Low dietary protein resilience is an indicator of the relative protein efficiency of individual dairy cows

E. Liu and M. J. VandeHaar*

Department of Animal Science, Michigan State University, East Lansing 48824

ABSTRACT

Our objectives were to determine (1) the sources of variation in cow responses to dietary protein reduction, and (2) the association of low dietary protein resilience (LPR) with protein efficiency. Lactating Holstein cows in peak lactation ($n = 166$; 92 primiparous, 77 multiparous) with initial milk yield 41 ± 9.8 kg/d were fed high-protein (HP) or low-protein (LP) diets in 4-wk periods in a crossover design with half the cows fed LP first and half fed HP first. The study was repeated for 69 of these cows (42 primiparous, 27 multiparous) 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 but inadequate rumen-undegradable protein for cows with average production in this study to maintain their production. High-protein diets were 18% CP in peak lactation and 16% CP in late lactation and contained extra expeller soybean meal to meet metabolizable protein requirements. Protein efficiency was defined as the protein captured in milk or in both milk and body tissues per unit of consumed protein. Low dietary protein resilience was calculated for each cow in peak and late lactation based on actual intake, production, and body weight measures. The ability of a cow to maintain total protein captured in milk and body gain when fed less protein varied considerably and the variation was mostly explained by a cow's captured protein per kilogram of metabolic body weight when fed HP, her parity, treatment sequence, and experiment. Protein efficiency was moderately repeatable across diets within lactation stage. Milk urea nitrogen was not associated with protein efficiency in individual cows within a diet and lactation stage. Cows with greater dietary protein resiliency (higher LPR) had similar protein efficiency

on the HP diet as cows with lower LPR, but higher protein efficiency on the LP diet. In conclusion, cows generally maintained their protein efficiency rankings when switching diets between sufficient or insufficient protein; however, some high-producing cows are better able to maintain high production when fed less protein. We define this ability as LPR and suggest it might be useful for identifying cows that use protein more efficiently to enhance dairy sustainability.

Key words: low protein, resilience, protein efficiency, protein response

INTRODUCTION

Dairy cattle convert protein in feeds (many of which have little direct value for human nutrition) into milk protein and dairy products that have provided high-quality protein for human consumption for centuries (Broderick, 2018). Improving the efficiency of protein use has been the goal of many studies in the past 40 yr and dairy cattle in North America are generally fed lower protein diets today than they were 30 yr ago. Lower dietary protein with the same milk protein output increases protein efficiency and profitability. However, reduced production is a potential risk of feeding diets with less protein (NRC, 2001), so feeding less protein may reduce the energetic and economic efficiency of producing milk. Thus, the emerging challenge is to identify ways to feed less protein while maintaining or enhancing milk production to meet the dietary protein needs of a growing human population (Huhtanen et al., 2008; Sinclair et al., 2014).

Direct genetic selection for protein efficiency is challenging because it requires knowledge of individual cow feed intake, which is not known on commercial farms. Milk urea nitrogen is an indicator of protein efficiency when cows are fed diets varying in protein sources and content; its measurement requires no knowledge of feed intake (Broderick and Clayton, 1997). Milk urea nitrogen varies for cows within a herd and has been suggested as a means of selecting for protein efficiency (Wattiaux et al., 2005; Stoop et al., 2007), although the idea of using MUN as a proxy for protein efficiency

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*Corresponding author: mikevh@msu.edu

was challenged by Sebek et al. (2007). Another challenge for selecting cows based on protein efficiency is that if a cow is fed more protein than she requires, she is essentially forced to use protein less efficiently than her innate ability would allow. In fact, as shown by Moraes et al. (2018), the efficiency of converting MP to milk protein decreases as the MP supply available for milk increases; thus, ranking cows for protein efficiency may have little meaning unless each cow is fed protein exactly at her requirement. We suggest that one way to examine potential differences among cows for their ability to efficiently use protein is to feed diets that are marginally low in protein. Whether the ranking of individual lactating cows for protein efficiency is repeatable across diets that are high or low in protein content and whether MUN is a meaningful indicator of protein efficiency for cows across diets high and low in protein is not clear.

Nutritionists typically examine the average response to diet interventions, and variation in the response among cows to protein reduction or supplementation has not been extensively studied. Some cows need less protein to meet their requirement because they produce less milk even when fed well; these cows maintain production when fed lower protein diets, but they are not more efficient or desirable. Some cows, however, may be able to maintain high production when fed lower protein diets because they metabolically process protein more efficiently than their cohorts. We will define these cows that can tolerate lower protein diets and still maintain protein output as having low dietary protein resiliency. One advantage of this trait, low dietary protein resiliency (**LPR**), is that it could be measured in cows without knowledge of individual feed intake. Whereas we expect that LPR might be correlated with protein efficiency, this is not known. Thus, our objectives were to determine (1) the sources of variation in cow responses to dietary protein reduction, and (2) the association of LPR with protein efficiency.

MATERIALS AND METHODS

Data

Experimental procedures were approved by the Institutional Animal Care and Use Committee of Michigan State University. Data from 166 lactating Holstein dairy cows were used in this study. Among the 166 cows, 69 were studied in both peak and late lactations. Data of milk yield (**MY**), milk components (milk protein and MUN), BW, and hip height were collected in the experiments. These are the same animals as in Liu and VandeHaar (2020). In brief, lactating Holstein cows ($n = 166$; 92 primiparous, 77 multiparous) with

initial MY 41.3 ± 9.8 kg/d were included in the 7 cross-over experiments with 2 treatments (high-protein diets, **HP**; low-protein diets, **LP**) and 2 periods of 28 to 35 d each; within each experiment, half of the cows received LP first, whereas the other half received HP first and then changed diets in period 2. Production of 69 of the 166 cows (42 primiparous, 27 multiparous) was also measured in the 4 late-lactation experiments. Diets for these studies were described in Liu and VandeHaar (2020). Briefly, the LP diet for peak lactation contained 31% NDF, 32% starch, and 14% CP, whereas the HP diet contained 29% NDF, 30% starch, and 18% CP; both diets contained at least 9.8% RDP (DM basis) to promote rumen function (NRC, 2001). For late lactation, the LP diet contained 40% NDF, 26% starch, and 13% CP, whereas the HP diet contained 38% NDF, 24% starch, and 16% CP; both diets contained at least 9.0% RDP. The extra protein of the HP diets was achieved by replacing soybean hulls and ground corn with expeller soybean meal. Based on NRC (2001), both HP diets contained more MP than required for cows of average BW and milk production in this study, whereas the LP diets supplied 83% and 95% of the required MP in peak and late lactation, respectively. Cows were milked 2 times daily; DMI and MY were recorded daily. Milk composition was measured over 4 consecutive milkings weekly and BW was measured 3 times weekly after the afternoon milkings.

Calculations

For cows >190 d pregnant, BW was corrected for conceptus weight (**CW**) for use in the calculation of protein change of body tissues. Conceptus weight was calculated based on 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.

After plotting the BW and DMI data along with the experimental day in the peak-lactation cows (data not shown), we did not have evidence to prove that BW change was not related to DMI difference when cows switched diets; in other words, we suspected that some BW change in the current study might be due to gut fill. In an attempt to measure true BW change more accurately, empty BW (**EBW**) was calculated for each cow based on Gibb et al. (1992) and Andrew et al. (1994) as

$$EBW = BW - 5.2 \times DMI - CW,$$

where DMI was the daily DMI when BW was measured.

Mean daily EBW gain (**EBG**; 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.

