NEW APPROACHES TO ASSESS AND IMPROVE PROTEIN EFFICIENCY IN LACTATING DAIRY COWS

By

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ABSTRACT

NEW APPROACHES TO ASSESS AND IMPROVE PROTEIN EFFICIENCY IN LACTATING DAIRY COWS

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The long-term goal of the work is to improve protein efficiency in lactating dairy cows. To achieve this goal, four specific objectives were proposed: 1) determine the relationship of residual feed intake (RFI) to protein efficiency in lactating Holstein cows fed high or low protein diets, 2) determine whether low protein resilience (LPR) is an indicator of protein efficiency in individual dairy cows, 3) examine the association of digestibility with RFI and LPR in lactating dairy cows, and 4) quantify the importance of including body weight (BW) change in the cow response to decreased dietary protein content and develop models for predicting BW change when dietary protein is altered. Lactating Holstein cows (n= 166; 92 primiparous, 77 multiparous) with initial milk yield (MY) of 41 ± 9.8 kg/d were fed high (HP) and low (LP) protein diets in crossover experiments of two 28-35 d periods. Experiments were repeated in 69 of the 166 cows (42 primiparous, 27 multiparous) in late lactation. Low protein diets were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate rumen-degraded protein to maintain rumen function. Expeller soybean meal was added to formulate the HP diet, which contained 18% CP in peak lactation and 16% CP in late lactation. Cows were milked twice daily; DMI and MY were recorded once daily. Milk composition was measured over 4 consecutive milkings weekly, and BW was measured 3 times weekly. Samples of feed ingredients, orts and feces were collected in the last 5 days of each period and analyzed to determine digestibilities of DM, NDF, and CP for each cow on each diet. Fixed effects of diet, parity, treatment sequence nested in experiment, treatment period nested in experiment,

interaction of parity and diet, and random effects of experiment and cow nested within experiment were included in models to compare production of cows fed different levels of CP. Protein efficiency was calculated for each cow on each diet in both peak lactation and late lactation. Residual feed intake was estimated for each cow on each treatment based on the actual intake, milk energy output, metabolic BW, and body energy change (estimated from BW change and BCS). Low protein resilience was estimated for each cow in peak lactation and also late lactation, based on protein captured in milk and body tissue when fed the LP vs HP diet. A negative correlation was observed between RFI and protein efficiency in cows fed the HP and LP diets in peak lactation and cows fed the HP diet in late lactation. Cows with higher LPR values had similar protein efficiency on the HP diet but significantly higher protein efficiency on the LP diet. Neither RFI nor LPR was correlated with digestibility regardless of diets or lactation stages. When dietary protein content was reduced, 40-50 % of the total energy loss, 10-20 % of total protein loss, and 15-25% of total income loss were due to BW loss, indicating that considering only changes in milk production underestimates the impact of dietary protein changes. In conclusion, 1) cows with lower RFI values utilized protein more efficiently, and protein efficiency will be improved in the process of selecting dairy cattle for low RFI, 2) cows with higher LPR values are better able to maintain production and have higher protein efficiency to adapt to low-protein feeding conditions, 3) variation in digestibility cannot explain the variations of RFI or LPR among lactating dairy cows, and we suggest that post-absorptive metabolism explains most of the variation in RFI and LPR when lactating cows are fed diets with minimal NDF in peak lactation and 40% NDF in late lactation, and 4) body reserve mobilization should not be neglected when assessing the cow response to changes in dietary protein

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AA = amino acids

AMP = adenosine diphosphate

mRNA = messenger RNA, or message Ribonucleic acid

ATP = adenosine triphosphate

BodyE = energy expended for body tissue gain

BodyP = protein captured for body tissue gain

BUN = body urea nitrogen

BW = body weight

CapE = total energy capture

CapP = total protein capture

CBW = calf birth weight

CP = crude protein

CW = conceptus weight

d = day

dBW = mean daily BW change

dEBW = mean daily change of empty body weight

DHIA = dairy herd improvement association

DietNE_L = apparent diet energy content

DIM = days in milk

DM = dry matter

DMI = dry matter intake

EAA = essential amino acids

EBW = empty body weight

ECM = energy corrected milk

BCS = body condition score

ECM: feed = energy-corrected milk per unit of feed

Exp = experiment

GE = gross energy

GPE = gross protein efficiency

h = hours

HH = hip height

HLPR = high LPR group

HP = high protein

HRFI = high RFI group

IOFC = income over feed cost

LifeNitroEff = lifetime nitrogen efficiency

LLPR = low LPR group.

LP = low protein

LPR = low Protein Resilience

LRFI = low RFI group

MBW = metabolic body weight

ME = metabolizable energy

MCP = ruminal microbial protein

Milk: feed = milk to feed ratio

MilkE = milk energy output

MilkP: FeedP = milk protein efficiency

MLPR = medium LPR group

MNE = milk nitrogen efficiency

MP = metabolizable protein

MPE = milk protein efficiency

MRFI = medium RFI group

mTOR = mammalian target of rapamycin

MUN = milk urea nitrogen

MY = milk yield

N = nitrogen

NDF = neutral detergent fiber

NE = net energy

Par = parity

PDV = portal drained viscera

PregE = energy expended for pregnancy

PregP = protein captured for pregnancy

RDP = rumen degradable protein

RE = retained energy

REI = residual energy intake

RFI = residual feed intake

RUP = rumen undegradable protein

VIF = variance inflation factors

CHAPTER 1

LITERATURE REVIEW

FEED EFFICIENCY AND RESIDUAL FEED INTAKE

Feed Efficiency in Dairy Industry: From Milk: Feed to Residual Feed Intake

Many terms have been used to define feed efficiency in the dairy industry. The earliest and simplest definition is milk to feed ratio (milk: feed), or the amount of milk output per unit of feed (Hooven et al., 1972). Although milk: feed is straightforward and easy to understand, it does not account for milk components in the milk output. Milk: feed does not differentiate cows with different yields of milk protein and fat if milk yield is similar. To address this issue, Grieve et al. (1976) and Custodio et al. (1983) revised the definition of feed efficiency to the amount of energy-corrected milk per unit of feed (ECM: feed) by adjusting milk production based on yields of milk protein and fat. ECM accounts for the difference in milk energy, thus is more appropriate to measure feed efficiency in the modern dairy industry. However, ECM: feed is still limited as it does not account for body reserve mobilization. In lactating dairy cows, body reserve is generally mobilized to support milk production, especially in the early lactation. Excessive body reserve mobilization can lead to metabolic disorders and impair future production performance (NRC, 2001). Thus, cows that produce more milk at the expense of excessive body reserve mobilization should not be considered efficient. Gross efficiency is defined as the energy captured in both milk and body tissue divided by feed energy intake (VandeHaar and St-Pierre, 2006); gross efficiency can distinguish cows that convert more feed to product (milk and body reserve), from ones that mobilize more body reserve to milk. However, gross efficiency also has limitations. In the dairy industry, milk production is more valuable than body tissue gain. Moreover, for cows with BCS over 3.5, body tissue gain is not desirable (NRC, 2001). Thus, cows that convert more

feed to milk rather than body reserve gain are preferable. Also, BW change is difficult to measure and usually not known; even under the condition that BW change is known, it is still challenging to compute GPE correctly since there is likely genetic variability between cows in how cows convert feed to BW gain.Therefore, compared to milk: feed and ECM: feed, gross efficiency is not commonly used to define efficiency in the current dairy industry.

Essentially, efficiency consists of two aspects:1) more energy directed towards milk (and body tissue) than to maintenance; 2) better ability to convert gross energy (GE) to net energy (NE). As modern dairy cows consume at intake levels of four to five times maintenance, marginal benefits from further increasing production are decreasing (VandeHaar, 1998). Future improvements in feed efficiency can be attainable by selecting cows that can convert GE to NE more efficiently. Residual feed intake (RFI), calculated as the difference between the actual feed intake and the predicted feed intake, is an alternative definition for feed efficiency in livestock. Cattle consuming less than expected will be assigned negative RFI values and considered more efficient in converting GE to NE. RFI was first proposed by Koch et al. (1963) as a direct measurement of energy efficiency in beef heifers and bulls. Over the years, RFI has been established, evaluated, and validated in the beef industry (Herd et al., 2004; Durunna et al., 2011); RFI was proposed for the dairy industry in the 1990s (Veerkamp et al., 1995), and has received more attention in the past 10 years (Connor, 2015). Different equations were used to estimate RFI in dairy heifers (Rius et al., 2012) and lactating dairy cows (Tempelman et al., 2015; Potts et al., 2017). Although the equations were different, there are commonly accepted ground rules when estimating RFI. That is, all production variables, including energy partitioned towards milk, maintenance, and BW change, should be taken into account when estimating RFI (Connor, 2015). This avoids bias regarding body size and milk production level when comparing

cows in the same cohort. As milk production and maintenance are already adjusted in the calculation of RFI, RFI is independent of predicted maintenance energy based on $BW^{0.75}$ and milk energy output (Pryce et al., 2012; Connor, 2015).

Genetic Selection for Residual Feed Intake

The heritability of RFI in beef cattle has been repeatedly shown to be between 0.38 and 0.62 (Archer et al., 1997; Schenkel et al., 2004). However, in dairy cattle, there are limited data available and the estimated heritability of RFI varied widely from 0.01 to 0.38 (Veerkamp et al., 1995; Vallimont et al., 2011; Pryce et al., 2012). Recently, a large data set was pooled through collaboration across institutes, making it possible to more accurately estimate RFI heritability in dairy cows. Based on the data from 5000 Holstein cows across countries, the estimated RFI heritability for dairy cows was about 0.17 (Tempelman et al., 2015). With this level of heritability, genetic selection for reduced feed intake with no loss in milk should be fruitful (Tempelman et al., 2015). Given all the benefits that come with using RFI when selecting efficient dairy cows, the breeding programs in New Zealand and Netherlands have already incorporated RFI into the selection index. In the next 3-5 years, RFI will be included in the breeding programs in the U.S. as well.

Repeatability of Residual Feed Intake across Diets and Physiological State

By definition, repeatability is a measure of the strength of relationship between repeataed records for a trait in a population (Boake, 1989). Repeatability across diets for a selection index is essential for the genetic selection to be effective. High repeatability across diets can assure that the values obtained from the test population will apply to cows fed with various diets. In lactating dairy cattle, RFI is repeatable across diets that vary in starch and NDF contents (Potts et al., 2015; Mangual et al., 2016). However, no studies have examined RFI repeatability across

dietary protein content in dairy cows. Most diets in previous RFI studies contained adequate or even excessive protein (Mangual et al., 2016; Potts et al., 2015; Tempelman et al., 2015). However, as ~42% of the total feed cost for a lactating cow is associated with protein (St-Pierre, 2012), feeding diets with less protein is becoming more and more common. For example, from 2004 to 2010, there was a 1.1%-unit reduction of CP content in dairy rations in Wisconsin (Shaver, 2010). Thus, it is important to understand whether RFI remains the same across diets with high- versus low- protein diets. Using RFI to determine protein efficiency will be misleading if it is not repeatable across protein contents.

Moreover, a useful breeding index should also be repeatable across lactation stages. However, the repeatability of RFI across physiological stages is still inconclusive in cattle. RFI in beef cattle appears to be repeatable over different stages of production cycles. In Hurley et al. (2017), the estimated repeatability of residual energy intake (REI) across lactation stages ranges from 0.19 to 0.23 in a sample of 1290 Holstein-Friesian cows. 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). Thus, more effort is needed for us to better understand the reason for lack of repeatability of RFI across lactation stages before using it for genetic selection; otherwise, RFI information obtained from cows in a certain period of lactation can be misleading.

Sources of Variation for Residual Feed Intake

Richardson and Herd (2004) listed various sources of variation in RFI in beef cattle which included feeding behavior, body composition, protein turnover and tissue metabolism, heat increment of feeding, digestive efficiency, and physical activity. Among these sources, 12%

of the RFI variation was from feeding behavior and physical activity, 10% was from digestibility, and 37% was from protein turnover and tissue metabolism (Figure 1. 1; Richardson and Herd, 2004). Considering that 27% of RFI variation in Richardson and Herd (2004) was unknown and might be measurement errors, the contribution of the sources mentioned above (feeding behavior and physical activity, digestibility, and protein turnover and tissue metabolism) is even greater. Thus, the discussion of possible sources of RFI variation among dairy cows will focus on these sources.

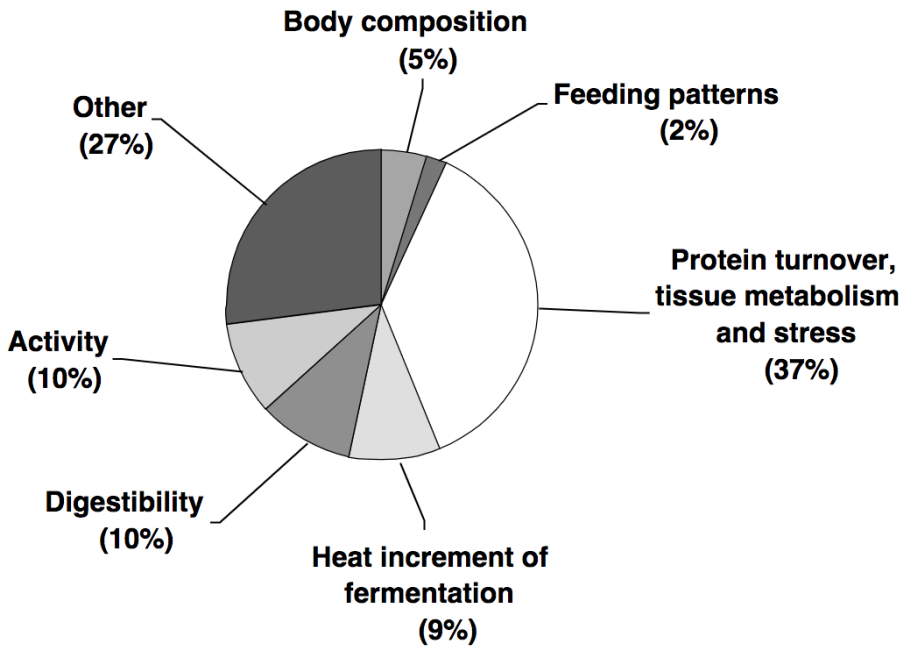

Figure 1.1 Contributions of biological mechanisms to variation in residual feed intake as determined from experiments on divergently selected cattle (Richardson and Herd, 2004)

One source of RFI variation among dairy cows can be feeding behavior and general activity. For example, studies have shown that, compared to high-RFI dairy heifers, low-RFI dairy heifers eat less frequently and spend less time eating per meal (Durunna et al., 2011; Green et al., 2013; Fitzsimons et al., 2014). Later McGee et al. (2014) found that heifers fed with different levels of concentrate had varying feeding behaviors which were associated with varying

RFI across the diets. More studies are needed before any conclusion can be drawn regarding the association between RFI and feeding behavior. In addition to the feeding behavior, difference in general activity may also contribute to the RFI variation. Gomes et al. (2013) demonstrated that low- RFI steers tend to spend more time lying and less time standing. As a result, the energy expense in feeding behavior and activity for low-RFI cattle is less than those with high-RFI values.

