Relationship of residual feed intake and protein efficiency in lactating cows fed high- or low-protein diets

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ABSTRACT

Our objectives were to determine the repeatability of residual feed intake (RFI) across dietary protein levels and to determine the association between RFI and protein efficiency in lactating cows. Holstein cows ($n = 166$; 92 primiparous, 74 multiparous) with initial milk yield 41.3 ± 9.8 kg/d were fed diets with high or low protein in peak lactation. Experiments were conducted as crossovers with 2 treatment periods of 28 to 35 d. Production of 69 of the 166 cows (42 primiparous, 27 multiparous) was also measured in late lactation. Low-protein diets were 14% crude protein (CP) in peak lactation and 13% CP in late lactation and were formulated to contain adequate rumen-degradable protein to maintain rumen function. High-protein diets were 18% CP in peak lactation and 16% CP in late lactation and contained extra expeller soybean meal to increase absorbed protein. Cows were milked twice daily; DMI and milk yield were recorded daily. Milk composition was measured over 4 consecutive milkings weekly, and body weight (BW) was measured 3 times weekly. Fixed effects of diet, parity, and treatment period, interaction of parity and diet, and random effects of experiment and cow nested within experiment were included in the model to compare intake and production performance between cows fed different levels of CP. The RFI value was calculated for each cow on each treatment based on the actual intake, milk energy output, metabolic BW, and body energy (calculated from BW change and body condition score over the treatment period) change. Ranking of cows for RFI was moderately repeatable across dietary protein in peak lactation ($r = 0.59$) but less repeatable in late lactation ($r = 0.41$). Negative correlation was observed between RFI and protein efficiency values (dietary protein captured in milk) for cows in both peak lactation ($r = -0.42$) and late lactation ($r = -0.24$), which suggested that cows with higher energy efficiency had greater protein ef-

iciency. In conclusion, RFI was repeatable across dietary protein levels within lactation stage, and cows with lower RFI values utilized protein more efficiently.

Key words: protein efficiency, residual feed intake

INTRODUCTION

Given that 40% of feed cost can be attributed to protein (St-Pierre, 2012), much effort has been made to improve protein efficiency in dairy cows. Many nutritional means have been explored to improve protein efficiency, such as altering protein sources, supplementing with non-protein nitrogen or specific amino acids, and lowering dietary protein levels (Sinclair et al., 2014; Broderick et al., 2015; Gidlund et al., 2015). However, the efficiency of converting feed protein to milk protein is still less than 30% in the modern dairy (Huhtanen and Hristov, 2009). We wondered whether genetic means could be used to further improve protein efficiency.

One possible way to enhance protein efficiency is to select cows based on residual feed intake (**RFI**). Calculated as the difference between actual feed intake and predicted feed intake, RFI is considered a direct measurement of energy efficiency (Koch et al., 1963) and has drawn considerable attention in the genetic improvement of dairy cattle (Connor, 2015; VandeHaar et al., 2016). The RFI takes into account all production variables and avoids bias caused by body size or milk production level when comparing efficiency between cows (Pryce et al., 2012; Connor, 2015). The RFI is usually calculated on an energy basis, and, although we expect that lower RFI would be associated with greater protein efficiency, this has not been demonstrated.

We also wondered whether RFI ranking among cows is repeatable across diets with varying protein levels. In lactating dairy cattle, RFI is repeatable across diets with varying starch and NDF contents (Potts et al., 2015; Carrasquillo-Mangual et al., 2016). However, no studies have reported the RFI ranking of cows across dietary protein levels. Most diets in previous RFI studies contained adequate or even excessive protein (Potts et al., 2015; Tempelman et al., 2015; Carrasquillo-

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Mangual et al., 2016). If RFI is not repeatable across protein levels, using the RFI information from cows fed excessive protein to determine protein efficiency might be misleading. Lack of repeatability might especially be a problem if cows are fed diets that limit protein to minimize nitrogen excretion. Thus, the objective of this study was to examine the repeatability of RFI across diets with high or marginally deficient protein levels and the relationship between RFI and protein efficiency. We hypothesized that (1) RFI was relatively repeatable across dietary protein levels, and (2) cows with lower RFI values would have higher protein efficiency.

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

Experimental procedures were approved by the Institutional Animal Care and Use Committee of Michigan State University. Data from 166 cows in 11 separate crossover experiments, with 7 studies containing cows in peak lactation and 4 studies containing cows in late lactation, were used to determine the RFI and protein efficiency of individual cows across diets that were high (HP) and low (LP) in protein at different lactation stages. The LP diets were formulated to be marginally deficient in protein so that milk production would likely drop.

In the 7 experiments containing 166 peak-lactation cows, all cows were 50 to 130 DIM at the beginning of each experiment. For all 7 experiments, the 2 experimental periods lasted 28 to 35 d per period. Within each experiment, cows were blocked based on their parity and productivity (milk energy per unit of metabolic BW) and then randomly assigned to 1 of the 2 treatment sequences (HP-LP, or LP-HP). For cows in peak lactation the LP diets (**LP_{peak}**) contained 31% NDF, 32% starch, and 14% CP, and the HP diets (**HP_{peak}**) contained 29% NDF, 30% starch, and 18% CP. Both diets contained at least 9.8% RDP (DM basis) to maintain adequate rumen function (NRC, 2001). The extra protein of **HP_{peak}** was achieved by replacing soybean hulls and ground corn with expeller soybean meal (Table 1).

In the 4 experiments containing 69 late-lactation cows, DIM was 190 to 250 d at the beginning of each experiment. Intake, BW, and milk production of the 69 cows were measured in both peak and late lactation. For all the 4 experiments, the 2 experimental periods lasted 28 to 35 d per period. Within each experiment, cows were blocked based on their parity and milk energy per unit of metabolic BW, and then randomly assigned to 1 of the 2 treatment sequences (HP-LP, or LP-HP). For cows in late lactation, the LP diets

(**LP_{late}**) contained 40% NDF, 26% starch, and 13% CP, and the HP diets (**HP_{late}**) contained 38% NDF, 24% starch, and 16% CP. Both diets contained at least 9.0% RDP (DM basis) for rumen function. The extra protein of **HP_{late}** was achieved by replacing soybean hulls and ground corn with expeller soybean meal (Table 1).