For multiparous cows, EBW change was considered to be all body condition; thus, protein captured for body tissue gained or lost with changes in EBW (**BodyP**, kg/d; derived from NRC, 2001, Tables 2–4) was calculated as

$$\text{BodyP} = (0.151 - 0.0268 \times \text{BCS}) \times \text{EBG},$$

For primiparous cows, we assumed that their mature BW would be 700 kg and they had to gain 0.14 kg of EBW/d of true growth across the first lactation to reach 92% of mature BW by their second calving (NRC, 2001). Based on NRC (2001) equations (11–1 and 11–2), 0.132 kg of protein per kg of EBG was assigned to 0.14 kg/d true growth. Any deviation in EBG from 0.14 kg/d was considered to be body condition gain or loss and the EBG associated with body condition change was the same as for multiparous cows ($0.151 - 0.0268 \times \text{BCS}$) kg of protein per kg of EBG. BodyP was estimated as

$$\text{BodyP} = \begin{cases} (0.151 - 0.0268 \times \text{BCS}) \times \text{EBG}, & \text{parity} > 1 \\ 0.132 \times 0.14 + [(0.151 - 0.0268 \times \text{BCS}) \times (\text{EBG} - 0.14)], & \text{parity} = 1 \end{cases}$$

Protein captured for pregnancy (**PregP**, kg/d) was calculated based on NRC (2001):

$$\text{PregP} = 0.00069 \times D - 0.0692 \times (\text{CBW}/45),$$

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

Total protein capture (**CapP**, kg/d) was calculated for each cow in each treatment (HP and LP) as

$$\text{CapP} = \begin{cases} \text{milk protein} + \text{BodyP}, & \text{DIM} < 200 \\ \text{milk protein} + \text{BodyP} + \text{PregP}, & \text{DIM} \geq 200 \end{cases}$$

After plotting the change of CapP from HP to LP (**dCapP**) along with the milk protein yield on the HP diet (Figure 1), we found that the cows that produced less milk on the HP diet were those that exhibited less of a drop in captured protein on the LP diet. This

decreased response to the LP diet would not appropriately indicate that a cow is more resilient.

To account for the difference in milk production on the HP diet and other factors that can potentially influence body protein mobilization and milk protein production, LPR was essentially calculated as the difference between the actual change of CapP and the predicted change of CapP, where larger numbers indicated better resilience. To calculate LPR, the initial full model was

$$\begin{aligned} \text{dCapP} = & \beta_0 + \beta_1 \times \text{CapP_MBW}_{\text{HP}} + \beta_2 \times \text{BCS}_{\text{HP}} \\ & + \beta_3 \times \text{MBW_HH}_{\text{HP}} + \beta_4 \times \text{dCP} + \beta_5 \times \text{CP}_{\text{HP}} + \beta_6 \\ & \times \text{DIM}_{\text{HP}} + \text{Par} (\text{Seq} \times \text{Exp}) + \text{Seq} (\text{Exp}) + \text{Exp} + e, \end{aligned}$$

where dCapP was the change of CapP from HP to LP ($\text{dCapP} = \text{CapP}_{\text{LP}} - \text{CapP}_{\text{HP}}$); $\text{CapP_MBW}_{\text{HP}}$ was the CapP per kg of metabolic BW when fed the HP diet; BCS_{HP} was the BCS when fed the HP diet; $\text{MBW_HH}_{\text{HP}}$ was the metabolic BW to height ratio when fed the HP diet; *Par* was parity (primiparous or multiparous); DIM_{HP} was the starting days in milk when fed the HP diet; *Seq* was treatment sequence (HP-LP or LP-HP); CP_{HP} was the actual CP% in the HP diet; dCP was the actual CP% change from HP to LP; *Exp* was experiment, and *e* was the residual term in the model. LPR was the residual term of the model. β_1 , β_2 , β_3 , β_4 , β_5 , and β_6 are the coefficients associated with the $\text{CapP_MBW}_{\text{HP}i}$, $\text{BCS}_{\text{HP}i}$, $\text{MBW_HH}_{\text{HP}i}$, dCP_{*i*}, CP_{*HPi*}, and DIM_{*HPi*} for the *i*th cow, respectively.

All covariates were jointly checked for multicollinearity through variance inflation factor analysis (REG, SAS 9.4, SAS Institute Inc., Cary, NC). No covariates had variance inflation factors greater than 10. Following that, the backward stepwise model selection was used to finalize the model (GLM, SAS 9.4). The reduced equation was used to determine an LPR value for each animal at each stage of lactation.

Cows were then grouped into high (**HLPR**), medium (**MLPR**), and low LPR (**LLPR**) groups. Cows >0.5 SD of the mean LPR were classified as HLPR, cows <−0.5 SD were classified as LLPR, and those between ±0.5 SD were classified as MLPR.

For each cow on each diet within each lactation stage, 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**).

Repeatability was used as the term to describe the correlation of a cow measurement across diets or lactation stages, because for traits to be valuable in comparing

Figure 1. Relationship between milk protein yield and cows response (and LPR, low dietary protein resilience) in peak-lactation cows. Open circles indicate primiparous cows, and solid triangles indicate multiparous cows. The correlation coefficient between milk protein yield for the high-protein (HP) diet and change of total protein capture from HP to low-protein (LP) diet (dCapP) was -0.41 in primiparous cows, and -0.45 in multiparous cows; dCapP was the change of CapP from HP to LP, where CapP (total protein capture, kg/d) was calculated for each cow in each treatment (HP and LP) as the sum of milk protein, body protein, and pregnancy protein. The correlation coefficient between milk protein yield for HP and LPR was 0.04 in primiparous cows and 0.03 in multiparous cows. Each data point represents one value ($n = 166$).

cows, they must be repeatable. Repeatability of MPE across dietary protein contents within each lactation stage was calculated as Pearson correlation coefficients using the GLM procedure of SAS (9.4), after accounting for the effects of parity, treatment sequence, and experiment. To further examine the MPE repeatability across dietary protein contents, 2 MPE values were calculated: MPE_{HP} and MPE_{LP} , where MPE_{HP} was the average MPE for the HP diets across lactation stages and MPE_{LP} was the average MPE for the LP diets across lactation stages. To examine the MPE repeatability across lactation stages, 2 MPE values were calculated: MPE_{peak} and MPE_{late} , where MPE_{peak} was the average

MPE across diets in peak lactation and MPE_{late} was the average MPE across diets in late lactation. Pearson correlation coefficients between MPE_{HP} and MPE_{LP} and between MPE_{peak} and MPE_{late} were calculated. Partial correlations accounting for the effects of parity and experiment were estimated using the GLM Procedure. Correlation was considered as significant at $P \leq 0.05$ and trends at $P \leq 0.10$.

Similar calculations and analyses were performed for GPE and MUN to determine repeatability of GPE and MUN across dietary protein contents and lactation stages, respectively. The change of MUN from the HP diet to the LP diet was calculated as dMUN. The

Table 1. Dry matter intake, milk production, and protein efficiency for cows fed treatment diets in peak and late lactation^{1,2}

Item	Treatment ³		SEM	Parity		SEM	P-value ⁴		
	HP	LP		Primiparous	Multiparous		TRT	Parity	TRT × parity
Peak-lactation cows	n = 166	n = 166		n = 184	n = 148				
DMI, kg/d	24.3	23.3	0.14	21.3	26.3	0.35	<0.01	<0.01	0.86
Milk protein, kg/d	1.21	1.07	0.01	1.01	1.28	0.02	<0.01	<0.01	0.18
Protein capture, ⁵ kg/d	1.24	1.09	0.01	1.04	1.30	0.03	<0.01	<0.01	0.86
MUN, mg/dL	15.1	9.2	0.13	11.6	12.7	0.20	<0.01	<0.01	0.16
Milk protein efficiency, ⁶ %	27.6	32.4	0.18	29.6	30.3	0.46	<0.01	0.11	0.31
Gross protein efficiency, ⁷ %	27.5	30.1	0.32	29.6	30.1	0.48	<0.01	0.39	0.25
Late-lactation cows	n = 69	n = 69		n = 84	n = 54				
DMI, kg/d	19.8	18.4	0.20	18.0	20.2	0.48	<0.01	0.32	<0.01
Milk protein, kg/d	0.80	0.68	0.01	0.77	0.72	0.04	<0.01	0.25	<0.01
Protein capture, kg/d	0.84	0.70	0.02	0.80	0.75	0.04	<0.01	<0.01	0.54
MUN, mg/dL	12.1	8.1	0.16	9.9	10.2	0.27	<0.01	0.28	0.41
Milk protein efficiency, %	26.0	28.8	0.43	29.8	25.0	1.10	<0.01	<0.01	0.84
Gross protein efficiency, %	26.2	27.8	0.56	29.0	25.0	1.11	0.01	<0.01	0.08