Another factor that has been investigated in explaining the variation in RFI is the variation in digestion. Prior work has examined the link between RFI variation and digestive variability among cattle; however, findings are inconclusive. Specifically, RFI was associated with digestibility in some studies (Richardson and Herd, 2004; Nkrumah et al., 2006; McDonald et al., 2010; Rius et al., 2012), but not others (Cruz et al., 2010; Lawrence et al., 2011). One possible explanation of the inconsistent findings might be due to the difference in diets across the studies (Rius et al., 2012). Potts et al. (2017) examined the association of RFI and nutrient digestibility across high- and low-starch diets, and found that RFI was only associated with digestibility in cows fed low-starch diets but not high-starch diets. Findings in Potts et al. (2017) supported the notion that the association between RFI and nutrient digestibility depended on the diet. However, it is still unclear what it is in the diet that influences the association between RFI and digestibility.

The next set of important factors that can impact RFI values in cattle are protein turnover and tissue metabolism. Protein turnover is energetically expensive in cattle; higher rates of protein turnover were shown to be related to higher maintenance expense (Castro Bulle et al., 2007), and thus leading to cows having higher RFI values. Richardson and Herd (2004) found that protein turnover and tissue metabolism contributed up to 37% of the RFI variation in beef

cattle. However, the link between RFI and protein turnover in dairy cattle is less clear. In one of the few studies exploring the relationship between protein turnover and RFI, Lawrence et al. (2012) did not find any association between them two. No other work has validated the results in other physiological states of dairy cows. If protein turnover is a significant contributor to RFI variation among beef cattle, it will likely explain at least part of the variation in RFI in dairy cows.

Linking Residual Feed Intake to Protein Efficiency

Protein turnover rates accounted for some of the variation in RFI (Richardson and Herd, 2004) and were also negatively associated with protein utilization efficiency in dairy cows (Herd et al., 2004; Castro Bulle et al., 2007). RFI is usually calculated on an energy basis, and, although we expect that RFI and protein efficiency are associated with each other to some extent, the direct link between RFI and protein efficiency has not been demonstrated. In lactating dairy cows, Xi et al. (2016) and Mangual et al. (2016) hypothesized an association between the two. There has been some existing work done in dairy heifers (Rius et al., 2012; Thornhill et al., 2014; Marett et al., 2017); however, no evidence supported such a link. No work has directly examined the link in the lactating dairy cows. As protein requirement and protein metabolism in lactating dairy cows were different from those in heifers, research work on lactating cows is in need before any conclusion can be drawn.

PROTEIN EFFICIENCY

Terms to Define Protein Efficiency

There are a number of different ways to define protein efficiency, and each one has its own advantages and limitations. Milk protein efficiency, milk protein yield per unit of feed

protein consumption (MilkP: FeedP), sometimes referred to as milk nitrogen efficiency (MNE), is the most commonly used term to describe protein efficiency in the dairy industry. According to a meta-analysis done by Huhtanen and Hristov (2009), using data from North America and Europe from 1979 to 2005, the average MNE was 25%, with a range from 10% to 40%. Although easier to calculate and commonly used, MNE is limited due to failing to consider body protein reserve mobilization. In addition, by including body protein capture in addition to milk protein output, the protein-efficiency measure can be applied to cows in other physiological states (e.g., dry cows, heifers, and 1st lactation animals that are not at mature BW). Dairy cows spend about half of their lives as replacement heifers and dry cows; thus, protein efficiency in those stages is definitely critical to measure. In addition, by considering BW change, the protein-efficiency term that includes body protein can also enable a comparison of protein efficiency among different production enterprises in the livestock industry, such as cattle, swine, and poultry. However, relative to a large body of literature on feed efficiency, few publications discussed this complete measure of protein efficiency that includes both milk production and body tissue gain. A term accounting for lifetime nitrogen efficiency (LifeNitroEff) has been proposed to measure the protein efficiency throughout the entire life cycle (Foskolos and Moorby, 2018). LifeNitroEff considers not only the milk protein production, but also the growth and body composition change, protein expenditure for reproduction, and immune responses. Due to the complexity of measurement, calculation, and modeling, LifeNitroEff has not been adopted for use by the dairy industry to date.

The next measure of protein efficiency is called human edible protein efficiency. As noted by Broderick (2017), ruminants convert human-inedible protein to human-edible protein, while monogastric animals competed with humans for feedstuff that is potential human food.

Thus, Broderick (2017) argued that it was not appropriate to directly compare protein efficiency between ruminants and monogastric animals. To address this, Broderick (2017) suggested using a term, human edible protein efficiency. Similar to MilkP: FeedP, human edible protein efficiency was calculated as the protein in the product per unit of human-edible dietary protein, which adjusts protein based on the feed source. After the adjustment, ruminants are much more efficient than swine and poultry (2.08 for milk, 1.19 for beef, 0.29 for pork, and 0.62 for poultry).

In addition to all the ratio terms mentioned above, a biological indicator, milk urea nitrogen (MUN), is also commonly used to indicate protein efficiency in the dairy industry. MUN was first proposed as an indicator of protein efficiency by Oltner and Wiktorsson (1983). When protein (especially rumen degradable protein; RDP) exceeds microbial needs, there will be a large amount of ammonia produced in the rumen. Ammonia is then converted to urea in the liver (Colmenero and Broderick, 2006), and urea equilibrates between body fluids (DePeters and Ferguson, 1992). A high concentration of MUN indicates high body urea nitrogen (BUN) and inefficient utilization of protein (Broderick and Clayton, 1997; Roseler et al., 1993; Gustafsson and Palmquist, 1993).

Improving Protein Efficiency by Nutritional Means

Many nutritional means have been explored to improve protein efficiency (Sinclair et al., 2014; Broderick et al., 2015; Gidlund et al., 2015). After years of examination, several critical areas of protein utilization in dairy cows have been identified to improve protein efficiency. These critical areas include protein degradation and synthesis in the rumen, digestion and absorption in the small intestine, absorbed amino acids (AA) passing through portal drained

viscera (PDV) and liver, and AA extraction and utilization in mammary glands. The discussion below will be focused on how nutrition practice can potentially impact these four areas.

Protein degradation and synthesis in the rumen. Over 30% of the total AA pool comes from dietary AA (Apelo et al., 2014). Thus, the efficiencies of protein digestion and AA absorption play important roles in determining overall protein efficiency. In rumen, the efficiency of protein degradation and ruminal microbial protein (MCP) synthesis are critical. It was commonly assumed that maximal microbial growth is equal to maximal nitrogen efficiency in the rumen. However, more nitrogen outflow is achieved per unit of of N consumed in low RDP diets. Clearly, maximal microbial growth cannot be achieved under this condition. Thus, to clarify, this section mainly reviews factors that can maximize MCP synthesis. Extensive work has been done to examine the impacts of diet and feed management factors on MCP synthesis. These factors include energy content and source, RDP content, matching content and source of carbohydrate and RDP content, forage source and length, and RDP source. Dietary energy level was initially considered as one of the most influential factors on MCP synthesis, because MCP synthesis improved significantly when increasing dietary starch level (Febel and Fekete,1996). However, in Russell and Wallace (1997), higher starch contents decreased rumen pH and fiber digestion, reducing the synthesis of de novo amino acids, and therefore depressed MCP synthesis. In Hoover and Stokes (1991), dietary RDP content was found to increase MCP synthesis, however, the optimal level of RDP also depended on the content and source of starch. Therefore, RDP content should be matched with energy level, otherwise protein will be wasted in the form of ammonia, or energy will be wasted in the form of heat (Kolver et al., 1998; Moharrery, 2004). In order to achieve maximal utilization efficiency of both RDP and starch, the ideal ratio of RDP to starch is 1: 4 (Yang et al., 2010), but the optimal ratio varies along with

other dietary factors (e.g., energy source and RDP source; Yang et al., 2010). Besides matching contents of energy and RDP, sources of energy and RDP should also be matched. Cone et al. (1989) and Chamberlain et al. (1993) showed that different energy sources have different effects on the MCP synthesis. The work done by Cone et al. (1989) showed that oat and barley fermented faster than corn, and negatively impacted the MCP synthesis. Chamberlain et al. (1993) found that when RDP was mostly from urea, supplementing soluble sugars (saccharose, lactose, and fructose) increased MCP synthesis, compared to supplementing cereals (high in starch). In addition to energy and RDP, forage can also affect MCP synthesis. The reason why forage source affects MCP synthesis is similar to that of starch source. Forage with slower digestion rates and longer particle sizes would maintain a more consistent rumen pH, a more functional rumen, and thus greater MCP synthesis. Lastly, RDP source can also impact MCP synthesis. Supplementing diets with RDP from non-urea sources, versus urea sources, increased ruminal microbial protein synthesis (Kertz, 2010). However, not all microbial species can efficiently use a sole source of RDP; to optimize growth of rumen microbes, a mix of urea, AA, and peptide is preferred. Additionally, supplementing certain AA (e.g., Phe, Leu, Ile) can inhibit microbial growth. Previous work has shown that, when supplementing Phe, Leu, and Ile, ruminal de novo AA synthesis fell up to 80% (Atasoglu et al., 1999). Besides MCP synthesis, nitrogen recycling can also impact rumen nitrogen utilization efficiency. According to Wallace and McPherson (1987), microbial nitrogen recycling significantly affect ruminal protein utilization and it is mainly associated with protozoal predation on ruminal bacteria, where protozoal predation mainly depends on the energy availability (Dijkstra et al., 1992). Also, urea in saliva circulating back to rumen contributes to the RDP pool and thus influences rumen nitrogen utilization efficiency. Based on the model in Dijkstra et al. (1992), saliva circulation is related to

DMI and dietary NDF content. Thus, the factors that can impact DMI (which will be discussed in the section "Other means to improve protein efficiency") can all potentially impact nitrogen recycling and overall rumen nitrogen efficiency. All the nutritional optimizations above are under the assumption that other nutrients (such as minerals and vitamins) are not limiting. In daily practice, minerals and vitamins should also be supplemented sufficiently.

Digestion and absorption in the small intestine. More absorbable rumen undegradable protein (RUP) and protected AA can enhance intestinal AA profile, increase milk protein yield, and increase protein efficiency in dairy cows (NRC, 2001). For example, supplementing methionine for soy-based diets, lysine for corn-based diets, and histidine for grass-based diets can improve milk yield and protein efficiency (Schwab and Broderick, 2017). However, as reviewed by Santos et al. (1997), the effect of protected AA supplements sometimes might be lower than expected. The discrepancy could be due to the following reasons: 1) supplementing RUP decreased MCP synthesis (Schwab, 1994), 2) the RUP source did not balance out the AA shortage in the base diets (e.g., supplementing corn gluten meal to the corn-based diets; Chandler, 1991), 3) the RUP sources (e.g., feather meal, meat and bone meal, and blood meal) had low intestinal digestibility (Schingoethe, 1991), and 4) RUP level in the base diets might already have been high enough.

Absorbed AA passing through portal drained viscera (PDV) and liver. 3-10% of the absorbed AA are catabolized when they first pass through PDV, and up to 50% are catabolized on a daily basis (Apelo et al., 2014). Based on Hanigan et al. (1998; 2004), AA not utilized by mammary glands would be catabolized in PDV, and the remaining AA that flow out from PDV would circulate back to mammary glands, becoming available for milk protein synthesis again. The catabolic rates of different AA are different in PDV. MacRae et al. (1997) found that 13-

25% of Leu, Ile, Val, Lys, Thr, and His were catabolized in PDV, while up to 54% of Phe was catabolized. This work suggested that the requirement of Phe was higher than other AA in PDV. Similar to the metabolism of PDV, AA were also catabolized and utilized when passing through liver (Lobley et al., 2000). The catabolized AA were utilized in several critical metabolic steps, including: 1) converting carbon skeleton of deaminated AA to glucose or lipids, and 2) synthesizing critical protein (e.g., albumin) from non-essential AA (Lobley et al., 2000). Similar to the AA metabolism in PDV, the AA catabolism in liver entirely depends on the AA availability in blood flow when passing through liver. The work done by Reynolds (2005; 2006) showed that, when protein synthesis in mammary glands decreased, plasma AA concentration increased, and more AA were removed by liver. Therefore, to minimize the AA catabolism in liver and PDV, maximizing protein utilization in mammary glands is required to minimize the AA concentration in blood flow.