All cows were housed in individual tiestalls and milked twice a day (0430 and 1530 h). Tiestalls were equipped with a double-cupped watering system to prevent contamination of feed with water and with side panels and a front gate to prevent other cows from stealing feed during cow movements. Water was available ad libitum. Cows were fed once a day (1200 h) at >110% of expected intake based on intake of the previous day, and orts were removed (1000 h) and weighed before feeding. Milk yield was recorded electronically at each milking, and milk samples were obtained from 4 consecutive milkings each week. Milk samples were analyzed for fat, protein, lactose, SCC, and MUN with infrared spectroscopy (AOAC, 1990; method 972.160) by Michigan DHIA (Grand Ledge, MI). Body weight for each cow was recorded 3 times per week, immediately after afternoon milking. At the beginning and end of each period, BCS was determined by 3 trained investigators and averaged for each cow on a 5-point scale, where 1 = thin and 5 = fat (Wildman et al., 1982).

Collection and analyses of diet ingredients were the same for all the experiments. During the last 5 d of experimental periods, samples of feed ingredients were obtained daily to determine the nutrient profile of the diets. All samples were frozen after collection until analysis. Samples were composited to obtain 1 sample per period and dried in a forced-air oven (57°C for >72 h) before grinding through a Wiley mill (5-mm and 1-mm screens; Arthur H. Thomas Co., Philadelphia, PA). Samples of feed were analyzed for CP, starch, NDF, and ether extract.

Calculations

Milk energy output (**MilkE**, Mcal/d) for individual cows was estimated using the following equation (NRC, 2001, Equation 2-15):

$$\text{MilkE} = [9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)}],$$

where each component was calculated as the average output of individual cows during the treatment period.

The milk-to-feed ratio for a cow during a period was determined as the average daily ECM yield [$\text{ECM} = 0.327 \times \text{milk (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{protein}$

(kg); Tyrrell and Reid, 1965] divided by the average daily DMI over the entire period.

For cows more than 190 d pregnant, BW was corrected for conceptus weight for use in the RFI equation and to calculate energy and protein change of body tissues. Conceptus weight (CW) was calculated using the following equation from NRC (2001):

$$CW = [18 + (D - 190) \times 0.665] \times (CBW/45),$$

where D was the day of gestation between 190 and 279, and CBW was the calf birth weight.

Metabolic BW (**MBW**) of a cow was estimated as $BW^{0.75}$, where BW was the mean measured BW for the cow during the treatment period.

Mean daily BW change (ΔBW , kg/d) was calculated for each cow within the treatment period by linear regression after 2 rounds of removing outliers in the data. An outlier was any BW >3.5 standard deviations (SD) from the regression line.

Energy expended for body tissue gain (ΔBodyE , Mcal/d) was estimated using an equation derived from NRC (2001, Table 2-5):

$$\Delta \text{BodyE} = (2.88 + 1.036 \times \text{BCS}) \times \Delta BW,$$

where BCS was the average BCS for a cow during the treatment period.

Energy expended for pregnancy (**PregE**, Mcal/d) was estimated using the following equation from NRC (2001, Equation 2-19):

$$\text{PregE} = [(0.00318 \times D - 0.0352) \times (CBW/45)]/0.218,$$

where D was the day of gestation between 190 and 279, and CBW was the calf birth weight.

Apparent diet energy content (DietNE_L , Mcal/kg) was calculated for each diet as the average NE_L re-

Table 1. Feed ingredients and nutrient composition of experimental diets¹

Item	Treatment			
	Peak lactation		Late lactation	
	HP _{peak}	LP _{peak}	HP _{late}	LP _{late}
Ingredient, % DM				
Corn silage	35.2	35.2	50.0	50.0
Alfalfa silage	15.7	15.7	18.2	18.2
Corn grain, ground	25.6	29.7	8.2	11.4
Soybean hulls	5.7	12.8	9.2	15.8
Soybean meal solvent extracted 48% CP	0.3	2.5	0.6	0.4
Expeller soybean meal ⁴	13.4	—	9.7	—
Vitamin and mineral mix ²	3.2	3.2	3.3	3.3
Urea	0.9	0.9	1.0	1.0
Forage:concentrate	51:49	51:49	68:32	68:32
Nutrient composition, % DM				
DM	47.1	47.1	37.9	37.9
NDF	29.4	31.3	38.0	40.2
Forage NDF	20.7	20.7	29.5	29.5
Starch	31.5	33.5	24.4	26.0
CP	18.0	14.3	15.9	12.8
RDP	10.3	9.8	10.0	9.0
RUP	7.7	4.5	6.0	3.5
Apparent NE_L ³ Mcal/kg	1.7	1.6	1.5	1.6

¹HP_{peak} and LP_{peak} were high- and low-protein diets fed to peak-lactation cows, and HP_{late} and LP_{late} were high- and low-protein diets fed to late-lactation cows. Experimental diets were fed to cows in crossover design with at least 28-d periods.

²Vitamin and mineral mix contained 24.8% ground corn grain, 21.5% dehydrated cane molasses, 11.2% limestone, 9.6% blood meal, 9.0% sodium bicarbonate, 6.6% dicalcium phosphate, 4.2% ReaShure Choline (Balchem Corp., New Hampton, NY), 3.1% magnesium sulfate, 2.8% salt, 2.0% vegetable oil, 1.5% niacin, 1.3% trace mineral mix, 0.95% biotin, 0.7% Yeast Plus (Chr. Hansen, Milwaukee, WI), 0.54% vitamin ADE premix, 0.32% selenium yeast, and 0.09% Rumensin 90 (Elanco, Animal Health, Indianapolis, IN).

³Mean apparent net energy concentration of diets (Diet NE_L), based on average cow performance. For each diet, Diet NE_L = the average of $(\text{MilkE} + 0.08 \times \text{MBW} + \Delta \text{BodyE})/\text{DMI}$ for all cows on the diet, where MilkE is net energy utilized for milk synthesis, MBW is metabolic body weight, and ΔBodyE is net energy captured in body tissue.

⁴SoyPlus, Landus Cooperative, Ames, IA.

quired by each cow divided by her average daily intake for the diet:

$$\text{DietNE}_L = \text{Average} [(\text{MilkE} + 0.08 \times \text{MBW} + \Delta\text{BodyE} + \text{PregE})/\text{DMI}],$$

where DMI was the average DMI for a cow during the treatment period.