¹Average DIM was 125 for primiparous cows in the HP_{peak} diet, 126 for primiparous cows in the LP_{peak} diet, 122 for multiparous cows in the HP_{peak} diet, and 121 for multiparous cows in the LP_{peak} diet; average DIM was 258 for primiparous cows in the HP_{late} diet, 257 for primiparous cows in the LP_{late} diet, 263 for multiparous cows in the HP_{late} diet, and 264 for multiparous cows in the LP_{late} diet. HP = high-protein diet; LP = low-protein diet.

²Average parity for multiparous cows was 2.94 in peak lactation and 3.12 in late lactation.

³Treatments contained 18% and 14% CP on a DM basis for peak-lactation cows, and 16% and 13% CP on a DM basis for late-lactation cows.

⁴P-value associated with treatment differences (HP vs. LP; TRT) and parity differences (primiparous vs. multiparous; parity).

⁵Total protein capture = milk protein + body protein, where body protein was estimated as BodyP = (0.151 - 0.0268 × BCS) × (EBG - 0.14), when parity > 1; BodyP = 0.132 × 0.14 + [(0.151 - 0.0268 × BCS) × (EBG - 0.14)], when parity = 1. EBG = empty BW gain.

⁶Milk protein efficiency, milk protein per unit of dietary protein intake.

⁷Gross protein efficiency, milk protein, and body protein capture per unit of dietary protein intake.

change of MPE and GPE were calculated as dMPE and dGPE, respectively, from the HP diet to the LP diet.

To quantify the association of LPR with various protein efficiency terms (MPE, GPE, MUN, dMPE, dGPE, and dMUN), Pearson correlation coefficients were obtained using the GLM procedure in SAS (9.4), after accounting for the effects of parity, treatment sequence, and experiment.

To determine the differences between the most and least resilient cows, production performance and protein efficiency of the cows from different LPR groups within each lactation stage were compared. The effect of LPR was determined using the GLM procedure of SAS according to the model $Y_i = \mu + \text{LPR}_i + e$, where μ was the overall mean, LPR_i was the fixed effect of LPR group, and e was the residual error.

Cow production performance and protein efficiency responses to diets within each lactation stage were analyzed using the HPMIXED procedure in SAS (9.4), with fixed effects of diet, parity, treatment sequence nested in experiment, period within experiment, 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

Cow Performance

As shown in Table 1, during peak lactation, the LP diet decreased milk protein yield ($P < 0.01$), CapP ($P < 0.01$), and MUN ($P < 0.01$); the LP diet also increased MPE ($P < 0.01$) and GPE ($P < 0.01$), compared with the HP diet. During late lactation, the LP diet decreased milk protein yield ($P < 0.01$), CapP ($P < 0.01$), and MUN ($P < 0.01$); the LP diet also increased MPE ($P < 0.01$) and GPE ($P = 0.01$), compared with the HP diet.

In peak lactation, compared with multiparous cows, primiparous cows had less DMI ($P < 0.01$), milk protein ($P < 0.01$), CapP ($P < 0.01$), and MUN ($P < 0.01$), with similar MPE ($P = 0.11$) and GPE ($P = 0.39$). In late lactation, compared with multiparous cows, primiparous cows had higher MPE ($P < 0.01$) and GPE ($P < 0.01$), with less DMI ($P < 0.01$), milk protein yield ($P = 0.25$), CapP ($P = 0.55$), and MUN ($P = 0.28$).

Cows with higher protein yield (kg/d) had greater protein efficiency. Protein efficiency (MPE or CPE) was correlated with protein output (MilkP or CapP) at $r = 0.67$ ($P < 0.01$) in peak lactation and $r = 0.86$ (P

< 0.01) in late lactation, after accounting for the effects of diet, parity, experiment, and treatment sequence (Supplemental Figure S1; <https://doi.org/10.3168/jds.2020-18143>).

Repeatability of Protein Efficiency Across Dietary Protein Contents and Lactation Stage

Protein efficiency was moderately repeatable across dietary protein contents in peak-lactation cows. The repeatability for MPE, GPE, and MUN across HP and LP diets was 0.76, 0.57, and 0.48, respectively. Protein efficiency was moderately repeatable across dietary protein contents in late-lactation cows. The repeatability for MPE, GPE, and MUN across HP and LP diets was 0.56, 0.48, and 0.66, respectively. Further details can be found in Supplemental Figures S2 and S3 (<https://doi.org/10.3168/jds.2020-18143>).

When averaged across both lactation stages, the MPE of individual cows fed HP diets was repeatable with their average MPE when fed LP diets ($r = 0.68$, $P < 0.05$); the same was true for GPE ($r = 0.43$, $P < 0.05$). When averaged across diets, the average MPE of individual cows in peak lactation was not repeatable with their average MPE in late lactation ($r = 0.21$, $P = 0.12$), and the same was true for GPE ($r = 0.16$, $P = 0.37$). In contrast, the average MUN was repeatable across both dietary protein contents ($r = 0.66$, $P < 0.05$) and lactation stages ($r = 0.55$, $P < 0.05$). Further details can be found in Supplemental Figures S4 and S5 (<https://doi.org/10.3168/jds.2020-18143>).

MUN and Protein Efficiency

In peak-lactation cows, GPE was highly correlated with MPE in both HP ($r = 0.93$, $P < 0.05$) and LP diets ($r = 0.86$, $P < 0.05$). In late-lactation cows, GPE was highly correlated with MPE in both HP ($r = 0.78$, $P < 0.05$) and LP diets ($r = 0.91$, $P < 0.05$). In contrast, MUN was not correlated with either measure of protein efficiency in the HP or LP diet in peak or late lactation (Supplemental Figures S6 and S7; <https://doi.org/10.3168/jds.2020-18143>). When averaged across dietary protein contents, MUN was not correlated with either measure of protein efficiency in either peak or late lactation. When averaged across lactation stage, MUN was not correlated with either measure of protein efficiency in either LP or HP diets (Supplemental Figure S8; <https://doi.org/10.3168/jds.2020-18143>).

Variation of LPR in Lactating Dairy Cows

The average dCapP in peak-lactation cows when switched from the HP to LP diet was -0.15 kg/d, with

a SD of 0.13 kg/d. The final model for LPR in peak-lactation cows was

$$\text{dCapP} = \beta_0 + \beta_1 \times \text{CapP_MBW}_{\text{HP}} + \text{Par}(\text{Seq} \times \text{Exp}) + \text{Seq}(\text{Exp}) + \text{Exp} + e.$$

Among the variables, 31% of the dCapP variation was explained by CapP_MBW_{HP}, with 9%, 7%, and 14% explained by parity, treatment sequence, and experiment, respectively; the remaining 39% was defined as LPR.

The average dCapP in late-lactation cows when switched from the HP to LP diet was -0.15 kg/d, with a SD of 0.14 kg/d. The final model for LPR in late-lactation cows was

$$\begin{aligned} \text{dCapP} = & \beta_0 + \beta_1 \times \text{CapP_MBW}_{\text{HP}} + \beta_2 \\ & \times \text{MBW_HH}_{\text{HP}} + \text{Par}(\text{Seq} \times \text{Exp}) \\ & + \text{Seq}(\text{Exp}) + \text{Exp} + e. \end{aligned}$$

Among the variables, 24% of the dCapP variation was explained by CapP_MBW_{HP}, with 7%, 15%, 2%, and 11% explained by MBW_HH_{HP}, parity, treatment sequence, and experiment, respectively; the remaining 40% was defined as LPR.