AA utilization in mammary glands. AA uptake and milk protein synthesis are the two critical steps in regulating protein utilization efficiency in mammary glands. Several factors alter AA uptake and milk protein synthesis; these included plasma AA concentration, blood flow rate, plasma energy/AA status, and factors influencing mammalian target of rapamycin (mTOR) pathways (e.g., certain EAA, insulin, prolactin, cortisol, AMP/ATP). Plasma AA concentrations can influence AA uptake by mammary glands. The AA that are taken up into mammary glands exhibit Michaelis-Menten kinetics, in other words, the amount of AA uptake is maximized at infinite concentrations of AA (Neal and Thornley, 1983). However, AA uptake efficiency, if calculated as moles removed per mole delivered to the tissue, would be maximized as supply approaches zero. An optimal range of AA should be the amount of each AA that results in maximal protein synthesis. Thus, an optimal range of AA is usually expressed as per unit of milk

protein output; AA concentration exceeding that optimal range will decrease efficiency while AA concentration below the optimum will not decrease efficiency. Another factor that influences protein synthesis and protein efficiency is the blood flow rate. In Rius et al. (2010), faster blood flow within mammary glands induced by arginine supplementation increased milk yield. This is consistent with previous studies showing a positive link between blood flow and milk yield/ protein efficiency (Cant and McBride, 1995; Hanigan et al., 2002). Lastly, nutrients and hormones influencing signaling pathways can also increase protein efficiency via activating translation of milk protein mRNA. mTOR is the most well-studied pathway among all the pathways that control the activity of milk protein synthesis. Activation of mTOR pathway enhances protein synthesis, and thus increases protein efficiency (Rius et al., 2010). There are several factors (e.g., EAA such as Leu/ Ile/ Thr, insulin, cortisol, and cellular energetic status such as AMP/ATP ratio) that can be manipulated to directly activate mTOR pathways (Apelo et al., 2014).

Other means to improve protein efficiency. Protein efficiency is defined as the ratio of milk protein yield (the numerator) to dietary protein intake (the denominator). Thus, theoretically, all the factors that can impact protein intake and milk protein output have the potential to impact protein efficiency. If this is the case, in addition to all the nutritional means mentioned above, management practices, such as grouping precise feeding diet reformulation frequency, forage harvest and ensiling process, feeding consistency, stocking density, barn temperature and humidity, improving cow comfort, and managing photoperiod, can also impact protein efficiency on herd level (Jonker et al., 2002).

Improving Protein Efficiency by Genetic Selection

Considerable effort has been made to explore the feasibility of using genetic selection to improve protein efficiency in dairy cattle. Although a medium level of heritability in protein efficiency was detected in lactating dairy cows (0.10- 0.31; Li et al.,1998; Zamani et al., 2011), a number of issues have been raised regarding selecting dairy cows based on the traditional protein efficiency term (milk protein per unit of dietary protein intake; Zetouni et al., 2017). Most of the doubt was due to the fact of protein efficiency being a ratio trait. The drawback of ratio traits is that they 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., protein efficiency) in genetic selection induces large error variance and unexpected results. Another issue for using MilkP: FeedP as a selection index is the reliability of the heritability calculated from the small data set. Protein efficiency data in individual cows is limited, as individual intake data is usually not available in commercial production settings. Accordingly, the data set size used to calculate the heritability of protein efficiency is commonly less than 600 cows (Zamani et al., 2011), which is generally much lower than the number required for an accurate heritability estimate (Misztal, 1997). Due to the two concerns mentioned above, MUN was proposed as an alternative selection index to indirectly improve protein efficiency (Wood et al., 2003). The variation in MUN within cow and herd has been widely recognized (Huhtanen et al., 2015). The heritability for MUN concentration ranges from 0.13 to 0.22 (Mitchell et al., 2005; Stoop et al., 2007; Bastin et al., 2009). The studies using MUN data suggest that there is a genetic component in the variation of protein efficiency. However, using MUN to represent protein efficiency is questionable. Nousiainen et al. (2004) observed a quadratic relationship between protein efficiency and MUN concentration in a meta-analysis. The work done by Nousiainen et al. (2004) suggested that MUN may not be a good

representation of the true protein efficiency when cows are fed excessive protein. As mentioned earlier, dairy cows are typically fed excessive protein on commercial farms to maximize production. As most of the MUN data used for calculating the genetic variation is from commercial farms (Michell et al., 2005; Stoop et al., 2007; Bastin et al., 2009), precaution should be taken when interpreting the results calculated from MUN data. Additionally, MUN concentration can be affected by many other factors: dehydration (Burgos et al., 2001; Weeth and Lesperance, 1965), the season of the year, time of sampling (Depeters and Cant, 1992; Kauffman and St-Pierre, 2001; Broderick and Clayton, 1997) and variable transport activities in kidney and rumen wall (Aguilar et al., 2012; Stewart and Smith, 2005). For example, MUN concentration can be elevated merely due to less blood urea transported to urine. According to Kohn et al. (2004), MUN values can be different between regions and milk analysis laboratories using different analysis methods. To sum, MUN can be used to monitor protein feeding in daily practice; however, it is not suitable to help define true protein efficiency in dairy cattle. Ranking cows for their protein efficiency based on MUN concentration can be misleading.

The Most Effective Nutritional Method: Lowering Dietary Protein Content?

Due to the drawbacks mentioned above in genetic selection for improving protein efficiency, nutritional manipulation might be a more effective way to improve protein efficiency. Among all nutritional means, the most effective and economical way is to lower dietary protein content (Huhtanen and Hristov, 2009). However, it is also well recognized that the reduction of protein intake could lead to reduced DMI, and consequently MY (Cantalapiedra-Hijar et al., 2014). The emerging challenge is to figure out ways to lower dietary protein intake while mitigating against the effects of low protein on DMI and milk production (Sinclair et al., 2014; Huhtanen et al., 2008; Ingvartsen and Andersen, 2000). If the protein shortage decreases milk

production, then the savings from feeding less protein is outweighed by the lost milk revenue. In this case, we need to identify cows with better ability to maintain their milk production and body reserve when fed low- protein diets.

LOW PROTEIN RESILIENCE

Resilience in Animal Science

The idea of resilience originated from developmental psychology (de Terte and Stephens, 2014). By definition, resilience is "the ability to mentally or emotionally cope with a crisis or to return to pre-crisis status quickly". In the animal production system, resilience can be defined as the ability of the animal to maintain its normal state after exposure to environmental disturbances, or the ability to quickly return to a normal state (Colditz and Hine, 2016). Several definitions of resilience and resilience- associated concepts (robustness, tolerance, resistance, plasticity, environmental sensitivity, canalization, and stability) have been discussed in the literature (Knap, 2005; Mulder et al., 2013; Colditz and Hine, 2016). "General" resilience is considered as a composite trait, consisting of different resilience to various environmental disturbances (Colditz and Hine, 2016; Elgersma et al., 2018), where disturbances are categorized in two groups: macro-environmental factors and micro- environmental factors (Falconer and Mackay, 1996; Mulder et al., 2013). Macro-environmental factors are environmental factors that impact the majority, if not all of the whole population (e.g., disease pressure, ambient temperature); while micro-environmental factors are the factors that only impact a minority of the whole population within that macro-environment (e.g., diseases, social interactions).

Low Protein Resilience in Dairy Cows

To mitigate the impact of low-protein diets on DMI and MY, identifying and selecting cows with better ability to maintain their protein output when fed low-protein diets are needed. To help the identification and selection process, we are proposing a term: Low Protein Resilience (LPR). We define LPR as the difference between individual cow decreases in protein output and the average decrease in protein output when cows are switched from high-protein diets to low-protein diets after adjusting for levels of MY, parity, DIM, etc. In pilot studies, we included cows in the crossover studies with 2 periods and 2 diets (14% and 18% CP) to examine the individual cow response to the low-protein diet. We found, on average, milk production and body weight were significantly decreased in cows fed 14% CP when switched from 18% CP. However, the response to the same 4% CP decrease varied a lot among cows. In the figure below, the differences in cows' milk protein yield as diets changing from 18% CP to 14% CP were plotted (Figure 1.2). For cows not differing in productive ability (ECM per kg MBW), the differences in the milk protein yield in response to the 4% dietary CP decrease was considerable. The findings suggest that there are individual differences in the ability of maintaining protein output when fed low protein diets among cows. The model can be further improved by using total protein captured in both milk and body tissues to avoid misleading information (Figure 1.3). Production level, BW, BCS, DIM, and parity need to be considered when modeling LPR, as all of these factors can impact the extent of protein mobilization and deposition. To our knowledge, no prior study examined the variation among cows in terms of their resilience to low-protein diets, and none explore the possible mechanisms to explain the resilience variation.

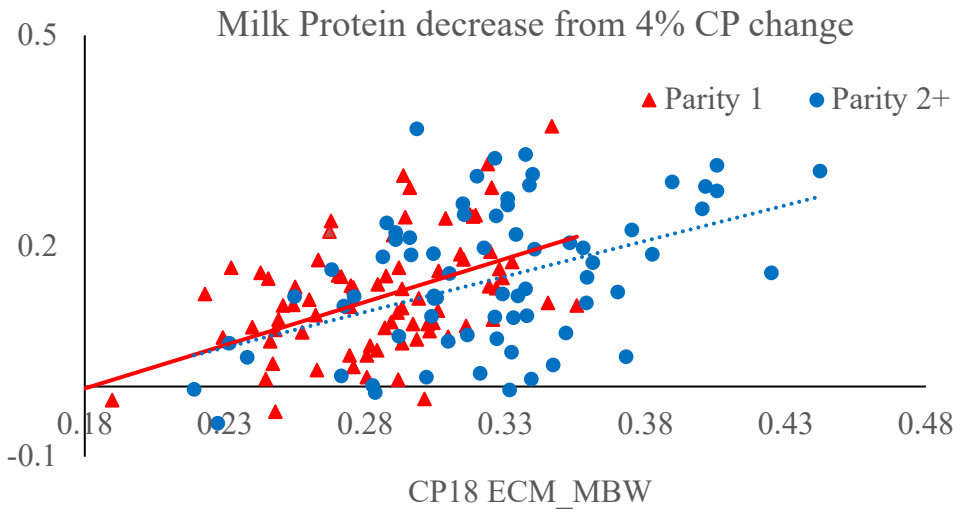

Figure 1.2 Change of milk protein yield from 14CP to 18CP as a function of ECM per kg MBW.
CP18 ECM_MBW is the energy corrected milk per kg metabolic body weight when cows on diets with 18% CP.

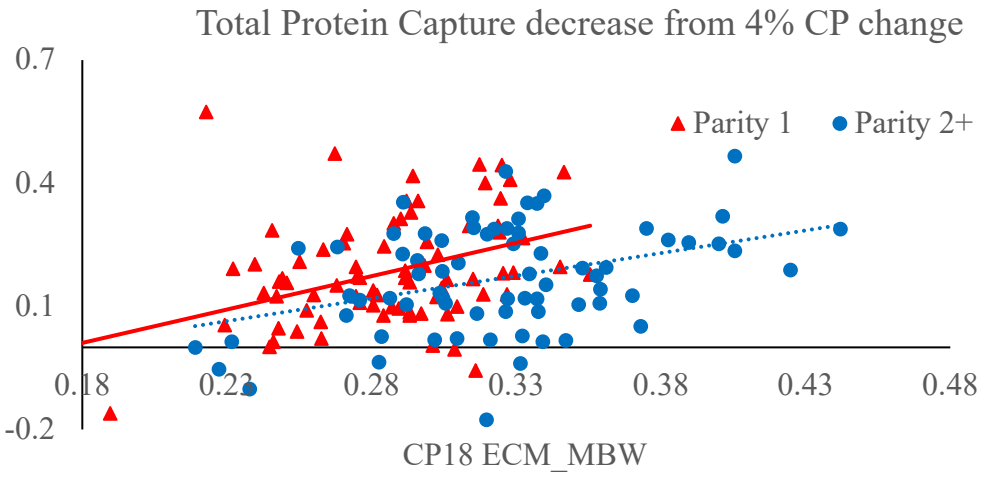

Figure 1.3 Change of total protein capture from 14CP to 18CP as a function of ECM per kg MBW
Total protein capture = milk protein + body protein gain, where body protein deposition was estimated as 0.07 × body weight change for multiparous cows (Parity 2+) and 0.12 × body weight change for primiparous cows (Parity 1). CP18 ECM_MBW is the energy corrected milk per kg metabolic body weight when cows on diets with 18% CP.

By definition, LPR should be independent of milk production, which provides the advantage of combining LPR with production traits to select cows with both high productivity and better resilience. In the genetic selection program, production traits and robustness traits (the traits that describe the resistance, tolerance, and resilience to various environmental stressors and challenges) are usually contraindicative to each other (Knap, 2005). That is, animals with higher production are commonly more vulnerable to environmental stressors (i.e., less resilient). In the classic dairy breeding program, milk yield and milk protein yield are the major focuses, while health traits are often neglected (Egger-Danner et al., 2015). As a result, modern dairy cattle having higher productivity, but are more susceptible to physiological and immunological imbalances (mastitis, heat stress, etc.; Rauw and Gomez-Raya, 2015). As animal welfare, production longevity and sustainability are given more attention, including robustness traits into genetic selection programs is being discussed (Calus et al., 2013). At first, heat stress indicators were the only traits included in the genetic selection programs aiming to improve dairy cow robustness; later on, other health traits were also included into the index, in order to select cows with better resistance to infectious and non-infectious diseases (König and May, 2019). However, as discussed before, selecting robust cows might outweigh efforts in improving milk production. Given that there might be some common genetic factors in animals' resilience to various disturbances (disease, temperature, social stress; Mulder et al., 2013), the new definition of resilience, LPR, provides a potential solution to incorporate both production traits and resilience traits into one genetic selection program, and select more resilient cows among high-producing ones.