Models and Statistics

The RFI for each cow on each diet was calculated as the residual term in the prediction of DMI. The DMI for an individual cow during each period was regressed as a function of major energy sinks using the GLM Procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). To define RFI for cows in peak lactation, DMI was modeled as follows:

$$\begin{aligned} \text{DMI} = & \beta_0 + \beta_1 \times \text{MilkE} + \beta_2 \times \text{MBW} \\ & + \beta_3 \times \Delta\text{BodyE} + \beta_4 \times \text{DIM} + \beta_5 \times \text{DIM} \\ & \times \text{DIM} + \beta_6 \times \text{BCS} + \text{Parity} \\ & + \text{Experiment} + \text{Cohort}(\text{Experiment}) \\ & + \text{Diet}(\text{Cohort} \times \text{Experiment}) + e, \end{aligned}$$

where DMI was the observed DMI, MilkE was the observed milk energy output, MBW was the average BW^{0.75}, ΔBodyE was the predicted change in body energy based on measured BW and BCS, DIM was the average DIM during each treatment period, and BCS was the average BCS during each treatment period. Parity (1 or 2+), experiment (1 through 7), cohort nested within experiment, and diet nested within cohort and experiment were fixed effects, where a cohort is a group of cows that ate the same diet at the same time. The RFI was defined as the residual term (e) in the model.

For cows in late lactation, DMI was modeled as follows:

$$\begin{aligned} \text{DMI} = & \beta_0 + \beta_1 \times \text{MilkE} + \beta_2 \times \text{MBW} \\ & + \beta_3 \times \Delta\text{BodyE} + \beta_4 \times \text{PregE} + \beta_5 \times \text{DIM} \\ & + \beta_6 \times \text{BCS} + \text{Parity} + \text{Experiment} \\ & + \text{Cohort}(\text{Experiment}) \\ & + \text{Diet}(\text{Cohort} \times \text{Experiment}) + e, \end{aligned}$$

where PregE was the energy expended for pregnancy, and RFI remained the residual term (e) in the model.

To determine the number of animals that changed their efficiency classification when they were switched from one diet to the other, cows were grouped into high- (HRFI), medium- (MRFI), and low- (LRFI) RFI groups. Cows >0.5 SD of the mean RFI for a cohort were classified as HRFI, cows < -0.5 SD were classified as LRFI, and those ± 0.5 SD were classified as MRFI.

Repeatability of RFI across dietary protein levels was calculated using Pearson correlation coefficients in the CORR procedure of SAS. Two RFI values within each lactation stage for each cow ($\text{RFI}_{\text{HPpeak}}$ vs. $\text{RFI}_{\text{LPpeak}}$; $\text{RFI}_{\text{HPlate}}$ vs. $\text{RFI}_{\text{LPplate}}$) were included in the analyses. To examine the RFI repeatability across lactation stages, 2 RFI values were calculated: RFI_{peak} and RFI_{late} , where RFI_{peak} was the average RFI across diets in peak lactation, and RFI_{late} was the average RFI across diets in late lactation. Pearson correlation coefficients for RFI_{peak} and RFI_{late} were calculated. To further examine the RFI repeatability across dietary protein levels, 2 RFI values were calculated: RFI_{high} and RFI_{low} , where RFI_{high} was the average RFI for high-protein diets across lactation stage, and RFI_{low} was the average RFI for low-protein diets across lactation stage. Pearson correlation coefficients for RFI_{high} and RFI_{low} were calculated. Correlation was considered as significant at $P \leq 0.05$, and trends were designated at $P \leq 0.10$.

For each cow in each diet, protein efficiency was calculated as dietary protein captured in milk protein (milk protein efficiency, **MPE**), and dietary protein captured in milk protein and body tissues (gross protein efficiency, **GPE**), respectively. Protein captured for body tissue gain (BodyPro, kg/d) was calculated using the following equations, which were derived as averages for BCS of 3.0 based on NRC (2001):

$$\text{BodyPro} = 0.12 \times \Delta\text{BW for primiparous cows};$$

$$\text{BodyPro} = 0.07 \times \Delta\text{BW for multiparous cows}.$$

To quantify the association among RFI, MPE, and GPE, Pearson correlation coefficients were obtained using the CORR procedure in SAS. Partial correlations accounting for effects of parity, cohort, and experiment were estimated using the PARTIAL option in the CORR procedure. To further determine the differences in protein efficiency between the most and least efficient cows, cows with different RFI for each diet (HP or LP) in each lactation stage across all 11 experiments were compared. The effect of RFI was determined using the GLM procedure of SAS, according to the model $Y_i = \mu + R_i + e$, where Y_i is the dependent variable, μ was the overall mean, R_i was the fixed effect of RFI group, and e was the residual error.

Production, efficiency, and energy partitioning responses to diets with each lactation stage were analyzed using the MIXED procedure in SAS, with fixed effects of diet, parity, and period, interaction of parity and diet, and the random effects of experiment and cow nested within experiment. Significance was considered at $P \leq 0.05$ and tendency at $P \leq 0.10$. Interactions were considered significant at $P \leq 0.10$ and trends at $P \leq 0.15$.

RESULTS

Animal Performance

Cows fed low protein, in general, ate less, produced less milk, and gained less BW than cows fed high protein, in both peak and late lactations. As shown in Tables 2 and 3, LP_{peak} decreased DMI ($P < 0.01$), milk yield ($P < 0.01$), milk fat yield ($P < 0.01$), milk protein yield ($P < 0.01$), milk lactose yield ($P < 0.01$), milk protein percentage ($P < 0.01$), milk lactose percentage ($P < 0.01$), and MUN ($P < 0.01$). For these cows, LP_{peak} also decreased BW ($P < 0.01$), BW gain ($P < 0.01$), BCS ($P = 0.04$), and change in BCS ($P = 0.06$). In peak lactation, LP_{peak} also decreased ECM per kilogram of DMI ($P < 0.01$), milk energy ($P < 0.01$), and estimated retained energy ($P < 0.01$). As shown in Tables 4 and 5, LP_{late} decreased DMI ($P < 0.01$), milk yield ($P < 0.01$), 3.5% FCM ($P < 0.01$), milk fat yield

($P < 0.01$), milk protein yield ($P < 0.01$), milk lactose yield ($P < 0.01$), and MUN ($P < 0.01$). For these cows, LP_{late} also decreased BW ($P < 0.01$), non-pregnant BW ($P < 0.01$), BW gain ($P < 0.01$), non-pregnant BW gain ($P < 0.01$), and BCS ($P = 0.04$). We further found that LP_{late} also decreased ECM per kilogram of DMI ($P < 0.01$), milk energy ($P < 0.01$), maintenance energy ($P < 0.01$), and estimated retained energy ($P < 0.01$).