As shown in Tables 2 and 3, among peak-lactation cows, when fed the HP diet, HLPR cows had similar DMI, MY, ECM, MBW, BCS, protein intake, milk protein yield, CapP, MPE, and MUN, with lower BodyP and GPE, compared with LLPR cows. When switched to the LP diet, HLPR cows had higher DMI, MY, ECM, protein intake, milk protein yield, BodyP, CapP, and GPE, compared with LLPR cows. Among late-lactation cows, when fed the HP diet, HLPR cows had similar DMI, MY, ECM, BCS, protein intake, milk protein yield, CapP, MPE, GPE, and MUN, with lower EBG and BodyP, compared with LLPR cows. When switched to the LP diet, HLPR cows had higher DMI, MY, ECM, EBG, protein intake, milk protein yield, BodyP, CapP, MPE, and GPE.

LPR and Protein Efficiency

As shown in Table 4, LPR in peak-lactation cows was correlated with dMPE ($r = 0.19$, $P < 0.05$), GPE_{LP} ($r = 0.38$, $P < 0.05$), and dGPE ($r = 0.53$, $P < 0.05$); LPR was not correlated with MPE_{HP}, MPE_{LP}, GPE_{HP}, MUN_{HP}, MUN_{LP}, or dMUN.

As shown in Table 4, LPR in late-lactation cows was correlated with MPE_{LP} ($r = 0.34$, $P < 0.05$), dMPE ($r = 0.38$, $P < 0.05$), GPE_{LP} ($r = 0.55$, $P < 0.05$), and dGPE ($r = 0.67$, $P < 0.05$); LPR was not correlated with MPE_{HP}, MUN_{HP}, GPE_{HP}, MUN_{LP}, or dMUN.

Table 2. Comparisons of production parameters of high-, medium-, and low-LPR (low dietary protein resilience) cows in peak and late lactation¹

Item ²	HLPR	MLPR	LLPR
Peak-lactation cows (n = 166)			
LPR value, kg/d	0.096 ^a ± 0.006	0.004 ^b ± 0.004	-0.103 ^c ± 0.006
DMI for HP diets, kg/d	24.8 ^a ± 0.59	23.8 ^a ± 0.64	24.1 ^a ± 0.44
DMI for LP diets, kg/d	24.5 ^a ± 0.58	22.7 ^b ± 0.62	22.4 ^b ± 0.43
Change in DMI, kg/d	-0.48 ^a ± 0.25	-1.21 ^b ± 0.26	-2.19 ^c ± 0.18
Milk yield for HP diets, kg/d	42.3 ^a ± 1.18	40.9 ^a ± 0.87	41.6 ^a ± 1.26
Milk yield for LP diets, kg/d	39.8 ^a ± 1.32	35.3 ^b ± 0.98	35.7 ^b ± 1.42
Change in milk yield, kg/d	-2.47 ^a ± 0.50	-5.67 ^b ± 0.37	-5.44 ^b ± 0.54
ECM for HP diets, ³ kg/d	41.7 ^a ± 1.17	40.6 ^a ± 0.87	40.9 ^a ± 1.26
ECM for LP diets, kg/d	38.6 ^a ± 1.03	35.8 ^b ± 0.76	34.9 ^b ± 1.11
Change in ECM, kg/d	-2.89 ^a ± 0.45	-3.87 ^b ± 0.34	-5.18 ^c ± 0.49
Metabolic BW for HP diets ⁴	129 ^a ± 1.83	128 ^a ± 1.35	130 ^a ± 1.97
Metabolic BW for LP diets	129 ^a ± 1.79	128 ^a ± 1.32	129 ^a ± 1.92
Change in metabolic BW/28 d	-0.48 ^a ± 0.56	-0.25 ^a ± 0.42	-0.91 ^a ± 0.61
BCS for HP diets	3.12 ^a ± 0.05	3.29 ^a ± 0.04	3.26 ^a ± 0.05
BCS for LP diets	3.10 ^a ± 0.05	3.27 ^a ± 0.04	3.23 ^a ± 0.05
Change in BCS/28 d	-0.018 ^a ± 0.031	-0.016 ^a ± 0.024	-0.017 ^a ± 0.029
Empty BW gain for HP diets (EBG _{HP}), ⁵ kg/d	0.16 ^a ± 0.11	0.43 ^{ab} ± 0.09	0.61 ^b ± 0.11
Empty BW gain for LP diets (EBG _{LP}), kg/d	0.49 ^a ± 0.12	0.18 ^b ± 0.09	-0.32 ^c ± 0.12
Change in empty BW gain, kg/d	0.33 ^a ± 0.16	-0.27 ^b ± 0.12	-0.92 ^c ± 0.12
Captured protein per unit of metabolic BW for HP diets ⁶	0.0095 ^a ± 0.0002	0.0095 ^a ± 0.0002	0.0095 ^a ± 0.0002
Captured protein per unit of metabolic BW for LP diets	0.0092 ^a ± 0.0002	0.0077 ^b ± 0.0002	0.0082 ^c ± 0.0002
Change in captured protein per unit of metabolic BW	-0.0002 ^a ± 0.0001	-0.0011 ^b ± 0.0001	-0.0019 ^c ± 0.0001
Late-lactation cows (n = 69)			
LPR value, kg/d	0.082 ^a ± 0.008	0.001 ^b ± 0.006	-0.096 ^c ± 0.009
DMI for HP diets, kg/d	19.9 ^a ± 0.61	19.8 ^a ± 0.43	19.4 ^a ± 0.67
DMI for LP diets, kg/d	19.2 ^a ± 0.57	18.0 ^{ab} ± 0.41	17.8 ^{ab} ± 0.63
Change in DMI, kg/d	-0.72 ^a ± 0.41	-1.80 ^{ab} ± 0.29	-1.60 ^{ab} ± 0.45
Milk yield for HP diets, kg/d	26.1 ^a ± 1.35	26.0 ^a ± 0.96	26.3 ^a ± 1.48
Milk yield for LP diets, kg/d	23.1 ^a ± 1.23	23.5 ^a ± 0.88	18.4 ^b ± 1.34
Change in milk yield, kg/d	-2.99 ^a ± 0.91	-2.38 ^a ± 0.65	-7.88 ^b ± 0.98
ECM for HP diets, ³ kg/d	26.9 ^a ± 1.51	27.2 ^a ± 1.07	26.2 ^a ± 1.66
ECM for LP diets, kg/d	24.4 ^a ± 1.34	24.6 ^a ± 0.95	20.7 ^b ± 1.41
Change in ECM, kg/d	-2.53 ^a ± 0.86	-2.38 ^a ± 0.61	-5.46 ^b ± 0.94
Metabolic BW for HP diets ⁴	138 ^a ± 3.37	133 ^a ± 2.39	136 ^a ± 3.71
Metabolic BW for LP diets	137 ^a ± 3.35	132 ^a ± 2.39	136 ^a ± 3.67
Change in metabolic BW/28 d	-0.74 ^a ± 0.74	-0.98 ^a ± 0.41	-0.54 ^a ± 0.63
BCS for HP diets	3.78 ^a ± 0.10	3.62 ^a ± 0.08	3.73 ^a ± 0.10
BCS for LP diets	3.62 ^a ± 0.10	3.46 ^a ± 0.08	3.77 ^{ab} ± 0.10
Change in BCS/28 d	-0.17 ^a ± 0.047	-0.16 ^a ± 0.041	0.04 ^b ± 0.051
Empty BW gain for HP diets (EBG _{HP}), ⁵ kg/d	0.41 ^a ± 0.16	0.54 ^a ± 0.12	0.80 ^{ab} ± 0.17
Empty BW gain for LP diets (EBG _{LP}), kg/d	0.73 ^a ± 0.13	0.11 ^b ± 0.10	0.09 ^b ± 0.15
Change in empty BW gain, ⁵ kg/d	0.32 ^a ± 0.22	-0.43 ^b ± 0.16	-0.72 ^c ± 0.24
Captured protein per unit of metabolic BW for HP diets ⁶	0.0063 ^a ± 0.0004	0.0064 ^a ± 0.0003	0.0063 ^a ± 0.0004
Captured protein per unit of metabolic BW for LP diets	0.0058 ^a ± 0.0002	0.0055 ^b ± 0.0002	0.0046 ^b ± 0.0003
Change in captured protein per unit of metabolic BW	-0.0005 ^a ± 0.0002	-0.0010 ^b ± 0.0001	-0.0017 ^b ± 0.0002

^{a-c}Different superscripts indicate significant differences in means ($P < 0.05$).