MODELING COW RESPONSE TO DIETARY PROTEIN

In a typical U.S. farm, 42% of the total feed cost is spent on protein (St-Pierre, 2012). Therefore, optimizing protein feeding could significantly improve farm profitability. One way to improve profitability is to decrease feed cost by lowering dietary protein content. However, protein reduction could decrease milk production and in turn milk revenue, and the savings from feeding less protein may be outweighed by the lost milk revenue. Researchers have been examining the trade-off between dietary protein contents and milk production for a long time. One of the best studies examining this trade-off was conducted 20 years ago by Wu and Satter (2000) at Wisconsin. They measured the response of lactating dairy cows to different amounts of dietary protein and concluded that feeding cows 17-19% CP before week 30 and 16% CP after week 30 optimized milk production. Since then, many researchers have continued to study the effects of dietary protein content on milk production (Broderick, 2003; Lammers and Heinrichs, 2000), and documented a negative link between dietary protein reduction and milk production. Following that, many researchers have attempted to work out the optimal protein content by modeling cows' response to dietary protein. For example, Hristov et al. (2005) added initial BW in the response model to account for available body reserves for milk production, and significantly improved the overall model fit. Brun-Lafleur et al. (2010) found that parity explained significant variation in the model, and that the response curve for primiparous cows was much different from that for multiparous cows. They speculated that the differential MY response for primiparous cows versus multiparous cows might be because it was easier for multiparous cows to mobilize body reserves to support milk production. Furthermore, Moraes et al. (2018) acknowledged that body reserves would significantly impact the milk protein yield, especially body protein mobilization in early lactation and deposition in late lactation. However, it could not be captured in their analyses given the design and scope of the studies included in the

paper. Thus, although the effect of body reserves mobilization on milk production has been widely accepted by researchers, body reserves change has not been properly incorporated in prior models for various reasons (Hristov et al., 2005; Brun-Lafleur et al., 2010; Moraes et al., 2018). Ignoring body reserves change significantly impeded the accuracy of the prediction of cow response to dietary protein. If milk loss is consistently accompanied by body weight loss when fed low-protein diets, then the loss in protein capture and the loss from feeding less protein is underestimated.

Additionally, researchers have been working on profit response to dietary protein for years. Yet, findings on the effect of dietary protein reduction on farm profitability are inconclusive. For example, Stewart et al. (2012) did not find any financial penalty or benefit when reducing dietary CP from 18 to 16.5% whereas Phuong et al. (2013) noted in their study that the loss of milk income as a result of decreased dietary protein content (from 19% to 15%) greatly exceeded the extra cost of feeding excessive protein. More recently, contrary to findings in Phuong et al. (2013), Fadul-Pacheco et al. (2017) found in a sample of Eastern Canadian dairy herds in 2011 that improving protein efficiency by reducing dietary protein contents from 16.5% to 15% significantly increased income over feed cost (IOFC) from Can$14.3 to Can$18.2/ cow per day. It is likely that 16.5% CP in Stewart et al. (2012) has met or exceeded the genetic capacity of cows to generate protein output; thus, the first 1- 2% units reduction of CP might be simply removing the excess above requirement. In addition, the inconsistent findings in previous studies could also be partly due to the differential economic conditions over the years and not accounting for body reserve mobilization and response variation among cows. In Potts et al. (2015), IOFC was found to be higher for low-RFI cows than high-RFI cows only when cows were fed high-starch diets but not low-starch diets. Taken together, these findings suggested an

interaction between animal and nutrient content, meaning that the effect of nutrient content may vary across cows.

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CHAPTER 2

RELATIONSHIP OF RESIDUAL FEED INTAKE TO PROTEIN EFFICIENCY IN LACTATING COWS FED HIGH OR LOW PROTEIN DIET

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ABSTRACT

Our objectives were to determine the repeatability of residual feed intake (RFI) across dietary protein contents 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 (MY) 41.3±9.8 kg/d were fed diets with high or low protein (HP or LP) in peak lactation. Experiments were conducted as crossovers with two treatment periods of 28-35 d. Production of 69 of the 166 cows (42 primiparous, 27 multiparous) was also measured in late lactation. Low-protein diets were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP 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 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. Fixed effects of diet, parity, treatment sequence, and treatment period, interaction of parity and diet, interaction of diet and period, 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. 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 BCS 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). A 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 efficiency. In conclusion, RFI was repeatable across dietary protein contents within lactation stage, and cows with lower RFI values utilized protein more efficiently.

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 contents (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 if 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 as 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). 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). 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 contents. In lactating dairy cattle, RFI is repeatable across diets with varying starch and NDF contents (Potts et al., 2015; Mangual et al., 2016). However, no studies have reported the RFI ranking of cows across dietary protein contents. Most diets in previous RFI

studies contained adequate or even excessive protein (Mangual et al., 2016; Potts et al., 2015; Tempelman et al., 2015). If RFI is not repeatable across protein contents, 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 N excretion. Thus, the objective of this study was to examine the repeatability of RFI across diets with high or marginally deficient protein contents and the relationship between RFI and protein efficiency. We hypothesized that 1) RFI was relatively repeatable across dietary protein contents, 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 cross-over 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 in different lactation stages. The LP diet were formulated to be marginally-deficient in protein so that milk production would likely drop.

In the 7 experiments containing 166 peak lactation cows, days in milk (DIM) was 50 to 130 d for all cows 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 one of the two treatment sequences (HP-LP, or LP-HP). For cows in peak lactation, the LP_{peak} diet (LP diet for peak lactation cows) contained 31% NDF, 32% starch and 14% CP, and the HP_{peak} diet (the HP diet for peak lactation cows) 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 2.1).

Table 2.1 Feed Ingredients and Nutrient Composition of Experimental Diets [1,2]

	Treatments			
	Peak lactation		Late lactation	
Ingredient, % DM	HP_{peak}	LP_{peak}	HP_{late}	LP_{late}
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
Solvent extracted soybean meal	0.3	2.5	0.6	0.4
Expeller soybean meal	13.4	----	9.7	----
Vitamin and mineral mix[3]	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, Mcal/kg[4]	1.7	1.6	1.5	1.6

[1]HP_{peak} and LP_{peak} diet were high-protein diets and low-protein diets fed to peak lactation cows, and HP_{late} and LP_{late} diet were high-protein diets and low-protein diets fed to late-lactation cows.
[2]Experimental diets were fed to cows in crossover design with at least 28-d periods
[3]Vitamin 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, 3.1% magnesium sulfate, 2.8% salt, 2.0% vegetable oil, 1.5% niacin, 1.3% trace mineral mix, 0.95% biotin, 0.7% YeastPlus, 0.54% vitamin ADE premix, 0.32% selenium yeast, and 0.09% Rumensin 90.
[4]Mean apparent net energy concentration of diets, based on average cow performance. For each diet, Diet NE_L= the average of (MilkE + 0.08 × MBW + BodyE)/ 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.

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 one of the two treatment sequences (HP-LP, or LP-HP). For cows in late lactation, the LP_{late} diet (LP diet for late lactation cows) contained 40% NDF, 26% starch and 13% CP, and HP_{late} diet (HP diet for late lactation cows) 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 2.1).

All cows were housed in individual tie stalls and milked twice a day (0430 and 1530). Tie stalls 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) at > 110% of expected intake based on intake of the previous day, and orts were removed (1000) and weighed prior to feeding. Milk yield was recorded electronically at each milking, and milk samples were obtained from 4 consecutive milkings each wk. Milk samples were analyzed for fat, protein, lactose, somatic cell count, 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 the afternoon milkings. 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 one 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 screen; 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 by 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: feed ratio for a cow during a period was determined as the average daily energy-corrected milk yield (**ECM**; $\text{ECM} = [0.327 \times \text{milk (kg)} + 12.95 \times \text{fat (kg)} + 7.20 \times \text{protein (kg)}]$; Tyrell and Reid, 1965) divided by the average daily dry matter intake (**DMI**) over the entire period.

For cows > 190 d pregnant, body weight (**BW**) was corrected for conceptus weight (**CW**) for use in the RFI equation and to calculate energy and protein change of body tissues. CW was calculated using the 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 (**dBW**; kg/d) was calculated for each cow within the treatment period by linear regression after two rounds of removing outliers in the data; an outlier was any BW > 3.5 SD from the regression line.

Energy expended for body tissue gain (**BodyE**; Mcal/d) was estimated by an equation derived from NRC (2001; Table 2-5):

$$\Delta BodyE = (2.88 + 1.036 \times BCS) \times dBW,$$

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

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

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

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

$$DietNE_L = Average\ [(MilkE + 0.08 \times MBW + \Delta BodyE + PregE) / DMI],$$

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. DMI for an individual cow during each period was regressed as a function of major energy sinks using GLM Procedure in SAS (9.4). To define RFI for cows in the peak lactation, DMI was modeled as:

$$DMI = \beta_0 + \beta_1 \times MilkE + \beta_2 \times MBW + \beta_3 \times \Delta BodyE + \beta_4 \times DIM + \beta_5 \times DIM \times DIM + \beta_6 \times BCS + Parity + Experiment + Cohort(Experiment) + Diet(Cohort \times Experiment) + e,$$

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 (1-7), 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. RFI was defined as the residual term (e) in the model. For cows in the late lactation, DMI was modeled as::

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

where PregE was the energy expended for pregnancy, and RFI was still 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 contents was calculated using Pearson correlation coefficients by CORR procedure of SAS (9.4). Two RFI values within each lactation stage for each cow (RFI_{HPpeak} vs. RFI_{LPpeak}; RFI_{HPlate} vs. RFI_{LPlate}) were included in the analyses. To examine the RFI repeatability across lactation stages, two 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 coefficient for RFI_{peak} and RFI_{late} was calculated. To further examine the RFI repeatability across dietary protein contents, two 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 diets lactation stage. Pearson correlation coefficient for RFI_{high} and RFI_{low} was calculated. Correlation was considered as significant at $P \leq 0.05$ and trends at $P \leq 0.10$.

For each cow on 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

(BodyP; kg/d) was calculated using the following equations, which were derived as averages for BCS of 3.0 based on NRC (2001):

BodyP = 0.12 × dBW for primiparous cows,

BodyP = 0.07 × dBW for multiparous cows.

To quantify the association among RFI, MPE, and GPE, Pearson correlation coefficients were obtained using the CORR Procedure in SAS (9.4). 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 eleven experiments were compared. The effect of RFI was determined using the GLM Procedure of SAS according to the model $Yi = \mu + R_i + e$, where 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 (9.4), with fixed effects of diet, parity, treatment sequence, period, interaction of parity and diet, interaction of diet and period, 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 Table 2.2 and Table 2.3, LP_{peak} decreased DMI ($P< 0.01$), MY ($P< 0.01$), 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 kg DMI ($P< 0.01$), milk energy ($P< 0.01$), and estimated retained energy ($P< 0.01$). As shown in Table 2.4 and Table 2.5, 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 ($P= 0.04$). LP_{late} also decreased ECM per kg 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 Table 2.2 and Table 2.3, among peak lactation cows, compared to multiparous cows, primiparous cows had less DMI ($P< 0.01$), MY ($P< 0.01$), 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 to multiparous cows. As shown in Table 2.4 and Table 2.5, among all late lactation cows, compared to 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 to multiparous cows.

Table 2.2 Dry matter intake, milk production, milk components and feed efficiency for cows fed treatment diets in peak lactation[1,2]

	Treatments[3]			Parity[4]			P-value[5]		
	HP_{peak} n=166	LP_{peak} n=166	SEM	Primi. n=184	Multi. n=148	SEM	TRT	Parity	TRT × Parity[8]
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

[1]Average DIM was 125 for primiparous cows in HP_{peak} diet, 126 for primiparous cows in LP_{peak} diet, 122 for multiparous cows in HP_{peak} diet, and 121 for multiparous cows in LP_{peak} diet.

[2]Average parity for multiparous cows was 2.94 in peak lactation.

[3] Treatments contained 18% and 14% crude protein on a DM basis for peak lactation cows.

[4] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[5] *P*-value associated with treatment differences (HP_{peak} vs. LP_{peak}; TRT) and parity differences (Primi vs. Multi.; Parity) in peak lactation cows.

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

[7] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

[8] Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

Table 2.3 Body weight, body condition score and calculated energy values for cows fed experimental diets in peak lactation

	Treatments[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, kg/ d[4]	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 NEL of diet, Mcal/kg	1.74	1.58	0.64	1.67	1.65	0.85	<0.01	0.61	0.39
Apparent NEL, 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

[1] Treatments contained 18% and 14% crude protein on a DM basis for peak lactation cows.

[2] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[3] *P*-value associated with treatment differences (HP_{peak} vs. LP_{peak}; TRT) and parity differences (Primi vs. Multi.; Parity) in peak lactation cows.

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

[5] Milk (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= $BW^{0.75}$

Table 2.4 Dry matter intake, milk production, milk components and feed efficiency for cows fed treatment diets in late lactation[1,2]

	Treatments[3]			Parity[4]			P-value[5]		
	HP_{late} n=69	LP_{late} n=69	SEM	Primi. n=84	Multi. n=54	SEM	TRT	Parity	TRT × Parity[8]
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

[1]Average DIM was 258 for primiparous cows in HP_{late} diet, 257 for primiparous cows in LP_{late} diet, 263 for multiparous cows in HP_{late} diet, and 264 for multiparous cows in LP_{late} diet.

[2]Average parity for multiparous cows was 3.12 in late lactation.

[3] Treatments contained 16% and 13% crude protein on a DM basis for late lactation cows.

[4] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[5] *P*-value associated with treatment differences (HP_{late} vs. LP_{late}; TRT) and parity differences (Primi vs. Multi.; Parity) in late lactation cows.