Primiparous cows in general ate less and produced less milk but with greater milk component concentration, in both peak and late lactations. As shown in Tables 2 and 3, among peak-lactation cows, compared with multiparous cows, primiparous cows had lower DMI ($P < 0.01$), milk yield ($P < 0.01$), FCM ($P < 0.01$), milk fat yield ($P < 0.01$), milk protein yield ($P < 0.01$), MUN ($P < 0.01$), and milk lactose yield ($P < 0.01$), with higher milk protein percentage ($P = 0.05$) and milk lactose percentage ($P < 0.01$). Primiparous cows also had less milk energy ($P < 0.01$) and maintenance energy ($P < 0.01$) compared with multiparous cows. As shown in Tables 4 and 5, among all late-lactation cows, compared with multiparous cows, primiparous cows had higher milk fat yield ($P = 0.08$), milk fat percentage ($P = 0.02$), and milk lactose percentage ($P < 0.01$). Primiparous cows also had lower BW ($P < 0.01$), non-pregnant BW ($P < 0.01$), BCS ($P < 0.01$), change in BW ($P = 0.07$), and maintenance energy ($P < 0.01$) compared with multiparous cows.

Table 2. Dry matter intake, milk production, milk components, and feed efficiency for cows fed experimental diets in peak lactation¹

Item	Treatment ²			Parity ³			P-value ⁴		
	HP _{peak} n = 166	LP _{peak} n = 166	SEM	Primi. n = 184	Multi. n = 148	SEM	TRT	Parity	TRT × parity ⁵
DMI, kg/d	24.3	23.3	0.14	21.3	26.3	0.35	<0.01	<0.01	0.86
Milk yield, kg/d									
Milk	41.2	37.3	0.23	33.7	44.7	0.96	<0.01	<0.01	0.01
ECM ⁶	41.0	37.2	0.53	34.8	43.3	0.87	<0.01	<0.01	0.19
3.5% FCM ⁷	40.6	36.7	0.25	33.7	43.7	0.84	<0.01	<0.01	0.16
Milk components									
Fat, kg/d	1.41	1.27	0.01	1.18	1.50	0.03	<0.01	<0.01	0.59
Fat, %	3.46	3.49	0.02	3.53	3.43	0.06	0.13	0.13	0.30
Protein, kg/d	1.21	1.07	0.01	1.01	1.28	0.02	<0.01	<0.01	0.18
Protein, %	2.97	2.94	0.01	2.99	2.91	0.03	<0.01	0.05	0.07
Lactose, kg/d	2.07	1.84	0.01	1.71	2.20	0.05	<0.01	<0.01	0.01
Lactose, %	5.01	4.99	0.01	5.07	4.94	0.02	0.01	<0.01	0.14
MUN, mg/dL	15.1	9.2	0.13	11.6	12.7	0.20	<0.01	<0.01	0.16
ECM/DMI	1.70	1.62	0.03	1.65	1.66	0.03	<0.01	0.87	0.37

¹Average DIM was 125 for primiparous cows in HP_{peak} diets, 126 for primiparous cows in LP_{peak} diets, 122 for multiparous cows in HP_{peak} diets, and 121 for multiparous cows in LP_{peak} diets. Average parity for multiparous cows was 2.94 in peak lactation.

²Treatments contained 18% CP (HP_{peak}) and 14% CP (LP_{peak}) on a DM basis for peak-lactation cows.

³Primi. = primiparous cows; multi. = multiparous cows.

⁴P-value associated with treatment differences (HP_{peak} vs. LP_{peak}; TRT) and parity differences (primi. vs. multi.; parity) in peak-lactation cows.

⁵Values within each TRT × parity interaction are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17567>).

⁶ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)]; Tyrrell and Reid (1965).

⁷3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

Table 3. Body weight, body condition score, and calculated energy values for cows fed experimental diets in peak lactation

Item	Treatment ¹			Parity ²			P-value ³		
	HP _{peak} n = 166	LP _{peak} n = 166	SEM	Primi. n = 184	Multi. n = 148	SEM	TRT	Parity	TRT × parity
BW, kg	658	653	1.04	596	714	8.86	<0.01	<0.01	0.16
BCS, unit	3.23	3.19	0.02	3.23	3.20	0.05	0.04	0.41	0.54
Change in BW, ⁴ kg/d	0.57	0.20	0.10	0.38	0.40	0.10	<0.01	0.88	0.23
Change in BCS, unit/28 d	0.07	0.02	0.03	0.04	0.05	0.03	0.06	0.83	0.10
Calculated energy values ⁵									
Apparent NE _L of diet, Mcal/kg	1.74	1.58	0.64	1.67	1.65	0.85	<0.01	0.61	0.39
Apparent NE _L , Mcal/d	42.1	36.7	0.03	35.4	43.4	0.03	<0.01	<0.01	0.51
Milk, Mcal/d	28.1	25.1	0.18	23.4	29.9	0.57	<0.01	<0.01	0.15
Body tissue gain, Mcal/d	3.64	1.26	0.63	2.38	2.52	0.66	<0.01	0.82	0.30
Maintenance, Mcal/d	10.4	10.3	0.01	9.6	11.0	0.10	<0.01	<0.01	0.10

¹Treatments contained 18% CP (HP_{peak}) and 14% CP (LP_{peak}) on a DM basis for peak-lactation cows.

²Primi. = primiparous cows; multi. = multiparous cows.

³P-value associated with treatment differences (HP_{peak} vs. LP_{peak}; TRT) and parity differences (primi vs. multi.; parity) in peak-lactation cows.

⁴Determined by linear regression using BW measurements throughout the period.