¹Cows were grouped into high-, medium-, and low-LPR groups. Cows >0.5 SD of the mean LPR were classified as high LPR (HLPR), cows <-0.5 SD were classified as low LPR (LLPR), and those ± 0.5 SD were classified as medium LPR (MLPR). In peak lactation, LPR was defined as the residual term in the model: $dCapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + Par (Seq \times Exp) + Seq (Exp) + Exp + e$, where $CapP_MBW_{HP}$ was the total protein capture (CapP) per kilogram of metabolic BW when fed the HP; Par was parity; Seq was treatment sequence (HP-LP or LP-HP); Exp was experiment, and e was the residual term in the model. In late lactation, the LPR model was $dCapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + \beta_2 \times MBW_HH_{HP} + Par (Seq \times Exp) + Seq (Exp) + Exp + e$, where MBW_HH_{HP} was the metabolic BW to height ratio when fed the HP. HP = high-protein diet; LP = low-protein diet.

²Upper values within a row = LSM of the group. Lower values within a row = SE of the LSM.

³Energy-corrected milk = $[(0.327 \times \text{kg of milk}) + (12.95 \times \text{kg of milk fat}) + (7.20 \times \text{kg of milk protein})]$ (Tyrrell and Reid, 1965).

⁴Metabolic BW = $BW^{0.75}$.

⁵Change in empty BW (EBW) per day through the period, determined by linear regression using EBW measurements throughout the period, where EBW was the adjusted BW after accounting for the gut fill effect.

⁶Captured protein per unit of metabolic BW, where captured protein = milk protein + protein captured in body tissues + pregnancy protein.

Table 3. Comparisons of protein output and protein efficiency parameters of high-, medium-, and low-LPR (low dietary protein resilience) cows in peak and late lactation¹

Item ²	HLPR	MLPR	LLPR
Peak-lactation cows (n = 166)			
LPR value, kg/d	0.096 ^a ± 0.006	0.004 ^b ± 0.004	-0.103 ^c ± 0.006
Dietary CP content for HP diets, ³ %	17.9 ^a ± 0.03	17.9 ^a ± 0.03	17.9 ^a ± 0.02
Dietary CP content for LP diets, %	14.2 ^a ± 0.05	14.2 ^a ± 0.04	14.2 ^a ± 0.05
Change in dietary CP content, %	-3.71 ^a ± 0.06	-3.74 ^a ± 0.05	-3.73 ^a ± 0.06
CP intake for HP diets, kg/d	4.45 ^a ± 0.11	4.28 ^a ± 0.08	4.38 ^a ± 0.12
CP intake for LP diets, kg/d	3.48 ^a ± 0.08	3.23 ^b ± 0.07	3.18 ^b ± 0.08
Change in CP intake, kg/d	-0.97 ^a ± 0.047	-1.05 ^a ± 0.035	-1.20 ^b ± 0.051
Milk protein yield for HP diets, ⁴ kg/d	1.23 ^a ± 0.035	1.19 ^a ± 0.026	1.23 ^a ± 0.038
Milk protein yield for LP diets, kg/d	1.14 ^a ± 0.031	1.03 ^b ± 0.023	1.02 ^b ± 0.033
Change in milk protein yield, kg/d	-0.083 ^a ± 0.013	-0.130 ^b ± 0.011	-0.209 ^c ± 0.015
Body protein gain for HP diets, ⁵ kg/d	0.014 ^a ± 0.007	0.031 ^b ± 0.005	0.040 ^{bc} ± 0.007
Body protein gain for LP diets, kg/d	0.035 ^a ± 0.008	0.016 ^b ± 0.006	-0.017 ^c ± 0.008
Change in body protein gain, kg/d	0.021 ^a ± 0.010	-0.014 ^b ± 0.008	-0.058 ^c ± 0.010
Captured protein for HP diets, ⁶ kg/d	1.24 ^a ± 0.035	1.20 ^a ± 0.026	1.27 ^a ± 0.038
Captured protein for LP diets, kg/d	1.18 ^a ± 0.037	1.05 ^b ± 0.023	1.00 ^c ± 0.033
Change in captured protein, kg/d	-0.062 ^a ± 0.016	-0.145 ^b ± 0.012	-0.266 ^c ± 0.017
Milk protein efficiency for HP diets, ⁷ %	27.6 ^a ± 0.43	27.2 ^a ± 0.34	27.9 ^a ± 0.46
Milk protein efficiency for LP diets, %	32.8 ^a ± 0.50	32.0 ^a ± 0.37	32.1 ^a ± 0.54
Change in milk protein efficiency, %	5.23 ^a ± 0.35	4.88 ^a ± 0.26	4.05 ^b ± 0.36
MUN for HP diets	14.9 ^a ± 0.28	15.2 ^a ± 0.21	14.9 ^a ± 0.30
MUN for LP diets	9.15 ^a ± 0.22	9.31 ^a ± 0.17	8.97 ^a ± 0.24
Change in MUN	-5.84 ^a ± 0.25	-5.91 ^a ± 0.20	-6.00 ^a ± 0.27
Gross protein efficiency for HP diets, ⁸ %	27.9 ^a ± 0.44	27.9 ^a ± 0.32	29.0 ^{ab} ± 0.47
Gross protein efficiency for LP diets, %	34.0 ^a ± 0.52	32.6 ^b ± 0.39	31.5 ^c ± 0.57
Change in gross protein efficiency, %	6.11 ^a ± 0.43	4.65 ^{ab} ± 0.32	2.52 ^c ± 0.47
Late-lactation cows (n = 69)			
LPR value, kg/d	0.082 ^a ± 0.008	0.001 ^b ± 0.006	-0.096 ^c ± 0.009
Dietary CP content for HP diets, ³ %	15.8 ^a ± 0.003	15.8 ^a ± 0.003	15.8 ^a ± 0.004
Dietary CP content for LP diets, %	12.9 ^a ± 0.001	12.9 ^a ± 0.001	12.9 ^a ± 0.001
Change in dietary CP content, %	-2.93 ^a ± 0.001	-2.93 ^a ± 0.001	-3.00 ^a ± 0.001
CP intake for HP diets, kg/d	3.15 ^a ± 0.09	3.14 ^a ± 0.06	3.06 ^a ± 0.10
CP intake for LP diets, kg/d	2.50 ^a ± 0.08	2.32 ^a ± 0.06	2.30 ^{ab} ± 0.09
Change in CP intake, kg/d	-0.65 ^a ± 0.067	-0.82 ^a ± 0.047	-0.77 ^{ab} ± 0.073
Milk protein yield for HP diets, ⁴ kg/d	0.829 ^a ± 0.045	0.849 ^a ± 0.032	0.832 ^a ± 0.049
Milk protein yield for LP diets, kg/d	0.731 ^a ± 0.039	0.712 ^a ± 0.028	0.606 ^b ± 0.043
Change in milk protein yield, kg/d	-0.098 ^a ± 0.026	-0.136 ^a ± 0.019	-0.237 ^b ± 0.029
Body protein gain for HP diets, ⁵ kg/d	0.027 ^a ± 0.012	0.037 ^a ± 0.008	0.061 ^b ± 0.013
Body protein gain for LP diets, kg/d	0.059 ^a ± 0.012	-0.001 ^b ± 0.008	0.007 ^b ± 0.013
Change in body protein gain, kg/d	0.032 ^a ± 0.018	-0.038 ^b ± 0.019	-0.055 ^b ± 0.012
Pregnancy protein gain for HP diets, ⁹ kg/d	0.014 ^a ± 0.005	0.009 ^a ± 0.004	0.013 ^a ± 0.005
Pregnancy protein gain for LP diets, kg/d	0.011 ^a ± 0.005	0.009 ^a ± 0.005	0.005 ^a ± 0.005
Change in pregnancy protein gain, kg/d	-0.002 ^a ± 0.007	-0.001 ^a ± 0.005	-0.008 ^a ± 0.007
Captured protein for HP diets, ⁶ kg/d	0.823 ^a ± 0.048	0.886 ^a ± 0.034	0.844 ^a ± 0.053
Captured protein for LP diets, kg/d	0.790 ^a ± 0.035	0.712 ^{ab} ± 0.025	0.613 ^c ± 0.039
Change in captured protein, kg/d	-0.033 ^a ± 0.027	-0.174 ^b ± 0.019	-0.230 ^b ± 0.029
Milk protein efficiency for HP diets, ⁷ %	26.4 ^a ± 1.22	26.9 ^a ± 0.87	26.6 ^a ± 1.34
Milk protein efficiency for LP diets, %	29.4 ^a ± 1.29	30.7 ^a ± 0.92	26.1 ^b ± 1.31
Change in milk protein efficiency, %	2.98 ^a ± 0.98	3.76 ^a ± 0.68	-0.49 ^b ± 1.05
MUN for HP diets	12.52 ^a ± 0.35	11.92 ^a ± 0.25	12.41 ^a ± 0.38
MUN for LP diets	8.80 ^a ± 0.29	8.67 ^a ± 0.20	8.44 ^a ± 0.30
Change in MUN	-3.72 ^a ± 0.31	-3.27 ^a ± 0.22	-3.97 ^a ± 0.33
Gross protein efficiency for HP diets, ⁸ %	26.1 ^a ± 1.35	28.2 ^a ± 0.96	27.6 ^a ± 1.48
Gross protein efficiency for LP diets, %	32.0 ^a ± 1.16	30.7 ^a ± 0.92	26.5 ^c ± 1.26
Change in gross protein efficiency, %	5.87 ^a ± 0.86	2.47 ^b ± 0.61	-1.14 ^c ± 0.93