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

[7] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

[8] Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

Table 2.5 Body weight, body condition score and calculated energy values for cows fed experimental diets in late lactation

	Treatments[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
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[4], 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[5]									
Apparent NEL of diet, Mcal/kg	1.64	1.46	0.04	1.62	1.48	0.04	<0.01	<0.01	0.06
Apparent NEL, 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[6]
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

[1] Treatments contained 16% and 13% crude protein on a DM basis for late lactation cows.

[2] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[3] *P*-value associated with treatment differences (HP_{late} vs. LP_{late}; TRT) and parity differences (Primi vs. Multi.; Parity) in late lactation cows.

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

[5] Milk (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= $BW^{0.75}$

[6] Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

Repeatability of Residual Feed Intake across Protein Contents and Lactation Stages

In the RFI model in 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 R^2 and root mean square error were 0.87 and 1.50, respectively. In the RFI model in 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 2.6.

Table 2.6 Partial regression coefficients of the RFI models in peak- and late- lactation cows

	Peak lactation					Late lactation				
				Contribution to DMI					Contribution to DMI	
	Coefficient	SEM	P-value	Mean	SD	Coefficient	SEM	P-value	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				

[1]Milk energy (Mcal/d)= [9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)].

[2] Metabolic BW (Kg) = $BW^{0.75}$

[3] Energy utilized in body tissue gain (Mcal/d) = [(2.88+1.036 × BCS) × ΔBW].

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

Regarding the RFI repeatability, in general, cows with lower RFI values when fed high-protein diets still had low RFI when switched to low-protein diets. In peak lactation, RFI was moderately repeatable across high and low protein diets (r= 0.59, $P< 0.01$). Figure 2.1 illustrates the relationship between RFI in HP_{peak} and RFI in LP_{peak}. In late lactation, RFI was less repeatable across protein contents as it was in peak lactation (r= 0.41, $P= 0.03$). Figure 2.2 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 diet (r= 0.11, $P= 0.39$), but moderate across lactation stage for the LP diet (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 2.7 and Figure 2.3.

Figure 2.1 Repeatability of residual feed intake (RFI) across dietary protein contents in peak lactation cows

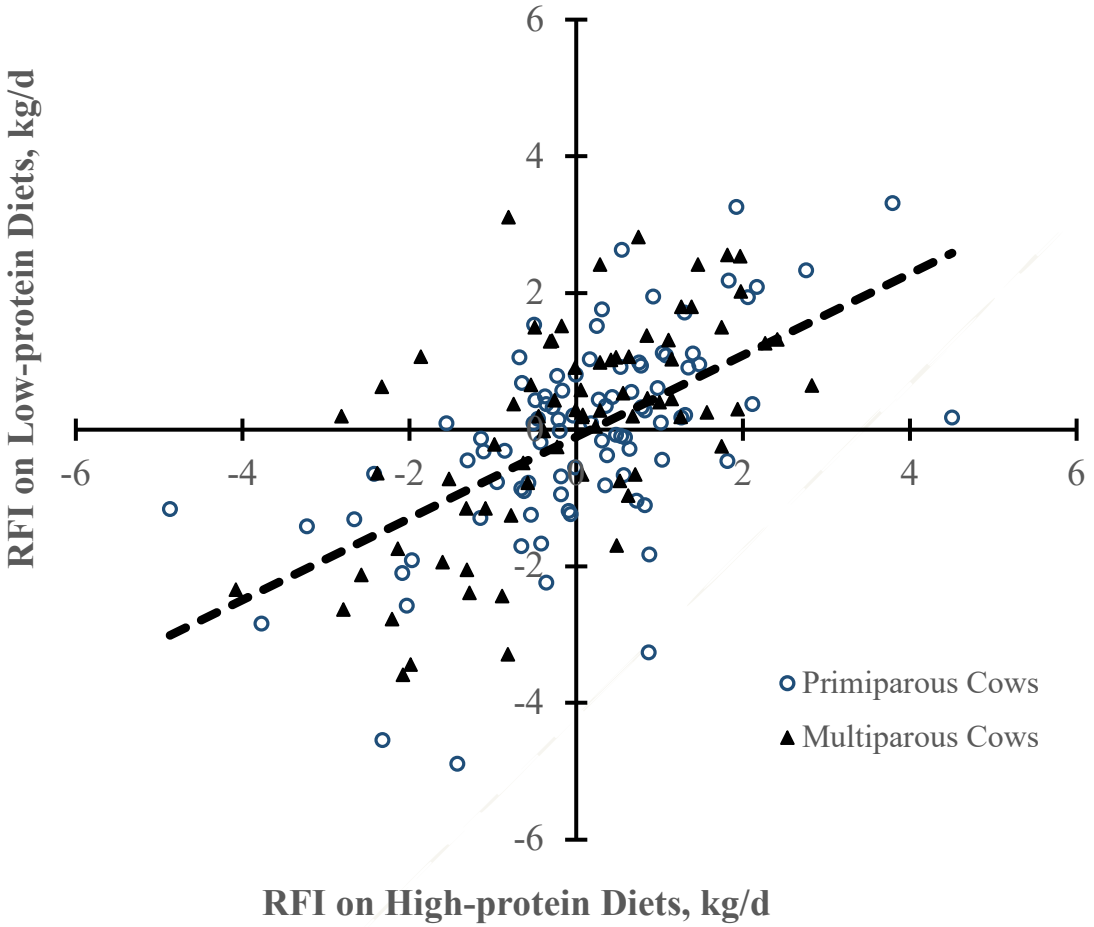

Figure 2.1 Repeatability of residual feed intake (RFI) across dietary protein contents in peak lactation cows (n=166). Repeatability of RFI across dietary protein contents was r= 0.59. RFI on low-protein diets could be predicted using RFI on high-protein diets as Y= 0.628 (± 0.062; *P*< 0.01) × X – 0.000 (± 0.09). Each data point represents one cow's RFI value for each diet (n=166). Open circles indicate primiparous cows (n=92), and filled triangles indicate multiparous cows (n=74).

Figure 2.2 Repeatability of residual feed intake (RFI) across dietary protein contents in late lactation cows

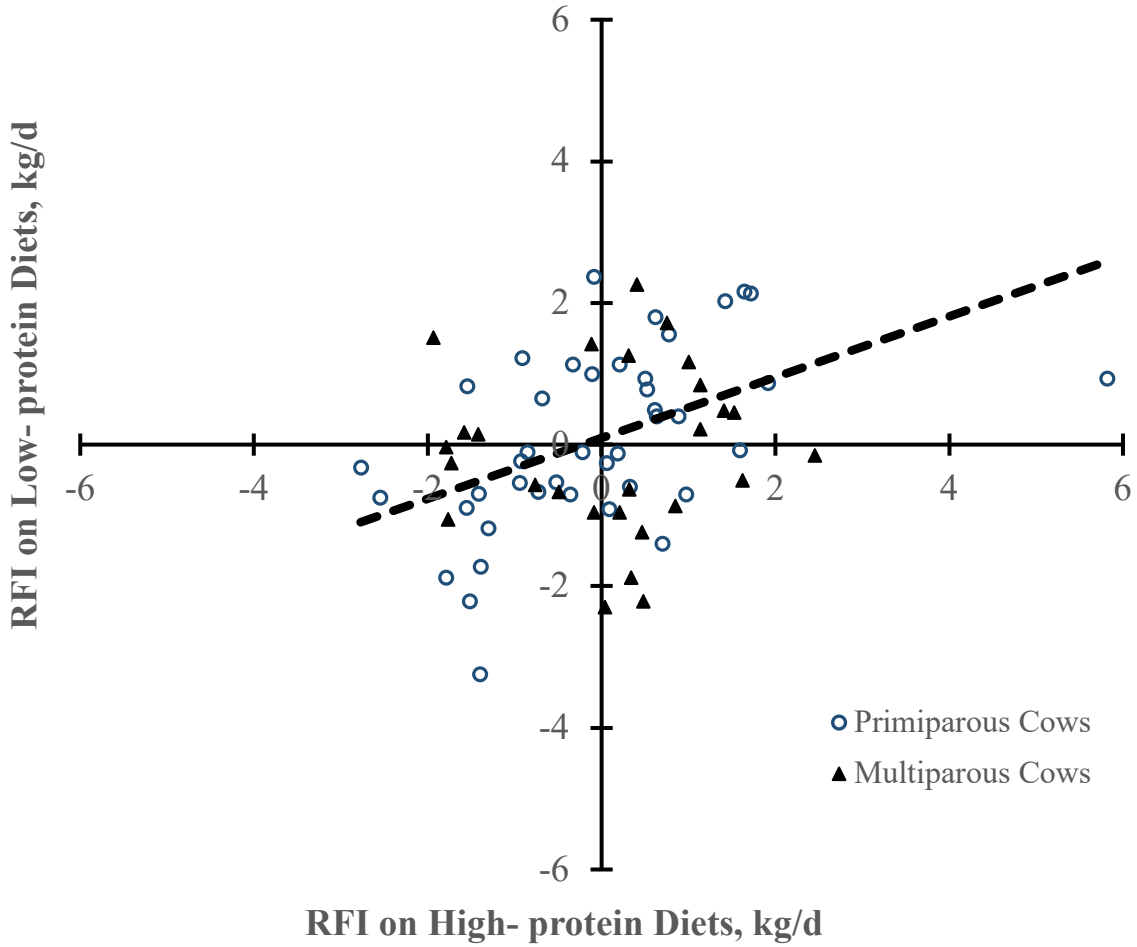

Figure 2.2 Repeatability of residual feed intake (RFI) across dietary protein contents in late lactation cows (n=69). Repeatability of RFI across dietary protein contents was r= 0.41. RFI on low-protein diets could be predicted using RFI on high-protein diets as Y= 0.387 (± 0.101; *P*< 0.01) × X – 0.000 (± 0.14). Each data point represents one cow's RFI value for each diet (n=69). Open circles indicate primiparous cows (n=42), and filled triangles indicate multiparous cows (n=27).

Figure 2.3 Repeatability of residual feed intake (RFI) across lactation stages in lactating cows

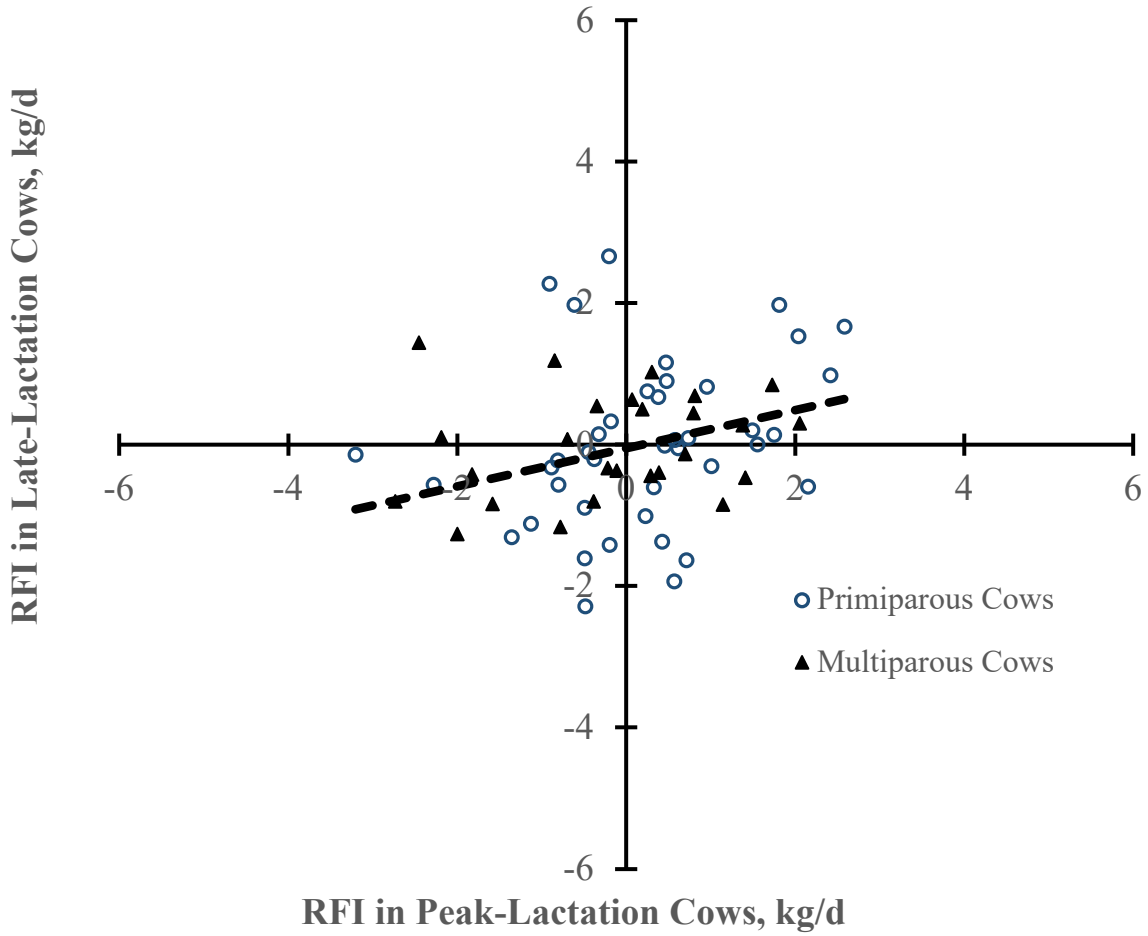

Figure 2.3 Repeatability of residual feed intake (RFI) across lactation stages in lactating cows (n=69). Repeatability of RFI across lactation stages was r= 0.25. RFI in late-lactation cows could be predicted using RFI in peak-lactation cows as Y= 0.198 (± 0.097; *P*= 0.02) × X – 0.009 (± 0.12). Each data point represents one cow's RFI value in each stage (n=69). Open circles indicate primiparous cows (n=42), and filled triangles indicate multiparous cows (n=27).