⁵Milk (MilkE) = [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)]; Body tissue gain (ΔBodyE) = [(2.88 + 1.036 × BCS) × ΔBW]; maintenance = 0.08 × MBW, where MBW = metabolic body weight, BW^{0.75}.

Repeatability of RFI Across Dietary Protein Levels and Lactation Stages

In the RFI model among peak-lactation cows, the coefficients for the major energy sinks were 0.44 ($P < 0.01$) for MilkE, 0.06 ($P < 0.01$) for MBW, and 0.03 ($P = 0.03$) for ΔBodyE. The model coefficient of determination (R^2) and root mean square error were 0.87 and 1.50, respectively. In the RFI model among late-

lactation cows, the coefficients for the major energy sinks were 0.37 ($P < 0.01$) for MilkE, 0.08 ($P < 0.01$) for MBW, 0.05 ($P = 0.13$) for ΔBodyE, and -0.04 ($P = 0.83$) for PregE. The model R^2 and root mean square error were 0.80 and 1.31, respectively. Further details are shown in Table 6.

Regarding RFI repeatability, in general, cows with lower RFI values when fed HP diets still had low RFI when switched to LP diets. In peak lactation, RFI was

Table 4. Dry matter intake, milk production, milk components and feed efficiency for cows fed experimental diets in late lactation¹

Item	Treatment ²			Parity ³			P-value ⁴		
	HP _{late} n = 69	LP _{late} n = 69	SEM	Primi. n = 84	Multi. n = 54	SEM	TRT	Parity	TRT × parity ⁵
DMI, kg/d	19.8	18.4	0.20	18.0	20.2	0.48	<0.01	0.32	<0.01
Milk yield, kg/d									
Milk	25.1	22.2	0.42	24.2	23.1	1.37	<0.01	0.42	0.06
ECM ⁶	27.8	24.4	0.45	27.2	25.1	1.53	<0.01	0.18	0.02
3.5% FCM ⁷	26.8	23.7	0.43	26.3	24.2	1.42	<0.01	0.15	0.03
Milk components									
Fat, kg/d	0.98	0.86	0.02	0.98	0.87	0.06	<0.01	0.08	0.02
Fat, %	3.92	4.05	0.03	4.13	3.85	0.12	<0.01	0.02	0.79
Protein, kg/d	0.80	0.58	0.01	0.77	0.72	0.04	<0.01	0.25	<0.01
Protein, %	3.23	3.21	0.01	3.23	3.21	0.04	0.34	0.79	0.10
Lactose, kg/d	1.21	1.05	0.02	1.21	1.06	0.14	<0.01	0.05	0.03
Lactose, %	4.79	4.79	0.03	4.99	4.58	0.05	0.90	<0.01	0.72
MUN, mg/dL	12.1	8.1	0.16	9.9	10.2	0.27	<0.01	0.28	0.25
ECM/DMI	1.41	1.32	0.02	1.51	1.22	0.06	<0.01	<0.01	0.06

¹Average DIM was 258 for primiparous cows in HP_{late} diets, 257 for primiparous cows in LP_{late} diets, 263 for multiparous cows in HP_{late} diets, and 264 for multiparous cows in LP_{late} diets. Average parity for multiparous cows was 3.12 in late lactation.

²Treatments contained 16% CP (HP_{late}) and 13% CP (LP_{late}) on a DM basis for late-lactation cows.

³Primi. = primiparous cows; multi. = multiparous cows.

⁴P-value associated with treatment differences (HP_{late} vs. LP_{late}; TRT) and parity differences (primi vs. multi.; parity) in late-lactation cows.

⁵Values within each TRT × parity interaction are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17567>).

⁶ECM = [(0.327 × kg of milk) + (12.95 × kg of milk fat) + (7.20 × kg of milk protein)]; Tyrrell and Reid (1965).

⁷3.5% FCM = [(0.4324 × kg of milk) + (16.216 × kg of milk fat)].

Table 5. Body weight, BCS, and calculated energy values for cows fed experimental diets in late lactation

Item	Treatment ¹			Parity ²			P-value ³		
	HP _{late} n = 69	LP _{late} n = 69	SEM	Primi. n = 84	Multi. n = 54	SEM	TRT	Parity	TRT × parity ⁴
BW, kg	702	693	1.52	623	772	14.5	<0.01	<0.01	0.98
Non-pregnant BW, kg	694	679	5.12	616	757	14.9	<0.01	<0.01	0.07
BCS, unit	3.62	3.56	0.03	3.44	3.74	0.09	0.04	<0.01	0.94
Change in BW, ⁵ kg/d	0.67	0.09	0.09	0.29	0.47	0.09	<0.01	0.07	0.18
Change in non-pregnant BW, kg/d	0.43	−0.05	0.09	0.14	0.23	0.09	<0.01	0.33	0.09
Change in BCS, unit/28 d	0.07	−0.45	0.37	−0.01	−0.37	0.37	0.16	0.34	0.14
Calculated energy values ⁶									
Apparent NE _L of diet, Mcal/kg	1.64	1.46	0.04	1.62	1.48	0.04	<0.01	<0.01	0.06
Apparent NE _L , Mcal/d	32.3	26.6	0.77	29.2	29.8	1.01	<0.01	0.55	0.05
Milk, Mcal/d	18.4	16.0	0.31	18.1	16.3	1.04	<0.01	0.09	0.02
Body tissue gain, Mcal/d	2.82	−0.34	0.60	0.89	1.59	0.61	<0.01	0.26	0.12
Maintenance, Mcal/d	10.8	10.6	0.06	9.9	11.5	0.18	<0.01	<0.01	0.09
Pregnancy, Mcal/d	0.34	0.37	0.11	0.27	0.45	0.13	0.77	0.17	0.72

¹Treatments contained 16% CP (HP_{late}) and 13% CP (LP_{late}) on a DM basis for late-lactation cows.

²Primi. = primiparous cows; multi. = multiparous cows.

³P-value associated with treatment differences (HP_{late} vs. LP_{late}; TRT) and parity differences (primi vs. multi.; parity) in late-lactation cows.

⁴Values within each TRT × parity interaction are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17567>).

⁵Determined by linear regression using BW measurements throughout the period.