^{a-c}Different superscripts indicate significant differences in means ($P < 0.05$).¹Cows were grouped into high, medium, and low LPR groups. Cows >0.5 SD of the mean LPR were classified as high LPR (HLPR), cows <-0.5 SD were classified as low LPR (LLPR), and those ± 0.5 SD were classified as medium LPR (MLPR). In peak lactation, LPR was defined as the residual term in the model: $dCapPro = \beta_0 + \beta_1 \times CapP_MBW_{HP} + Par (Seq \times Exp) + Seq (Exp) + Exp + e$, where $CapP_MBW_{HP}$ was the total protein capture (CapP) per kg of metabolic BW when fed the HP diet; Par was parity (primiparous or multiparous); Seq was treatment sequence (HP-LP or LP-HP); Exp was experiment, and e was the residual term in the model. In late lactation, the LPR model was $dCapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + \beta_2 \times MBW_HH_{HP} + Par (Seq \times Exp) + Seq (Exp) + Exp + e$, where MBW_HH_{HP} was the metabolic BW to height ratio when fed HP. HP = high-protein diet; LP = low-protein diet.²Upper values within a row = LSM of the group. Lower values within a row = SE of the LSM.³CP% of the diet, kg of CP per kg of DM.⁴Milk protein, kg/d.⁵Protein captured in body tissue gain: $BodyP = (0.151 - 0.0268 \times BCS) \times EBG$, when parity > 1 ; $BodyP = 0.132 \times 0.14 + [(0.151 - 0.0268 \times BCS) \times (EBG - 0.14)]$, when parity $= 1$; EBG = empty BW (EBW) gain per day through the period, determined by linear regression using EBW measurements throughout the period, where EBW was the adjusted BW after accounting for the gut fill effect.⁶Total protein captured: cows in the peak lactation: $CapP = milk\ protein + BodyP$; cows in the late lactation: $CapP = milk\ protein + BodyP + PregP$.⁷Milk protein efficiency, milk protein per unit of dietary protein intake.⁸Gross protein efficiency, milk protein, and body protein capture per unit of dietary protein intake.⁹Protein captured for pregnancy: $PregP = 0.00069 \times D - 0.0692 \times (CBW/45)$, where D was the day of gestation between 190 and 279, and CBW was the calf birth weight (NRC, 2001).

DISCUSSION

In general, the cows that produced less milk and had less total protein capture when fed the HP diet were those that exhibited less of a drop in captured protein when fed the LP diet (Figure 1). This was expected and this decreased response to the LP diet would not in any way indicate that a cow uses protein more efficiently—these cows needed less protein because they produced less. Our hypothesis was that some cows can tolerate lower protein to produce the same amount of milk because they are more efficient metabolically. To identify these more efficient cows, we tried to account for all factors that might explain differences in the protein response (such as CapP per kg of MBW); these factors are shown in Tables 2 and 3. After accounting for all factors not related to protein efficiency per se, LPR accounted for 40% of the variation in cow responses when switched from the HP to LP diet.

In the current study, when quantifying the total protein output in lactating dairy cows, we also considered protein captured in body tissues, as indicated by EBW change, in addition to milk protein output. Although EBW change might not be an accurate measure of body protein deposition/mobilization, it can still help provide a more complete measurement of protein capture and thus cows' resilience to the LP diet. When dietary protein is limited, cows would mobilize body protein to support milk protein production, especially in early lactation (Chilliard and Robelin, 1983). Thus, cows maintaining milk production under the LP diet through excessive body protein mobilization should not

be considered as resilient cows. Accordingly, our LPR model included all factors (production level, BW, BCS, DIM, parity, and so on) that may affect the level of protein mobilization and deposition in body tissues, as described by Bauman and Currie (1980) and Komaragiri and Erdman (1997). Furthermore, for late-lactation dairy cows, protein utilized for pregnancy was also included in our model.

Significant variation existed among cows in their ability to maintain protein capture in milk and body when fed the LP diet. Having explored an exhaustive list of phenotypic factors that may influence cows' response to the LP diet, our model explained ~60% of the variation in dCapP among cows. We hypothesize that some part of LPR may have a genetic basis. Indeed, genetic variation in resilience to various stressors has been well documented. For example, the resilience to mastitis is heritable and Scandinavian breeding program has been using it since the 1960s (Martin et al., 2018). Al-Kanaan (2016) showed that resilience to heat stress is a heritable trait for cattle. Furthermore, Mulder et al. (2013) suggested that genetic factors explain animals' resilience to various disturbances (e.g., disease, temperature, social stress). Because heritability studies require large numbers of animals, future collaborations should be encouraged to investigate the heritability of LPR to facilitate potential genetic selection.