Table 2.7 Repeatability of RFI across protein contents within lactation stage and across lactation stages (n = 69)

	RFI_{LPpeak}	RFI_{peak}[3]	RFI_{high}[4]	RFI_{HPlate}	RFI_{LPlate}	RFI_{late}[5]	RFI_{low}[6]
RFI_{HPpeak}	0.59[1] < 0.01[2]	0.84 < 0.01	0.77 < 0.01	0.11 0.39	0.11 0.39	0.13 0.31	0.40 < 0.01
RFI_{LPpeak}		0.87 < 0.01	0.46 < 0.01	0.20 0.10	0.28 0.02	0.28 0.02	0.86 < 0.01
RFI_{peak}[3]			0.84 < 0.01	0.23 0.06	0.23 0.05	0.25 0.04	0.73 < 0.01
RFI_{high}[4]				0.76 < 0.01	0.34 < 0.01	0.70 < 0.01	0.51 < 0.01
RFI_{HPlate}					0.41 < 0.01	0.85 < 0.01	0.36 < 0.01
RFI_{LPlate}						0.82 < 0.01	0.73 < 0.01
RFI_{late}[5]							0.64 < 0.01

[1] The Pearson correlation coefficient of the linear relationship between 2 variables

[2] The P value associated with the linear relationship between 2 variables

[3] Averaged RFI across the diets fed to cows in peak lactation

[4] Averaged RFI for the HP diet across lactation stages

[5] Averaged RFI across the diets fed to cows in late lactation

[6] Averaged RFI for the LP diet across lactation stages

Residual Feed Intake 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 the HP_{peak} diet and -0.41 ($P< 0.01$) in LP_{peak} diet. For cows in late lactation, the Pearson correlation coefficient between RFI and milk protein efficiency was -0.36 ($P= 0.02$) in the HP_{late} diet and -0.13 ($P= 0.34$) in LP_{late} diet. The correlation coefficient between RFI and milk protein efficiency across diets was -0.42 ($P< 0.01$; Figure 2.4) in peak lactation cows and -0.24 ($P= 0.06$; Figure 2.5) in 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 2.8, cows with lower RFI values in peak lactation had higher milk protein efficiency and gross protein efficiency regardless of protein content in the diet; however, cows with lower RFI values did not necessarily have lower MUN concentration. Similar trends were observed in cows fed the HP diet in late lactation. In contrast, when fed the LP diet, cows with lower RFI in late lactation did not exhibit greater protein efficiency (MPE, GPE) nor MUN.

Figure 2.4 Association between residual feed intake and milk protein efficiency in peak lactation cows

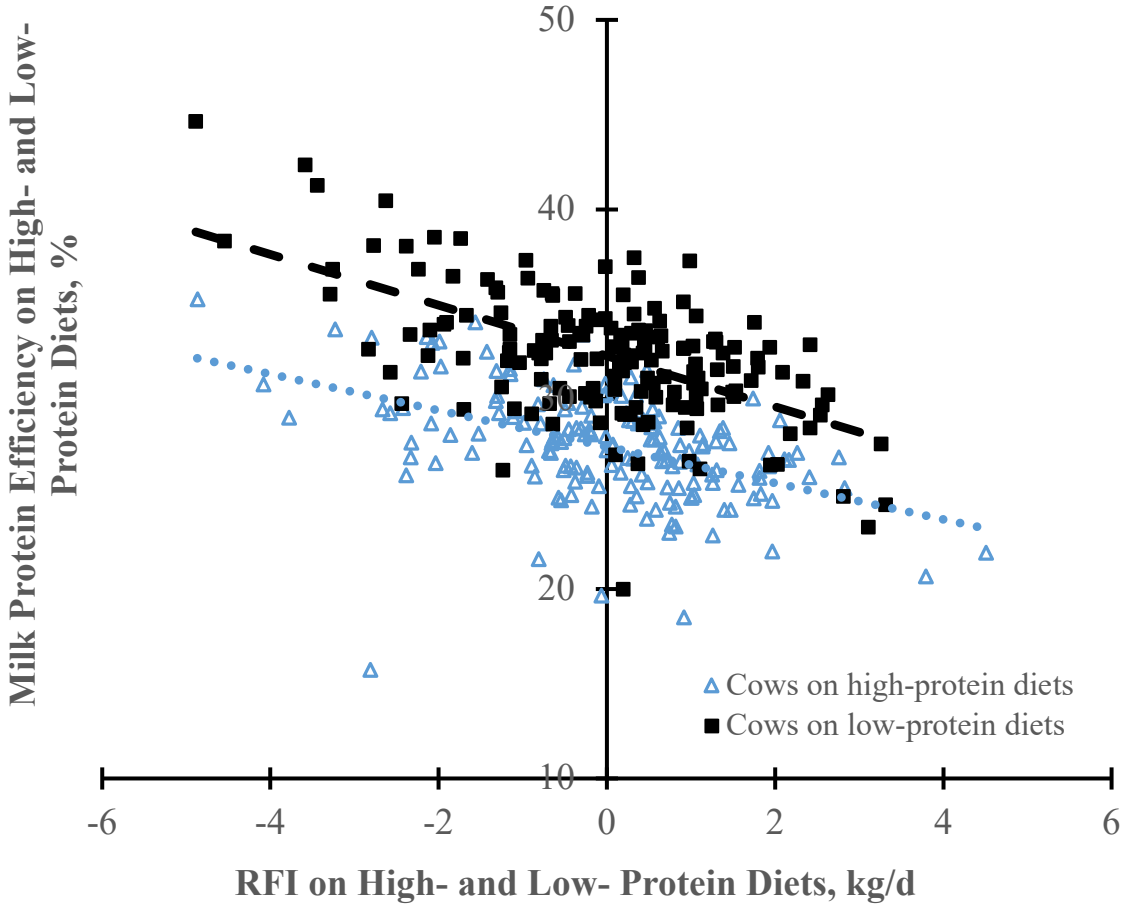

Figure 2.4 Association between residual feed intake and milk protein efficiency in peak lactation cows (n=332). In peak-lactation cows, correlation between residual feed intake (RFI) and milk protein efficiency (MPE) across high and low protein diets was -0.42. The correlation between RFI and MPE in high-protein diets was -0.59 (The equation was MPE= -0.96 (± 0.14; $P< 0.01$) × RFI + 27.5 (± 0.20; $P< 0.01$)), and the correlation between RFI and MPE in low-protein diets was -0.41 (The equation was MPE= -1.34 (± 0.14; $P< 0.01$) × RFI + 32.3 (± 0.21; $P< 0.01$)). Each data point represents one cow's RFI value for each diet. Open triangles indicate cows in high-protein diets (n=166), and filled squares indicate cows in low-protein diets (n=166).

Figure 2.5 Association between residual feed intake and milk protein efficiency in late lactation cows

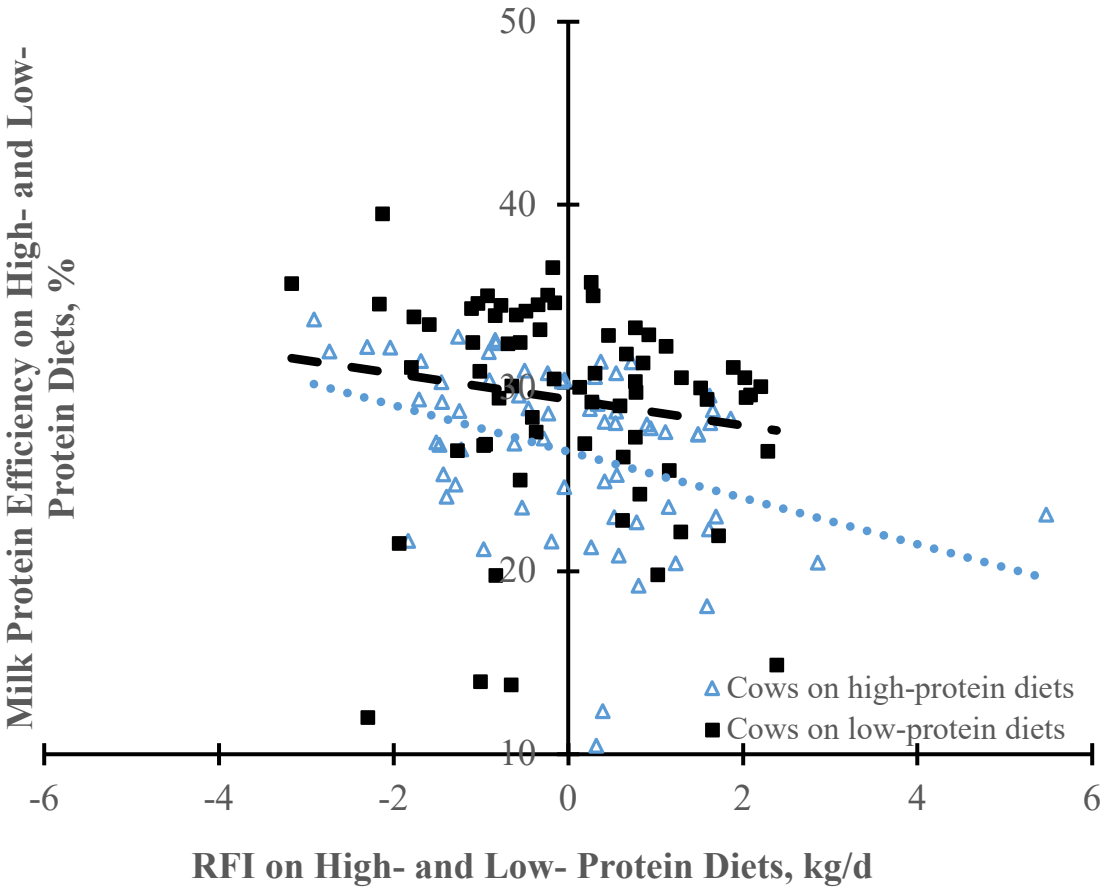

Figure 2.5 Association between residual feed intake and milk protein efficiency in late lactation cows (n=138). In late-lactation cows, correlation between residual feed intake (RFI) and milk protein efficiency (MPE) across high and low protein diets was -0.24. The correlation between RFI and MPE in high-protein diets was -0.36 (The equation was 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 (The equation was MPE= -0.71 (± 0.55; $P= 0.20$) × RFI + 29.4 (± 0.68; $P< 0.01$)). Each data point represents one cow's RFI value for each diet. Open triangles indicate cows in high-protein diets (n=138), and filled squares indicate cows in low-protein diets (n=138).

Table 2.8 Protein efficiency and MUN of high-, medium- and low-RFI cows fed high- and low- protein diets across lactation stage

	High-protein diets				Low Protein diets			
Variable	HRFI[1]	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[6], mg/dL	12.1	12.3	12.1	0.65	8.2	8.1	8.2	0.78

[1] 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.

[2] *P*-value associated with group difference

[3] MPE, milk protein efficiency, defined as the dietary protein captured in milk

[4] GPE, gross protein efficiency, defined as dietary protein captured in milk and body tissue

DISCUSSION

Animal Performance across Dietary Protein Contents and Lactation Stages

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

With the similar decrease in dietary protein content, the decrease of feed intake and milk production was similar between peak-lactation and late-lactation cows; however, late-lactation cows tended to lose more non-pregnant BW and BCS than peak-lactation cows (table 3 and table 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) described that the priority of nutrient partitioning in cattle was pregnancy, followed by milk production, and lastly body reserve gain; our data was consistent with this idea.

Repeatability of Residual Feed Intake across Dietary Protein Contents and Lactation Stages

Although production was significantly altered by the diets, RFI within cows was still repeatable across dietary protein contents within each lactation stage. The literature on RFI repeatability has predominantly been focusing on peak-lactation cows. The current study supports the 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 previous RFI repeatability studies, where RFI was

repeatable across starch contents (0.73; Potts et al., 2015) and forage NDF contents (0.54; 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 alter RFI significantly, 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 to Potts et al. (2015), might be related to the expected changes in protein metabolism when altering dietary protein. Lower RFI repeatability across dietary protein contents in late lactation, compared to peak lactation, was expected due to the uncertainty of pregnancy weight gain, which will be further discussed below.

RFI repeatability across physiological states, such as across lactation stages 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 peak-lactation, and 3) extra body fat storage 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 the energy sinks mentioned above can introduce errors in calculating RFI.

Errors in BW change could introduce significant bias in the RFI estimation (Potts et al., 2015). This becomes especially important when estimating RFI for late-lactation cows. BW change in 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 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 the errors in estimating energy utilized in pregnancy. As errors were introduced in the two primary energy sinks, we expected that the estimated value of RFI would be less accurate in late lactation cows. Indeed, due to the difficulty of estimating BW change, Prendiville et al. (2011) advised to estimate RFI based on data between DIM 150 and DIM 230 when tissue gain or loss was minimal, in order to generate the most accurate estimates of RFI.