⁶Milk (MilkE) = $[9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)}]$. Body tissue gain (ΔBodyE) = $[(2.88 + 1.036 \times \text{BCS}) \times \Delta\text{BW}]$, where ΔBW = change in BW. Maintenance = $0.08 \times \text{MBW}$, where MBW = metabolic BW ($\text{BW}^{0.75}$).

moderately repeatable across HP and LP diets ($r = 0.59$, $P < 0.01$). Figure 1 illustrates the relationship between RFI in HP_{peak} and RFI in LP_{peak}. In late lactation, RFI was less repeatable across protein levels than it was in peak lactation ($r = 0.41$, $P = 0.03$). Figure 2 illustrates the relationship between RFI in HP_{late} and RFI in LP_{late}. A moderate level of correlation between RFI_{high} and RFI_{low} was observed ($r = 0.51$, $P < 0.01$). The Pearson correlation coefficient across peak and late lactation was 0.52 ($P < 0.01$) for DMI, 0.05 ($P = 0.67$)

for MilkE, 0.05 ($P = 0.67$) for ΔBodyE , and 0.91 ($P < 0.01$) for MBW.

Based on the data from 69 cows examined in both peak and late lactations, the repeatability of RFI was low across lactation stage for the HP diets ($r = 0.11$, $P = 0.39$) but moderate across lactation stage for the LP diets ($r = 0.28$, $P = 0.02$). The correlation between RFI_{peak} and RFI_{late} for both diets combined was moderate ($r = 0.25$, $P = 0.04$). Further details are shown in Table 7 and Figure 3.

Table 6. Partial regression coefficients of the RFI models in peak- and late-lactation cows

Item	Peak lactation					Late lactation				
	Coefficient	SEM	P-value	Contribution to DMI		Coefficient	SEM	P-value	Contribution to DMI	
				Mean	SD				Mean	SD
Intercept	−3.22	2.48	0.18			2.26	2.46	0.36		
MilkE ¹	0.44	0.03	<0.01	10.8	2.18	0.37	0.03	<0.01	6.51	1.73
MBW ²	0.06	0.01	<0.01	8.99	0.85	0.08	0.02	<0.01	10.7	1.15
ΔBodyE ³	0.03	0.02	0.03	0.07	0.18	0.05	0.04	0.13	0.07	0.24
PregE ⁴						−0.04	0.19	0.83	0.08	0.19
BCS	0.45	0.31	0.16			−0.19	0.44	0.68		
Parity	−1.06	0.28	<0.01			−1.08	0.41	<0.01		
DIM	0.07	0.03	0.02			0.03	0.007	<0.01		
DIM × DIM	−0.0002	0.0001	0.04							
Experiment	0.13 to 3.03					−1.09 to −0.49				
Cohort	−1.49 to 1.65					−0.92 to −0.22				

¹MilkE = milk energy (Mcal/d) = $[9.29 \times \text{fat (kg)} + 5.63 \times \text{true protein (kg)} + 3.95 \times \text{lactose (kg)}]$.

²MBW = metabolic BW (kg) = $\text{BW}^{0.75}$.

³Energy utilized in body tissue gain (Mcal/d) = $[(2.88 + 1.036 \times \text{BCS}) \times \Delta\text{BW}]$.

⁴Pregnancy energy (Mcal/d) = $[(0.00318 \times \text{D} - 0.0352) \times (\text{CBW}/45)]/0.218$, where D was day of gestation between 190 and 279, and CBW was the calf birth weight.

RFI and Protein Efficiency

Overall, cows with lower RFI values exhibited higher protein efficiency. For cows in peak lactation, the Pearson correlation coefficient between RFI and milk protein efficiency was -0.59 ($P < 0.01$) in HP_{peak} diets and -0.41 ($P < 0.01$) in LP_{peak} diets. For cows in late lactation, the Pearson correlation coefficient between RFI and milk protein efficiency was -0.36 ($P = 0.02$) in HP_{late} diets and -0.13 ($P = 0.34$) in LP_{late} diets. The correlation coefficient between RFI and milk protein efficiency across diets was -0.42 ($P < 0.01$; Figure 4) among peak-lactation cows and -0.24 ($P = 0.06$; Figure 5) among late-lactation cows. Similar associations between RFI and gross protein efficiency were also observed in the current study, as MPE and GPE were highly correlated in both peak lactation ($r = 0.83$, $P < 0.01$) and late lactation ($r = 0.89$, $P < 0.01$).

As shown in Table 8, cows with lower RFI values during peak lactation had higher milk protein efficiency and gross protein efficiency regardless of protein con-

tent in the diet; however, cows with lower RFI values did not necessarily have lower MUN concentration. Similar trends were observed in cows fed HP diets in late lactation. In contrast, when fed LP diets, cows with lower RFI in late lactation did not exhibit greater protein efficiency (MPE, GPE) nor MUN.

DISCUSSION

Animal Performance Across Dietary Protein Levels and Lactation Stages

Overall, cows had lower feed intake and milk production when fed LP diets, regardless of lactation stage. Production differences between HP and LP cows were most likely the result of additional RUP supplementation in HP diets and, thus, inadequate metabolizable protein in the LP diets. The LP diets also decreased gains in BW and BCS. Significantly lower gains in BW than in body condition might be due to the slightly lower intakes of LP cows or might indicate that cows

Figure 1. Repeatability of residual feed intake (RFI) across dietary protein levels in peak-lactation cows ($n = 166$). Repeatability of RFI across dietary protein levels was $r = 0.59$. RFI on low-protein diets could be predicted using RFI on high-protein diets as $Y = 0.628 (\pm 0.062; P < 0.01) \times X - 0.000 (\pm 0.09)$. Each data point represents 1 cow's RFI value on each diet ($n = 166$). Open circles indicate primiparous cows ($n = 92$), and filled triangles indicate multiparous cows ($n = 74$).

