that cows in the most efficient quartile of RFI in mid lactation consumed 3.5 kg less DM in the first 15 wk of lactation than those in the least efficient quartile, a reduction of 15% without any changes in yields of milk, ECM, or milk components. It is well established that animals with negative RFI have less DMI, which has been shown for calves, heifers, lactating dairy cows, and beef cows (Richardson and Herd, 2004; Kelly et al., 2010; Williams et al., 2011; Connor et al., 2013, 2019). Nevertheless, it is important to note that the reductions in DMI observed herein, in either the first 5 or 15 wk of lactation, were unrelated to productive performance. In other words, the improvement in efficiency was caused by reduced DMI, suggesting that the more feed-efficient cows either have different requirements for maintenance or better extract or utilize nutrients for milk synthesis and maintenance of body tissues (Connor et al., 2013).

Residual DMI in mid lactation was correlated with RFI in early lactation. A potential concern about selecting cows for RFI is that reduced DMI in early lactation, which results in more negative NE\textsubscript{L} balance, might increase lipomobilization, which is often seen as detrimental to cow health. Stimulating early-lactation DMI is usually considered beneficial, because lower DMI in the first weeks postpartum is associated with
increased risk of some diseases (Pérez-Báez et al., 2019), and diseases themselves also are associated with lower DMI. It is no surprise that RFI has been reported to be strongly associated with NE\textsubscript{L} balance (r = 0.64; Seymour et al., 2020), because calculations of energy balance assume that the energy needs for maintenance and the efficiency of use of nutrients by dairy cows are fixed values; thus, cows that eat more for a given amount of milk or ECM produced should be in greater NE\textsubscript{L} balance than those that eat less. In fact, DM or NE\textsubscript{L} intake explains more than 50% of the variance in NE\textsubscript{L} balance in dairy cows (Santos et al., 2010). Because increments in DMI and NE\textsubscript{L} balance in early lactation have been associated with better health and reproduction in dairy cows (Butler, 2000; Pérez-Báez et al., 2019), concerns have been raised that improved feed efficiency using RFI in mid lactation might result in increased loss of BW or BCS in early lactation and negatively influence health in dairy cows (Dechow et al., 2017; Seymour et al., 2020). It is not uncommon to observe that cows in a prolonged negative NE\textsubscript{L} balance have increased risk of metabolic and infectious diseases in early lactation (Goff and Horst, 1997). Nevertheless, the current study demonstrated that measures of body energy and N mobilization in the first 5 wk of lactation did not differ according to quartile of RFI in mid lactation and the risk of common postpartum diseases or morbidity by 90 DIM, and survival up to 300 DIM was not associated with RFI in mid lactation. Interestingly, more feed-efficient cows in mid lactation had greater BCS throughout the first 105 DIM and had BW that did not differ from cows in the least efficient quartile of RFI. These differences were probably caused by the fact that cows in Q1 of RFI in mid lactation already had greater BCS and were heavier at calving than cows in Q4 of RFI. It is important to note that cows in the most efficient quartile for RFI had increased change in BW and body energy in the first 15 wk of lactation, which suggests either that they had less gut fill, resulting from less intake, or that they indeed mobilized more body tissue past the first 5 wk of lactation. From the present study, it is unknown whether body energy mobilization differences would perpetuate past 15 wk in lactation.

As expected, as RFI in early lactation increased, so did NE\textsubscript{L} balance in both primiparous and multiparous cows, which supports the notion that improving DMI benefits energy balance (Butler, 2000; Santos et al., 2010). Nevertheless, if nutritional cues that influence metabolism and reproduction in dairy cows depend on the energetic status of the animal (Butler, 2000), then changes in body energy might better reflect the energetic status of the cow because it accounts for changes in BW and BCS. Interestingly, the relationship between RFI and body energy changes in early lactation in primiparous and multiparous cows was nonexistent, suggesting that NE\textsubscript{L} balance might not accurately reflect how cows respond to diet or that dietary NE\textsubscript{L} content is not a static value and varies with individuals.