Residual Feed Intake and Protein Efficiency

No prior study has directly examined the relationship between RFI and protein efficiency in lactating Holstein cows; however, the relationship between RFI and protein efficiency in growing heifers was 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 when they were calves/heifers did not have higher nitrogen efficiency in the subsequent lactation. In contrast with the heifer studies, the results in 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 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). There is also work showing that greater protein turnover rates were related to higher RFI values in cattle (Richardson et al., 2004). Based on the prior work, a negative association between RFI and protein efficiency was expected. 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 the 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 medium level of heritability for protein efficiency existed in lactating dairy cows (0.10-0.31; Li et al.,1998; Zamani et al., 2011), directly selecting dairy cows based on traditional protein efficiency term was questioned. Most of the doubt was due to the drawbacks of protein efficiency being a ratio trait. 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., protein efficiency term) in genetic selection induces large error variance and unexpected results. In contrast, RFI, as a residual term, overcomes all the drawbacks in ratio traits and is favorable in cow selection. However, due to the complexity of collecting individual intake data in dairy cows, 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 contents; similarly, average RFI in high- and low-protein diets across lactation stages 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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APPENDIX

Supplementary Table 2.1 Dry matter intake, milk production, milk components, feed efficiency, body weight, body condition score and calculated energy for cows fed treatment diets in peak and late lactation[1][2]

	Peak-lactation cows				Late-lactation cows			
	Primi. on HP	Primi. on LP	Multi. on HP	Multi. on LP	Primi. on HP	Primi. on LP	Multi. on HP	Multi. on LP
DMI, kg/d					18.8	17.2	20.8	19.5
Milk, kg/d	35.4	32.1	47.0	42.5	26.1	22.4	24.2	22.1
ECM[3], kg/d					29.5	24.9	26.3	23.9
3.5% FCM[4], kg/d					28.4	24.2	25.3	23.1
Milk fat, kg/d					1.05	0.90	0.91	0.83
Milk protein, kg/d					0.84	0.69	0.76	0.68
Milk protein, %	3.01	2.96	2.92	2.90				
Milk lactose, kg/d	1.81	1.61	2.33	2.07	1.31	1.09	1.12	1.00
ECM/DMI					1.57	1.45	1.24	1.20
Non-pregnant BW, kg					619	614	769	745
Change in non-pregnant BW, kg/d					0.47	-0.18	0.40	0.07
Apparent NEL of diet, Mcal/kg					1.75	1.49	1.53	1.42
Apparent NEL, Mcal/d					32.8	25.5	31.9	27.7
Milk energy[5], Mcal/d					19.7	16.5	17.2	15.5
Maintenance energy[6], Mcal/d					9.9	9.9	11.7	11.4

[1] Treatments (HP vs. LP) contained 18% and 14% crude protein on a DM basis for peak lactation cows, and 16% and 13% crude protein on a DM basis for late lactation cows.

[2] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[3] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (Tyrrell and Reid, 1965).

[4] Fat-corrected milk; 3.5 % FCM = [(0.4324 × kg milk) + (16.216 × kg milk fat)].

[5] Milk (MilkE)=[9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg)].

[6] Maintenance=0.08 × MBW, where MBW= $BW^{0.75}$

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CHAPTER 3

LOW PROTEIN RESILIENCE IS AN INDICATOR OF RELATIVE PROTEIN EFFICIENCY OF INDIVIDUAL DAIRY COWS

A version of this manuscript has been submitted to *Journal of Dairy Science*

ABSTRACT

Our objectives were to determine 1) the sources of variation in cow responses to dietary protein reduction, and 2) the association of low protein resilience (LPR) with protein efficiency. Lactating Holstein cows (n= 166; 92 primiparous, 77 multiparous) with initial milk yield (MY) 41.3±9.8 kg/d were included in the crossover experiments with two treatments and two periods of 28-35 d each. Production of 69 of the 166 cows (42 primiparous, 27 multiparous) was also measured in late lactation. Low-protein diets (LP) were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function. High-protein diets (HP) were 18% CP in peak lactation and 16% CP in late lactation and contained extra expeller soybean meal to increase absorbed protein. Protein efficiency terms (MPE: dietary protein captured in milk; GPE: dietary protein captured in both milk and body tissues) were calculated for each cow on each diet in both peak lactation (n= 332) and late lactation (n= 138). Low protein resilience value was calculated for each cow in peak lactation (n= 166) and late lactation (n= 69). The ability to maintain total protein capture (CapP, milk protein + body protein gain) varied significantly among cows, and the variation was mostly explained by CapP per kg

metabolic body weight (MBW) on the HP diet , parity, treatment sequence (HP- LP, LP- HP), and experiment. Protein efficiency (MPE and GPE) was moderately repeatable across dietary protein contents regardless of lactation stages. Milk urea nitrogen (MUN) was not associated with MPE or GPE in individual cows after accounting for the diet effect. Compared to low- LPR cows, high- LPR cows had similar protein efficiency (GPE and MPE) on the HP diet, but significantly higher GPE on the LP diet. In conclusion, cows maintained their protein-efficiency rankings when switched from the HP to LP diet, or vice versa; however, using MUN to rank cows for their protein efficiency may be misleading. With similar milk production on the HP diet, high-LPR cows were better able to maintain production and utilize protein more efficiently to adapt to low- protein feeding conditions.

INTRODUCTION

Dairy cattle convert protein in feeds (many of which have little direct value for human nutrition) into milk protein, and dairy products 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 years, and dairy cattle in North America are generally fed lower protein diets today than they were 30 years ago. Lower dietary protein with the same milk protein output increases protein efficiency and profitability. However, protein-deficient diets can reduce DMI and thus milk yield (MY), which ultimately defeats the initial purpose of feeding less protein. 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 (Ingvartsen and Andersen, 2000; Huhtanen et al., 2008; Sinclair et al., 2014).

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 the requirement because their production level is low. For some cows, however, the lack of response to reduced protein may simply imply that they did not need as much protein to achieve their milk production potential because they were able to use protein more efficiently than the cohorts. We will define cows that can tolerate less protein to maintain protein output as being resilient to low protein. To our knowledge, no prior studies have quantified this resilience and its relationship with protein utilization efficiency.

It is widely recognized that protein utilization efficiency, represented by MUN, varies both within cow and within herd (Wattiaux et al., 2005; Stoop et al., 2007; Huhtanen et al., 2015). However, no prior work has directly examined the repeatability of protein efficiency for individual lactating cows across diets that are high or low in protein content. No existing literature examined whether cows that are more protein-efficient in general are also more resilient to low- protein diets. Thus, our objectives were to 1) determine 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 MY, milk components (milk protein and MUN), BW, and hip height were collected in the experiments. These are the same animal 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 crossover experiments with two treatments and two periods of 28-35 d. The two treatments were HP and LP. Production of 69 of the 166 cows (42 primiparous, 27 multiparous) also was measured in late lactation. For cows in peak lactation, the LP diet contained 31% NDF,

32% starch and 14% CP, and the HP diet 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). For cows in late lactation, the LP diet contained 40% NDF, 26% starch and 13% CP, and the HP diet 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 diet was achieved by replacing soybean hulls and ground corn with expeller soybean meal. 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.

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 using the 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.

After plotting the BW data along with the experimental day in the peak lactation cows (Supplementary Figure 1), we suspected that some of, if not all, BW change in the current study might be attributed to gut fill. In order to measure BW change more accurately, empty BW

(**EBW**) was calculated for each cow to adjust BW for the gut fill (Gibbs et al., 1992; Andrew et al., 1994),

EBW = BW – 5.2 × DMI - CW,

where DMI was the daily DMI when BW was measured.

Mean daily EBW change (**dEBW**; kg/d) was calculated for each cow within the treatment period by linear regression after two rounds of removing outliers in the data; an outlier was any BW > 3.5 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) was calculated using the following equations,

BodyP = $(0.151 - 0.0268 \times \text{BCS}) \times$ dEBW (derived from NRC 2001, Table 2-4)

For primiparous cows, we assumed their mature BW would be 700 kg and that they had to gain 0.14 kg 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 protein per kg dEBW was assigned to the 0.14 kg/d true growth. Any deviation in dEBW from 0.14 kg/d was considered to be body condition gain or loss, and the dEBW associated with body condition change was the same as for multiparous cows, (0.151- 0.0268 × BCS) kg protein per kg dEBW. BodyP was estimated as :

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

Protein captured for pregnancy (**PregP**; kg/d) was calculated using the equation from NRC (2001):

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

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 follows:

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

After plotting the dCapP (change of CapP from HP to LP) along with the milk protein yield on the HP diet (Figure 3. 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.

Figure 3.1 Relationship between milk protein yield and cows response (and LPR, low protein resilience) in peak- lactation cows

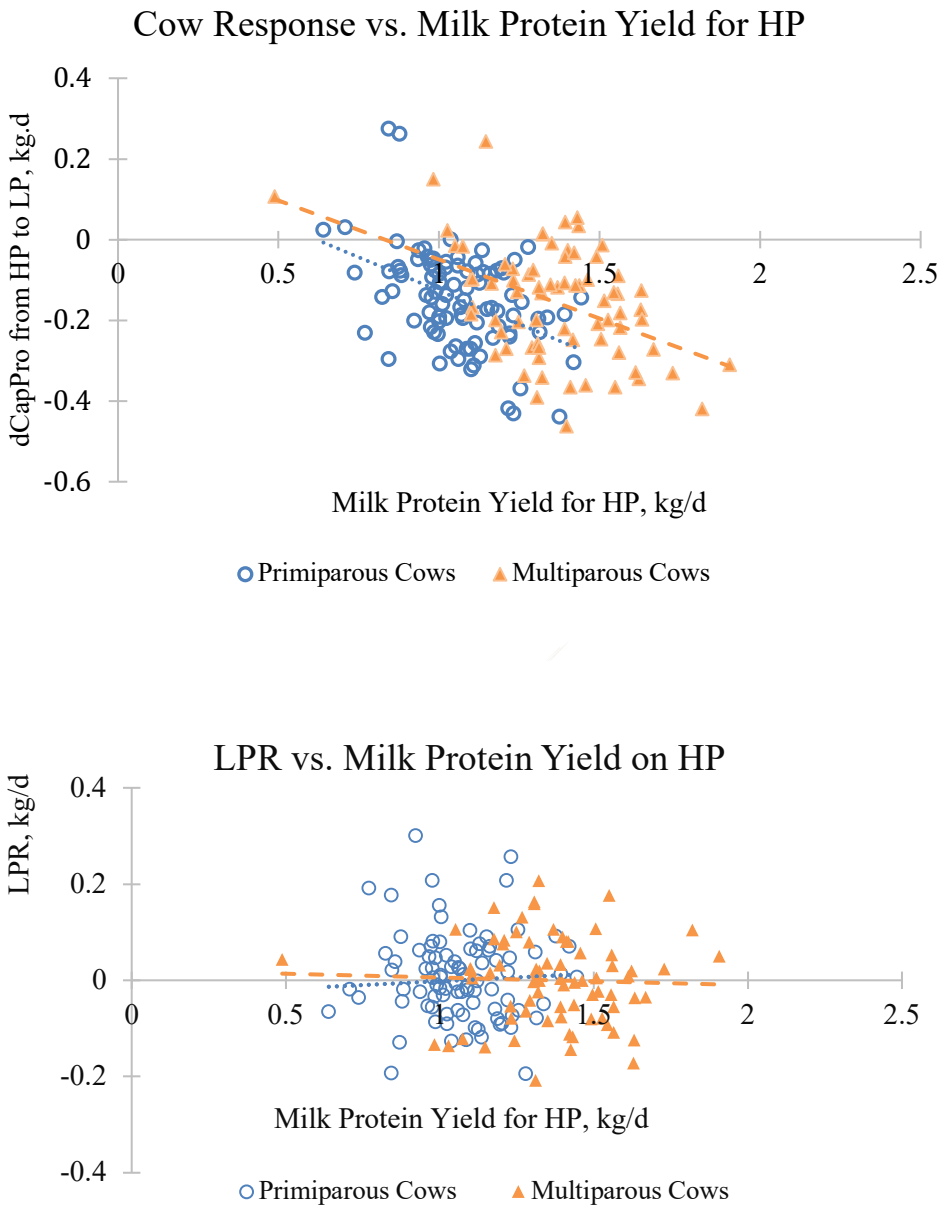

Figure 3.1 Relationship between milk protein yield and cows response (and LPR, low protein resilience) in peak- lactation cows. The correlation coefficient between milk protein yield for the HP diet and dCapP (change of total protein capture from HP to LP) was -0.41 in primiparous cows, and -0.45 in multiparous cows. 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). Open circles indicate primiparous cows, and solid triangles indicate multiparous cows.

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, low protein resilience (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 as:

$$dCapP = \beta_0 + \beta_1 \times CapP\_MBW_{HP} + \beta_2 \times BCS_{HP} + \beta_3 \times MBW\_HH_{HP} + \beta_4 \times dCP + \beta_5 \times CP_{HP} + \beta_6 \times DIM_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$$

where *dCapP was the change of CapP from HP to LP (dCapP= $CapP_{LP} - CapP_{HP}$);* $CapP\_MBW_{HP}$ was the CapP per kg metabolic BW when fed the HP diet; BCS_{HP} was the BCS when fed the HP diet; MBW_HH_{HP} was the metabolic body weight 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.

All covariates were jointly checked for multicollinearity through variance inflation factors (VIF) analysis (SAS, 9.4). No covariates had VIF greater than 10. Following that, the backward stepwise model selection was used to finalize the model (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 high LPR, cows < -0.5 SD were classified as Low LPR, and those between ± 0.5 SD were classified as medium LPR.

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 of MPE across dietary protein contents was calculated using GLM procedure of SAS (9.4) within each lactation stage, after accounting for effects of diet, parity, treatment sequence, and experiment. To further examine the MPE repeatability across dietary protein contents, two MPE values were calculated: MPE_{HP} and MPE_{LP}, where MPE_{HP} was the average MPE for the HP diet across lactation stages and MPE_{LP} was the average MPE for the LP diet across lactation stages. To examine the MPE repeatability across lactation stages, two 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 by GLM procedure after accounting for effects of parity and experiment. 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, in order to determine repeatability of GPE and MUN across dietary protein contents and lactation stages, respectively.

dMUN was calculated as the change of MUN from the HP diet to the LP diet. dMPE and dGPE were calculated as the change of MPE and GPE, 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 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 $Y_i = \mu + LPR_i + e$, 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 $P \leqslant 0.05$ and tendency at $P \leqslant 0.10$. Interactions were considered significant at $P \leqslant 0.10$ and trends at $P \leqslant 0.15$.

RESULTS

Cow Performance

As shown in Table 3.1, 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 to the HP diet. During late lactation, the LP diet decreased milk protein ($P < 0.01$), CapP ($P < 0.01$), and MUN ($P < 0.01$); the LP diet also increased MPE ($P < 0.01$) and GPE ($P = 0.01$), compared to the HP diet.