LPR, Protein Efficiency, and Other Production Traits

In this study, LPR was correlated with protein efficiency for the LP diet and also the difference of protein

Table 4. Pearson correlation coefficients between low dietary protein resilience (LPR) and various protein efficiency terms

Item ¹	Peak lactation		Late lactation	
	r	P-value	r	P-value
Milk protein efficiency for HP diet ²	0.01	0.83	0.04	0.72
Milk protein efficiency for LP diet ²	0.12	0.16	0.34	<0.05
Change in milk protein efficiency	0.19	<0.05	0.38	<0.05
Gross protein efficiency for HP diet ³	0.09	0.24	0.06	0.66
Gross protein efficiency for LP diet	0.38	<0.05	0.55	<0.05
Change in gross protein efficiency	0.53	<0.05	0.67	<0.05
MUN for HP diet	0.02	0.83	0.00	0.98
MUN for LP diet	0.02	0.82	0.07	0.58
Change in MUN	0.00	0.97	0.06	0.61

¹r stands for the Pearson correlation coefficient of the linear relationship between 2 variables (MPE vs. LPR, GPE vs. LPR, MUN vs. LPR); correlation was considered significant when $P < 0.05$. MPE = milk protein efficiency; GPE = gross protein efficiency.

²Milk protein efficiency is milk protein per unit of dietary protein intake. HP = high-protein diet; LP = low-protein diet.

³Gross protein efficiency is milk protein and body protein captured per unit of dietary protein intake. Protein captured in body tissue gain: $\text{BodyP} = (0.151 - 0.0268 \times \text{BCS}) \times \text{EBG}$, when parity > 1 ; $\text{BodyP} = 0.132 \times 0.14 + [(0.151 - 0.0268 \times \text{BCS}) \times (\text{EBG} - 0.14)]$, when parity = 1; EBG = empty BW (EBW) gain per day through the period, determined by linear regression using EBW measurements throughout the period, where EBW was the adjusted BW after accounting for the gut fill effect.

efficiency between HP and LP diets. In other words, cows with higher LPR might not be the most protein-efficient cows when fed the HP diet, but they are the more efficient cows when fed the LP diet. Interestingly, we also found that the HLPR cows, compared with LLPR cows, captured less body protein when fed the HP diet, but more when fed the LP diet. These results imply that cows with less body protein deposition in diets containing adequate protein were likely more resilient to diets deficient in protein.

Given that MY was similar between HLPR and LLPR cows (Table 2), LPR might be useful in a selection index. In the traditional dairy breeding programs, MY and milk protein yield were the major focus (Egger-Danner et al., 2015). As a result, modern dairy cows have higher production than cows 100 yr ago (VandeHaar et al., 2016). The tradeoff between promoting production and improving other traits, such as fertility, is currently considered in the Net Merit Index. Low dietary protein resilience provides a potential solution to incorporate a trait for protein efficiency into genetic selection programs.

Repeatability of Protein Efficiency Across Dietary Protein Contents and Lactation Stages

Overall, cows in the current study maintained their protein efficiency rankings across dietary protein contents but did not maintain their protein efficiency rankings across lactation stages. This is consistent with prior work (Zamani et al., 2011) demonstrating low repeatability for protein efficiency across lactation using monthly records on 500 dairy cows ($r = 0.12$). The low repeatability of protein efficiency across lactation stages could be due to shifts in nutrient partitioning between production and reproduction. In the last weeks of lactation, as pregnancy progressively takes priority over milk production, more protein is utilized to support fetal growth (NRC, 2001), and body deposition might become a more important priority (Friggens et al., 2013). Thus, cows producing more milk protein in peak lactation might not be the ones producing more milk protein in late lactation.

MUN and Protein Efficiency

We found no correlation between protein efficiency (both MPE and GPE) and MUN of individual cows within diets and lactation stages. That is, for cows fed the same dietary protein content in the same lactation stage, those with lower MUN values did not necessarily use protein more efficiently. Nousiainen et al. (2004) found a negative correlation between MUN concentration and protein efficiency when they did not add

dietary protein content in their model. Dietary protein content alters both MUN and protein efficiency simultaneously, as shown in the present study and previous studies (Huhtanen and Hristov, 2009), so it is not surprising that MUN was negatively correlated with protein efficiency across cows if the effect of diet was not removed. Indeed, in the current study, we found that (1) cows on the HP diet had higher MUN and lower protein efficiency (shown in Table 1), and (2) MUN was negatively correlated with MPE ($r_{\text{peak}} = -0.49$, $P < 0.05$; $r_{\text{late}} = -0.27$, $P < 0.05$) and GPE ($r_{\text{peak}} = -0.48$, $P < 0.05$; $r_{\text{late}} = -0.27$, $P < 0.05$), if the effect of diets was not removed. Once we accounted for diet, the correlation of MUN with MPE or GPE across cows was insignificant. Huhtanen et al. (2015) also found the relationship between protein efficiency and MUN was small when the effect of diet was removed from the analysis.

Milk urea nitrogen can be affected by many factors aside from diet: dehydration (Weeth and Lesperance, 1965), the season of the year (Fatehi et al., 2012), time of sampling (DePeters and Ferguson, 1992; Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001), and variable transport activities in kidney and rumen wall (Stewart and Smith, 2005; Aguilar et al., 2012). For example, one cow can have a higher MUN concentration merely due to less urea being excreted in urine, so that more urea is circulating in the body and being transported into milk. In addition, according to Kohn et al. (2004), MUN values can be different between regions and milk analysis laboratories if different analysis methods are used. Thus, we suggest that MUN can be a good indicator for whether dietary protein is adequate or in excess in daily practice; however, ranking cows for protein efficiency based on MUN may be misleading.

CONCLUSIONS

In general, when cows were fed the LP diet compared with the HP diet, production decreased and efficiency of protein use increased. In addition, cows maintained their protein efficiency ranking across HP and LP diets within lactation stage. Protein efficiency was poorly associated with MUN; thus, we suggest that using MUN to rank cows for protein efficiency within a diet may be misleading. Significant variation in response to a decrease in dietary protein existed among cows with some cows experiencing a little drop in protein output when fed the LP diet. We could predict the change in captured protein (in milk and body tissue) of individual cows when changing dietary protein content with 60% accuracy based on their performance when fed the HP diet. The remaining 40% could be considered LPR. We recognize that some portion of LPR could be due to

measurement error, but we suggest that some portion of LPR could be due to metabolic differences among cows that have a genetic basis. High-LPR cows had similar protein efficiency as LLPR cows when fed the HP diet, but as expected, higher protein efficiency when fed the LP diet. More work is needed to examine whether LPR is repeatable across other types of base diets and other protein or amino acid supplements and different lengths of time period (1 wk vs. 4 wk vs. 10 wk, and so on). If LPR is repeatable across diets and time, further work seems warranted to determine if it has a potential genetic basis. If LPR had a genetic basis, we suggest it might be worthy of consideration in selecting cows to improve protein efficiency. Use of commercial farm data to quantify this trait might be possible because individual feed intake is not needed.