The fact that low-RFI cows had reduced DMI but no differences in yields of milk or milk components, milk energy output, incidence of diseases, or survival enhances the attractiveness of selection for low RFI. Several plausible mechanisms could explain this greater efficiency of nutrient use. Studies have demonstrated relationship between RFI and increased nutrient digest-
ibility (Nkrumah et al., 2006). Xi et al. (2015) grouped cows based on RFI into efficient (RFI = −0.84 kg/d) or inefficient (RFI = +0.86 kg/d) and indicated that the molecular control of expression of genes associated with hormonal regulation and lipid metabolism differs according to RFI ranking. The authors’ findings included downregulation of genes such as LEP (leptin), JAK (Janus kinase 1), LEPR (leptin receptor), STAT3 (signal transducer and activator of transcription 3), and SOCS3 (suppressor of cytokine signaling 3) and upregulation of NPY (neuropeptide Y) in cows in the efficient RFI group. Dechow et al. (2017) analyzed metabolites and hormones from 393 cows in the first 60 DIM and observed that more efficient cows, those with negative RFI, also had lower concentrations of triiodothyronine. Triiodothyronine is associated with body heat production; thus it is possible that improved efficiency in cows with negative RFI might reflect less heat increment and improved use of ME. Body reserves management, rumen temperature, feeding behavior, and activity also contributed to explain the variation observed in RFI in lactating dairy cows (Fischer et al., 2018). Only cows that survived up to 13 wk were included the analyses of RFI in mid lactation; otherwise it would not be possible to measure DMI and calculate RFI. Thus, cows that developed severe disease in early lactation and were removed from the original experiments with no data collected during mid lactation were not included in the study. It is unclear whether those cows would have affected the results of the association between RFI and risk of diseases observed in the present study.

Improving use of dietary N has well-demonstrated benefits to reduce atmospheric and hydrospheric pollution (Castillo et al., 2000) and farm profitability (Fadul-Pacheco et al., 2017). Efficiency of dietary N use should consider the N incorporated into milk protein, the N accreted or released from tissues, and the N required as part of amino acids required for maintenance (Spanghero and Kowalski, 1997). In this study, RNI was calculated to account for the major N sinks. Interestingly, our results indicated that RNI is a repeatable trait across early and mid lactation. Moreover, RNI and RFI were moderately correlated between early and mid lactation and strongly correlated within the same stage of lactation. Collectively, these results indicate that phenotypic selection of cows for improved feed efficiency, using RFI in mid lactation, will also result in improved RNI. In fact, the quartile of most efficient cows, based on RFI in mid lactation, were 11.4% more efficient in utilizing dietary N for milk protein and body N synthesis than cows in the least efficient quartile for RFI. Liu and VandeHaar (2020) demonstrated that RFI was associated with milk protein efficiency, expressed as dietary protein captured in milk, in Holstein cows during peak or late lactation. They also observed that milk protein efficiency was repeatable across lactation stages. Reasons for improved N efficiency in cows with negative RFI might include increased apparent digestibility of N (Rius et al., 2012), more efficient synthesis of microbial protein, better recycling of N to the gut for use by microbes, reduced catabolism of N by tissues, or improved efficiency of amino acid absorption and transfer to the mammary gland. Regardless of the exact mechanisms, improved N efficiency was expected because excretion of fecal and urinary N increases with N intake, and the effect of N intake on urinary N excretion increases substantially when cows consume more than 400 g of N/d (Castillo et al., 2000).

CONCLUSIONS

Rankings of RFI intake and RNI were correlated within and between stages of lactation, and, thus, cows that were more feed efficient also had increased N efficiency, and these responses were observed in both early and mid lactation. A potential concern with selection for RFI in mid lactation is that reduced DMI might affect BW and increase body energy loss in early lactation; however, those differences were not observed in the first 5 wk of lactation. Indeed, more feed-efficient cows were able to maintain BW and BCS in the first 15 wk postpartum that were equal to or greater than the values observed for cows with positive RFI. In spite of the large differences in DMI observed among quartiles of RFI, measurements of RFI in mid lactation were not associated with productive performance, risk of diseases, or survival in dairy cows. Collectively, the findings from this study indicate that phenotypic selection for RFI improves feed efficiency without deleterious effects on performance, health, and survival in dairy cows, and selection for RFI in mid lactation improves RFI and RNI in early lactation. Further research is warranted to understand the biological processes associated with RFI and its association with reproductive performance in dairy cows.

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