Table 7. Repeatability of residual feed intake (RFI) across protein levels within lactation stage and across lactation stages (n = 69)

Item ¹	RFI _{LPpeak}	RFI _{peak} ²	RFI _{high} ³	RFI _{HPlate}	RFI _{LPate}	RFI _{late} ⁴	RFI _{low} ⁵
RFI _{HPpeak}	0.59	0.84	0.77	0.11	0.11	0.13	0.40
	<0.01	<0.01	<0.01	0.39	0.39	0.31	<0.01
RFI _{LPpeak}		0.87	0.46	0.20	0.28	0.28	0.86
		<0.01	<0.01	0.10	0.02	0.02	<0.01
RFI _{peak} ²			0.84	0.23	0.23	0.25	0.73
			<0.01	0.06	0.05	0.04	<0.01
RFI _{high} ³				0.76	0.34	0.70	0.51
				<0.01	<0.01	<0.01	<0.01
RFI _{HPate}					0.41	0.85	0.36
					<0.01	<0.01	<0.01
RFI _{LPate}						0.82	0.73
						<0.01	<0.01
RFI _{late} ⁴							0.64
							<0.01

¹Upper values within a row = Pearson correlation coefficient of the linear relationship between 2 variables. Lower values within a row = *P*-value associated with the linear relationship between 2 variables.

²Averaged RFI across the diets fed to cows in peak lactation.

³Averaged RFI in high-protein (HP) diets across lactation stages.

⁴Averaged RFI across the diets fed to cows in late lactation.

⁵Averaged RFI in low-protein (LP) diets across lactation stage.

Figure 2. Repeatability of residual feed intake (RFI) across dietary protein levels in late-lactation cows (n = 69). Repeatability of RFI across dietary protein levels was $r = 0.41$. RFI on low-protein diets could be predicted using RFI on high-protein diets as $Y = 0.387 (\pm 0.101; P < 0.01) \times X - 0.000 (\pm 0.14)$. Each data point represents 1 cow's RFI value on each diet (n = 69). Open circles indicate primiparous cows (n = 42), and filled triangles indicate multiparous cows (n = 27).

tended to gain less body protein than fat when fed LP diets.

With a similar decrease in dietary protein content, the decrease of feed intake and milk production was similar between peak- and late-lactation cows; however, late-lactation cows tended to lose more non-pregnant BW and BCS than peak-lactation cows (Tables 3 and 5). We suggest that nutrients were prioritized to pregnancy and milk synthesis instead of body tissue gain when protein was limiting in late lactation. Indeed, Bauman and Currie (1980) found that the priority of nutrient partitioning in cattle was pregnancy, followed by milk production, and lastly body reserve gain; our data were consistent with this idea.

Repeatability of RFI Across Protein Levels and Lactation Stages

Although production was significantly altered by the diets, RFI within cows was still repeatable across dietary protein levels within each lactation stage. The

existing literature on RFI repeatability has predominantly focused on peak-lactation cows. The current study supports previous studies on RFI repeatability and extends RFI repeatability across diets to late-lactation cows. Among peak-lactation cows, the moderate level of RFI repeatability found in the current study (0.59) was in line with the findings of previous RFI repeatability studies, where RFI was repeatable across starch contents (0.73; Potts et al., 2015) and forage NDF contents (0.54; Carrasquillo-Mangual et al., 2016). According to Richardson and Herd (2004), the major contributor to the variation of RFI in cattle is “tissue metabolism and protein turnover.” We expect that treatments altering these processes might significantly alter RFI and therefore alter RFI repeatability. Wessels et al. (1997) showed that supplementing amino acids alters protein turnover. Thus, the lower level of RFI repeatability in the current study, compared with the findings of Potts et al. (2015), might be related to the expected changes in protein metabolism when altering dietary protein. Lower RFI repeatability across

Figure 3. Repeatability of residual feed intake (RFI) across lactation stages in lactating cows ($n = 69$). Repeatability of RFI across lactation stages was $r = 0.25$. RFI in late-lactation cows could be predicted using RFI in peak-lactation cows as $Y = 0.198 (\pm 0.097; P = 0.02) \times X - 0.009 (\pm 0.12)$. Each data point represents 1 cow's RFI value in each stage ($n = 69$). Open circles indicate primiparous cows ($n = 42$), and filled triangles indicate multiparous cows ($n = 27$).

dietary protein levels in late lactation, compared with peak lactation, was expected due to the uncertainty of pregnancy weight gain, which will be further discussed.

Repeatability of RFI across physiological state, as across lactation stage in the current study, has been reported previously. Phenotypic correlation of RFI was low, either when compared across weaned beef heifers later tested as lactating cows (Archer et al., 2002) or when estimated in growing dairy heifers that were later tested during lactation (Nieuwhof et al., 1992; Williams et al., 2011; Waghorn et al., 2012). The work done by Liinamo et al. (2015) and Li et al. (2017) demonstrated that genetic RFI values estimated from various lactation stages were different, and the difference was extremely evident when comparing the RFI estimated from early lactation with that estimated from late lactation. Although the DMI was moderately repeatable ($r = 0.52$) across lactation stages in the current study, given the low repeatability of the major energy sinks, especially MilkE, the low RFI repeatability was fully

expected. The low RFI repeatability across lactation stage could be due to the following reasons: (1) mechanisms controlling energy efficiency (or partitioning) shifted as lactation proceeded, and (2) our estimates of body energy change were not accurate and were altered by lactation stage. Throughout lactation, dairy cows undergo physiological changes, including (1) body reserve mobilization in early lactation, (2) body tissue replenishment in mid-lactation, and (3) storage of extra body fat in late lactation. We used BW change and BCS to predict energy change; however, BW change also included change in gut content and pregnancy gain, and body composition could not be fully represented by BCS. Practically, RFI is an adjusted DMI after accounting for energy partitioning to milk, body tissue gain, maintenance, and pregnancy (in late lactation); thus, any errors in estimating these energy sinks can introduce errors in calculating RFI.

Errors in BW change could introduce significant bias in the RFI estimation (Potts et al., 2015). This

Figure 4. Association between residual feed intake (RFI) and milk protein efficiency (MPE) in peak-lactation cows ($n = 332$). In peak-lactation cows, correlation between RFI and MPE across high- and low-protein diets was -0.42 . The correlation between RFI and MPE in high-protein diets was -0.59 [$MPE = -0.96 (\pm 0.14; P < 0.01) \times RFI + 27.5 (\pm 0.20; P < 0.01)$], and the correlation between RFI and MPE in low-protein diets was -0.41 [$MPE = -1.34 (\pm 0.14; P < 0.01) \times RFI + 32.3 (\pm 0.21; P < 0.01)$]. Each data point represents 1 cow's RFI value on each diet. Open triangles indicate cows on high-protein diets ($n = 166$), and filled squares indicate cows on low-protein diets ($n = 166$).