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REFERENCES

- Aguilar, M., M. D. Hanigan, H. A. Tucker, B. L. Jones, S. K. Garbade, M. L. McGilliard, C. C. Stallings, K. F. Knowlton, and R. E. James. 2012. Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. *J. Dairy Sci.* 95:7261–7268. <https://doi.org/10.3168/jds.2012-5582>.
- Al-Kanaan, A. 2016. Heat stress response for physiological traits in dairy and dual purpose cattle populations on phenotypic and genetic scales. PhD thesis, Faculty of Organic Agriculture, University of Kassel, Kassel, Germany.
- Andrew, S. M., D. R. Waldo, and R. A. Erdman. 1994. Direct analysis of body composition of dairy cows at three physiological stages. *J. Dairy Sci.* 77:3022–3033. [https://doi.org/10.3168/jds.S0022-0302\(94\)77244-1](https://doi.org/10.3168/jds.S0022-0302(94)77244-1).
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529. [https://doi.org/10.3168/jds.S0022-0302\(80\)83111-0](https://doi.org/10.3168/jds.S0022-0302(80)83111-0).
- Broderick, G. A. 2018. Optimizing ruminant conversion of feed protein to human food protein. *Animal* 12:1722–1734. <https://doi.org/10.1017/S1751731117002592>.
- Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80:2964–2971. [https://doi.org/10.3168/jds.S0022-0302\(97\)76262-3](https://doi.org/10.3168/jds.S0022-0302(97)76262-3).
- Chilliard, Y., and J. Robelin. 1983. Mobilization of body proteins by early lactating cows measured by slaughter and D2O techniques. Pages 195–198 in IVth Int. Symp. Protein Metabolism and Nutrition. EAAP Publication no. 31, vol. 2. EAAP, Rome Italy.
- DePeters, E. J., and J. D. Ferguson. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. *J. Dairy Sci.* 75:3192–3209. [https://doi.org/10.3168/jds.S0022-0302\(92\)78085-0](https://doi.org/10.3168/jds.S0022-0302(92)78085-0).
- Egger-Danner, C., J. B. Cole, J. E. Pryce, N. Gengler, B. Heringstad, A. Bradley, and K. F. Stock. 2015. Invited review: Overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. *Animal* 9:191–207. <https://doi.org/10.1017/S1751731114002614>.
- Fatehi, F., A. Zali, M. Honarvar, M. Dehghan-Banadaky, A. J. Young, M. Ghiasvand, and M. Eftekhari. 2012. Review of the relationship between milk urea nitrogen and days in milk, parity, and monthly temperature mean in Iranian Holstein cows. *J. Dairy Sci.* 95:5156–5163. <https://doi.org/10.3168/jds.2011-4349>.
- Friggens, N. C., L. Brun-Lafleur, P. Faverdin, D. Sauvant, and O. Martin. 2013. Advances in predicting nutrient partitioning in the dairy cow: Recognizing the central role of genotype and its expression through time. *Animal* 7(s1):89–101. <https://doi.org/10.1017/S1751731111001820>.
- Gibb, M. J., W. E. Ivings, M. S. Dhanoa, and J. D. Sutton. 1992. Changes in body components of autumn-calving Holstein Friesian cows over the first 29 weeks of lactation. *Anim. Prod.* 55:339–360. <https://doi.org/10.1017/S0003356100021036>.
- Huhtanen, P., E. H. Cabezas-Garcia, S. J. Krizan, and K. J. Shingfield. 2015. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. *J. Dairy Sci.* 98:3182–3196. <https://doi.org/10.3168/jds.2014-8215>.
- Huhtanen, P., and A. N. Hristov. 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. *J. Dairy Sci.* 92:3222–3232. <https://doi.org/10.3168/jds.2008-1352>.
- Huhtanen, P., J. I. Nousiainen, M. Rinne, K. Kytölä, and H. Khalili. 2008. Utilization and partition of dietary nitrogen in dairy cows fed grass silage-based diets. *J. Dairy Sci.* 91:3589–3599. <https://doi.org/10.3168/jds.2008-1181>.
- Kauffman, A. J., and N. R. St-Pierre. 2001. The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and jersey cows. *J. Dairy Sci.* 84:2284–2294. [https://doi.org/10.3168/jds.S0022-0302\(01\)74675-9](https://doi.org/10.3168/jds.S0022-0302(01)74675-9).
- Kohn, R. A., K. R. French, and E. Russek-Cohen. 2004. A comparison of instruments and laboratories used to measure milk urea nitrogen in bulk-tank milk samples. *J. Dairy Sci.* 87:1848–1853. [https://doi.org/10.3168/jds.S0022-0302\(04\)73342-1](https://doi.org/10.3168/jds.S0022-0302(04)73342-1).
- Komaragiri, M. V., and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. *J. Dairy Sci.* 80:929–937. [https://doi.org/10.3168/jds.S0022-0302\(97\)76016-8](https://doi.org/10.3168/jds.S0022-0302(97)76016-8).
- Liu, E., and M. J. VandeHaar. 2020. Relationship of residual feed intake and protein efficiency in lactating cows fed high- or low-protein diets. *J. Dairy Sci.* 103:3177–3190. <https://doi.org/10.3168/jds.2019-17567>.
- Martin, P., H. W. Barkema, L. F. Brito, S. G. Narayana, and F. Miglior. 2018. Symposium review: Novel strategies to genetically improve mastitis resistance in dairy cattle. *J. Dairy Sci.* 101:2724–2736. <https://doi.org/10.3168/jds.2017-13554>.
- Moraes, L. E., E. Kebreab, J. L. Firkins, R. R. White, R. Martineau, and H. Lapierre. 2018. Predicting milk protein responses and the requirement of metabolizable protein by lactating dairy cows. *J. Dairy Sci.* 101:310–327. <https://doi.org/10.3168/jds.2016-12507>.
- Mulder, H. A., L. Rönnegård, W. F. Fikse, R. F. Veerkamp, and E. Strandberg. 2013. Estimation of genetic variance for macro- and micro-environmental sensitivity using double hierarchical generalized linear models. *Genet. Sel. Evol.* 45:23. <https://doi.org/10.1186/1297-9686-45-23>.
- Nousiainen, J., K. J. Shingfield, and P. Huhtanen. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *J. Dairy Sci.* 87:386–398. [https://doi.org/10.3168/jds.S0022-0302\(04\)73178-1](https://doi.org/10.3168/jds.S0022-0302(04)73178-1).
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

- Sebek, L., J. Vanriel, and G. Dejong. 2007. The breeding value for milk urea as predictor for the efficiency of protein utilization in dairy cows. Report 81 from Animal Sciences Group of Wageningen UR. Wageningen UR, Wageningen, the Netherlands.
- Sinclair, K. D., P. C. Garnsworthy, G. E. Mann, and L. A. Sinclair. 2014. Reducing dietary protein in dairy cow diets: Implications for nitrogen utilization, milk production, welfare and fertility. *Animal* 8:262–274. <https://doi.org/10.1017/S1751731113002139>.
- Stewart, G. S., and C. P. Smith. 2005. Urea nitrogen salvage mechanisms and their relevance to ruminants, non-ruminants and man. *Nutr. Res. Rev.* 18:49–62. <https://doi.org/10.1079/NRR200498>.
- Stoop, W. M., H. Bovenhuis, and J. A. M. Van Arendonk. 2007. Genetic parameters for milk urea nitrogen in relation to milk production traits. *J. Dairy Sci.* 90:1981–1986. <https://doi.org/10.3168/jds.2006-434>.
- Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of the milk. *J. Dairy Sci.* 48:1215–1223.
- VandeHaar, M. J., L. E. Armentano, K. Weigel, D. M. Spurlock, R. J. Tempelman, and R. Veerkamp. 2016. Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. *J. Dairy Sci.* 99:4941–4954. <https://doi.org/10.3168/jds.2015-10352>.
- Wattiaux, M. A., E. V. Nordheim, and P. Crump. 2005. Statistical evaluation of factors and interactions affecting dairy herd improvement milk urea nitrogen in commercial Midwest dairy herds. *J. Dairy Sci.* 88:3020–3035. [https://doi.org/10.3168/jds.S0022-0302\(05\)72982-9](https://doi.org/10.3168/jds.S0022-0302(05)72982-9).
- Weeth, H. J., and A. L. Lesperance. 1965. Renal function of cattle under various water and salt loads. *J. Anim. Sci.* 24:441–447. <https://doi.org/10.2527/jas1965.242441x>.
- Zamani, P., S. R. Miraei Ashtiani, D. Alipour, M. Tabatabaei, H. Aliarabi, A. A. Saki, and A. Abdolmohammadi. 2011. Statistical analysis of some factors affecting crude protein balance in lactating dairy cows. *J. Agric. Sci. Technol.* 13:1033–1043.

ORCIDS

- E. Liu <https://orcid.org/0000-0001-8608-9074>
 M. J. VandeHaar <https://orcid.org/0000-0002-8475-3493>