Table 8. Protein efficiency and MUN of high-, medium-, and low-residual feed intake (RFI) cows fed high- and low-protein diets in peak and late lactation

Variable	High-protein diet ¹				Low-protein diet ¹			
	HRFI	MRFI	LRFI	P-value ²	HRFI	MRFI	LRFI	P-value
Peak lactation	n _{peak} = 47	n _{peak} = 80	n _{peak} = 39		n _{peak} = 49	n _{peak} = 73	n _{peak} = 44	
MPE, ³ %	25.9	27.5	29.1	<0.01	30.5	32.1	34.6	<0.01
GPE, ⁴ %	26.9	28.7	30.9	<0.01	31.4	32.6	34.9	<0.01
MUN, mg/dL	14.7	15.1	15.3	0.76	9.1	9.1	9.5	0.47
Late lactation	n _{late} = 19	n _{late} = 28	n _{late} = 22		n _{late} = 22	n _{late} = 26	n _{late} = 21	
MPE, %	24.8	26.0	29.0	0.01	27.9	30.2	29.6	0.27
GPE, %	25.8	27.1	30.5	<0.01	27.9	29.7	30.8	0.29
MUN, mg/dL	12.1	12.3	12.1	0.65	8.2	8.1	8.2	0.78

¹Cows were grouped into high- (HRFI), medium- (MRFI), and low-RFI (LRFI) groups. Cows >0.5 SD of the mean RFI for a cohort were classified as HRFI, cows <-0.5 SD were classified as LRFI, and those ±0.5 SD were classified as MRFI.

²P-value associated with group difference.

³MPE = milk protein efficiency, defined as the dietary protein captured in milk.

⁴GPE = gross protein efficiency, defined as dietary protein captured in milk and body tissue.

becomes especially important when estimating RFI for late-lactation cows. Change in BW among late-lactation cows was calculated from adjusted BW after

deducting conceptus weight from measured BW. Given the difficulty in getting a precise estimate of conceptus weight, BW change in late-lactation cows could not

Figure 5. Association between residual feed intake (RFI) and milk protein efficiency (MPE) in late-lactation cows (n = 138). In late-lactation cows, correlation between RFI and MPE across high- and low-protein diets was -0.24. The correlation between RFI and MPE in high-protein diets was -0.36 [MPE = -1.27 (±0.42; *P* < 0.01) × RFI + 26.5 (±0.58; *P* < 0.01)], and the correlation between RFI and MPE in low-protein diets was -0.13 [MPE = -0.71 (±0.55; *P* = 0.20) × RFI + 29.4 (±0.68; *P* < 0.01)]. Each data point represents 1 cow's RFI value on each diet. Open triangles indicate cows on high-protein diets (n = 138), and filled squares indicate cows on low-protein diets (n = 138).

be quantified as accurately as it was in peak-lactation cows. Therefore, more errors could be introduced in estimating RFI among late-lactation cows. Additionally, the difficulty in assessing conceptus weight can also contribute to errors in estimating energy utilized in pregnancy. As errors were introduced in the 2 primary energy sinks, we expected that the estimated value of RFI would be less accurate among late-lactation cows. Indeed, due to the difficulty of estimating BW change, Prendiville et al. (2011) advised estimating RFI based on data between DIM 150 and DIM 230, when tissue gain or loss was minimal, to generate the most accurate estimates of RFI.

RFI and Protein Efficiency

No prior study has directly examined the relationship between RFI and protein efficiency among lactating Holstein cows; however, the relationship between RFI and protein efficiency in growing heifers has been examined. Rius et al. (2012) observed no difference in nitrogen efficiency between 2 groups of Holstein-Friesian heifers with divergent RFI values. Following that, the work done by Thornhill et al. (2014) and Marett et al. (2017) further showed that cows selected for lower RFI as calves or heifers did not have higher nitrogen efficiency in the subsequent lactation. In contrast with the heifer studies, the results of the current study suggested that RFI is strongly associated with protein efficiency in peak-lactation cows and also in late-lactation cows when protein is not limiting.

Xi et al. (2016) and Carrasquillo-Mangual et al. (2016) speculated that lactating cows with lower RFI values might have higher protein efficiency, as indicated by the lower MUN values in their low-RFI cows. Prior work showed that protein turnover rates could be negatively associated with protein utilization efficiency in dairy cows (Herd et al., 2004; Castro Bulle et al., 2007). Previous study also suggested that higher protein turnover rates were related to higher RFI values in cattle (Richardson et al., 2004). Based on these prior works, we expected a negative association between RFI and protein efficiency. Indeed, the current study directly proves that this negative relationship exists in most cases, unless protein is limiting for pregnant cows. This poor correlation could be due to the nutrient repartitioning to pregnancy when protein was limiting in pregnant cows. Therefore, when pregnancy does not take priority over milk synthesis, cows with lower RFI should utilize protein more efficiently.

The moderate correlation between RFI and protein efficiency provides a new means to genetically improve protein efficiency in dairy cattle. Although a moderate level of heritability for protein efficiency has been

shown to exist in lactating dairy cows (0.10 to 0.31; Li et al., 1998; Zamani et al., 2011), directly selecting dairy cows based on ratio traits (e.g., protein efficiency) has drawbacks. Ratio traits are usually not normally distributed. As a result, it is difficult to expect the selection response due to the disproportionate selection pressure on the component traits (Zetouni et al., 2017). In other words, using ratio traits (e.g., the protein efficiency term) in genetic selection induces large error variance and unexpected results. In contrast, RFI, as a residual term, overcomes all the drawbacks of ratio traits and is favorable in cow selection. However, due to the complexity of collecting individual intake data, estimating RFI is still difficult in dairy cows.

CONCLUSIONS

Low-protein diets significantly decreased feed intake, milk production, BW, energy captured in milk and body tissue, and feed efficiency in both peak- and late-lactation cows. Within each lactation stage, RFI was moderately repeatable across dietary protein levels; similarly, average RFI in HP and LP diets across lactation stage was also moderately repeatable. Thus, we expect that cows with lower RFI when fed diets with adequate protein, as is typical for North America, will still have lower RFI when fed diets marginally deficient in protein. Lastly, cows with lower RFI values utilized protein more efficiently. We suggest that protein efficiency will be improved in the process of selecting dairy cattle based on RFI.

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