In peak lactation, compared to 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 to multiparous cows, primiparous cows had higher MPE ($P < 0.01$) and GPE ($P < 0.01$), with similar DMI ($P = 0.32$), milk protein ($P = 0.25$), CapP ($P = 0.55$), and MUN ($P = 0.28$).

Table 3.1 Dry matter intake, milk production and protein efficiency for cows fed treatment diets in peak and late lactation[1,2]

	Treatments[3]			Parity			P-value[4]		
	HP	LP	SEM	Primi.	Multi.	SEM	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	0.25
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

[1]Average DIM was 125 for primiparous cows in HP_{peak} diet, 126 for primiparous cows in LP_{peak} diet, 122 for multiparous cows in HP_{peak} diet, and 121 for multiparous cows in LP_{peak} diet; average DIM was 258 for primiparous cows in HP_{late} diet, 257 for primiparous cows in LP_{late} diet, 263 for multiparous cows in HP_{late} diet, and 264 for multiparous cows in LP_{late} diet.

[2]Average parity for multiparous cows was 2.94 in peak lactation, and 3.12 in late lactation

[3] Treatments contained 18% and 14% crude protein on a DM basis for peak lactation cows, and 16% and 13% crude protein on a DM basis for late lactation cows

[4] *P*-value associated with treatment differences (HP vs. LP; TRT) and parity differences (Primi vs. Multi.; Parity). Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

[5] Total protein capture = milk protein + body protein, where body protein was estimated as: BodyP = (0.151 – 0.0268 × BCS) × dEBW, when Parity >1; BodyP = 0.132 × 0.14 + ((0.151- 0.0268 × BCS) × (dEBW – 0.14)), when Parity = 1

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

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

Repeatability of Protein Efficiency across Dietary Protein Contents and Lactation Stage

As illustrated in Figure 3.2, 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 were 0.72, 0.59, and 0.58, respectively. As illustrated in Figure 3.3, 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 were 0.70, 0.69, and 0.57, respectively.

As illustrated in Figure 3.4 and Figure 3.5, based on the average value across dietary protein contents and lactation stages, MPE was repeatable across dietary protein contents (r= 0.68, $P < 0.05$), but not across lactation stages (r= 0.19, $P = 0.12$). Similar trends were observed for GPE, with GPE repeatability being 0.58 ($P < 0.05$) across dietary protein contents, and 0.15 ($P = 0.21$) across lactation stages. In contrast, MUN was repeatable across both dietary protein contents (r= 0.68, $P < 0.05$) and lactation stages (r= 0.53, $P < 0.05$).

Figure 3.2 Repeatability of protein efficiency across dietary protein contents (HP vs. LP) in peak lactation cows

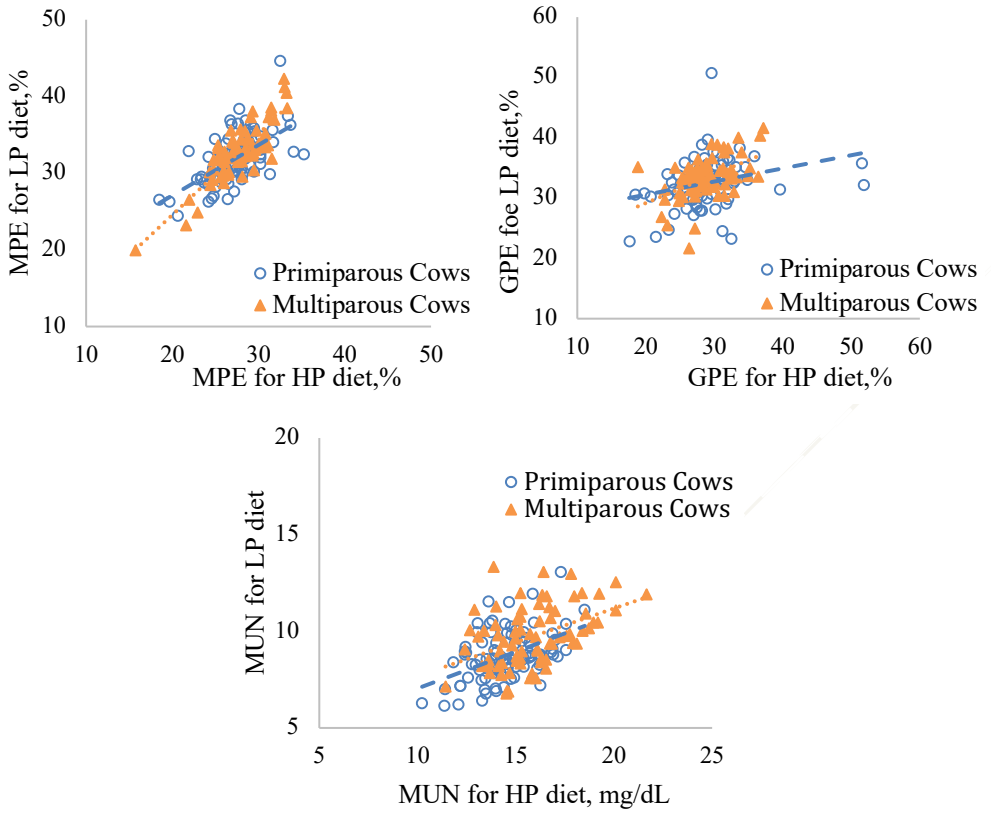

Figure 3.2 Repeatability of protein efficiency across dietary protein contents (HP vs. LP) in peak lactation cows (n=166). Repeatability of protein efficiency (MPE, GPE and MUN) across dietary protein contents were 0.72 for MPE (Y = 0.844 (± 0.063; $P < 0.01$) × X + 9.05 (± 1.73; $P < 0.01$)), 0.59 for GPE (Y = 0.680 (± 0.079; $P < 0.01$) × X + 13.55 (± 2.24; $P < 0.01$)), and 0.58 for MUN (Y = 0.409 (± 0.053; $P < 0.01$) × X + 3.01 (± 0.81; $P < 0.01$)). Each data point represents one cow's protein efficiency value for each diet (n=166). Open circles indicate primiparous cows (n=92), and solid triangles indicate multiparous cows (n=74). Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

Figure 3.3 Repeatability of protein efficiency (MPE, GPE, and MUN) across dietary protein contents (HP vs. LP) in late lactation cows

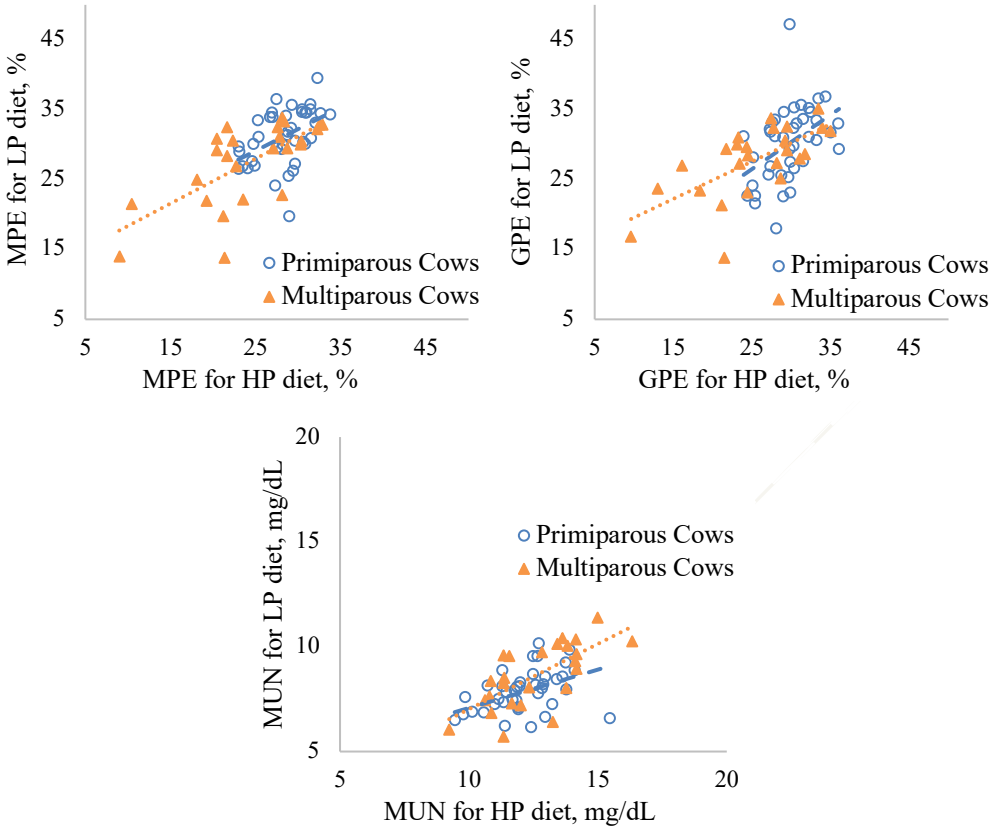

Figure 3.3 Repeatability of protein efficiency (MPE, GPE and MUN) across dietary protein contents (HP vs. LP) in late lactation cows (n=69). Repeatability of protein efficiency across dietary protein contents were 0.70 for MPE (Y = 0.784 (± 0.093; $P < 0.01$) × X + 8.56 (± 2.61; $P < 0.01$)), 0.69 for GPE (Y = 0.633 (± 0.081; $P < 0.01$) × X + 12.67 (± 2.28; $P < 0.01$)), and 0.57 for MUN (Y = 0.489 (± 0.086; $P < 0.01$) × X + 2.16 (± 1.06; $P = 0.04$)). Each data point represents one cow's protein efficiency value for each diet (n=69). Open cycles indicate primiparous cows (n=42), and solid triangles indicate multiparous cows (n=27). Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

Figure 3.4 Repeatability of protein efficiency (MPE, GPE, and MUN) for dietary protein contents (HP vs. LP) based on average values across lactation stages

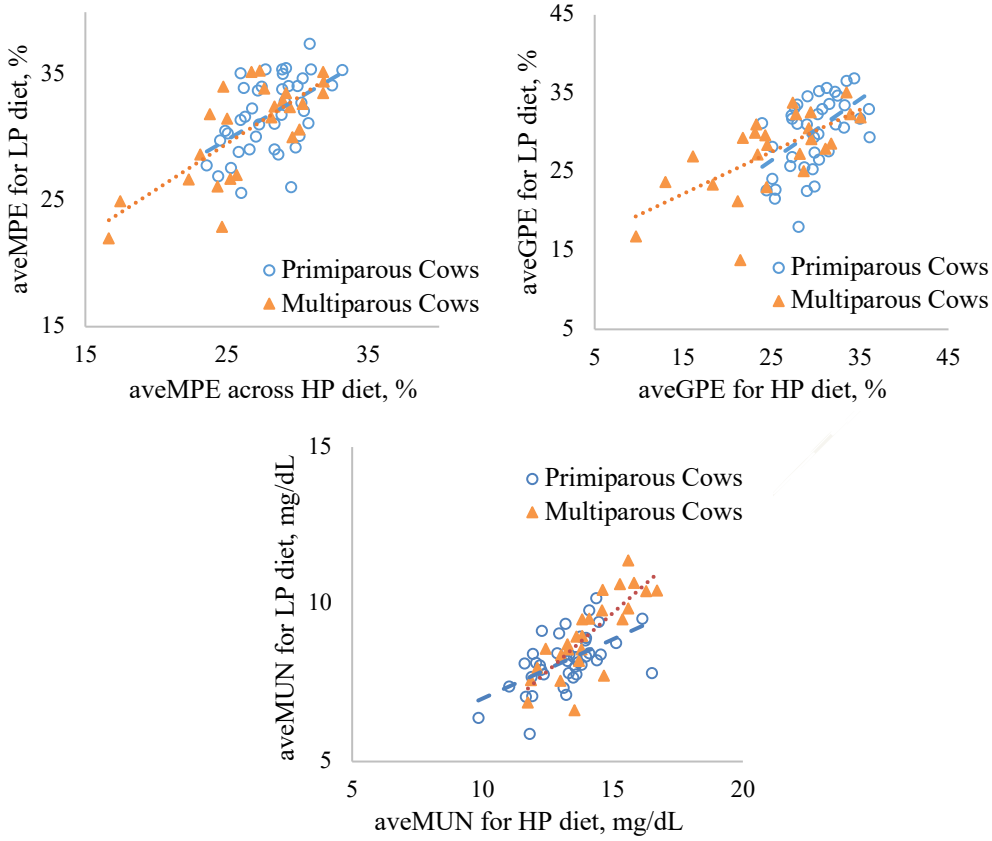

Figure 3.4 Repeatability of protein efficiency (MPE, GPE and MUN) for dietary protein contents (HP vs. LP) based on average values across lactation stage (n=69). Repeatability of protein efficiency across dietary protein contents were 0.68 for MPE (Y = 0.683 (± 0.080; $P < 0.01$) × X + 13.05 (± 2.73; $P < 0.01$)), 0.58 for GPE (Y = 0.454 (± 0.063; $P < 0.01$) × X + 2.55 (± 1.03; $P < 0.01$)), and 0.68 for MUN (Y = 0.644 (± 0.073; $P < 0.01$) × X + 12.05 (± 2.37; $P < 0.01$)). Each data point represents one cow's protein efficiency value for each diet (n=69). Open circles indicate primiparous cows (n=42), and solid triangles indicate multiparous cows (n=27). aveMPE is the average MPE for the HP diet across lactation stages. aveGPE is the average GPE for the HP diet across lactation stages. aveMUN is the average MUN for the HP diet across lactation stages. Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

Figure 3.5 Repeatability of protein efficiency (MPE, GPE, and MUN) across lactation stage based on average values across diets

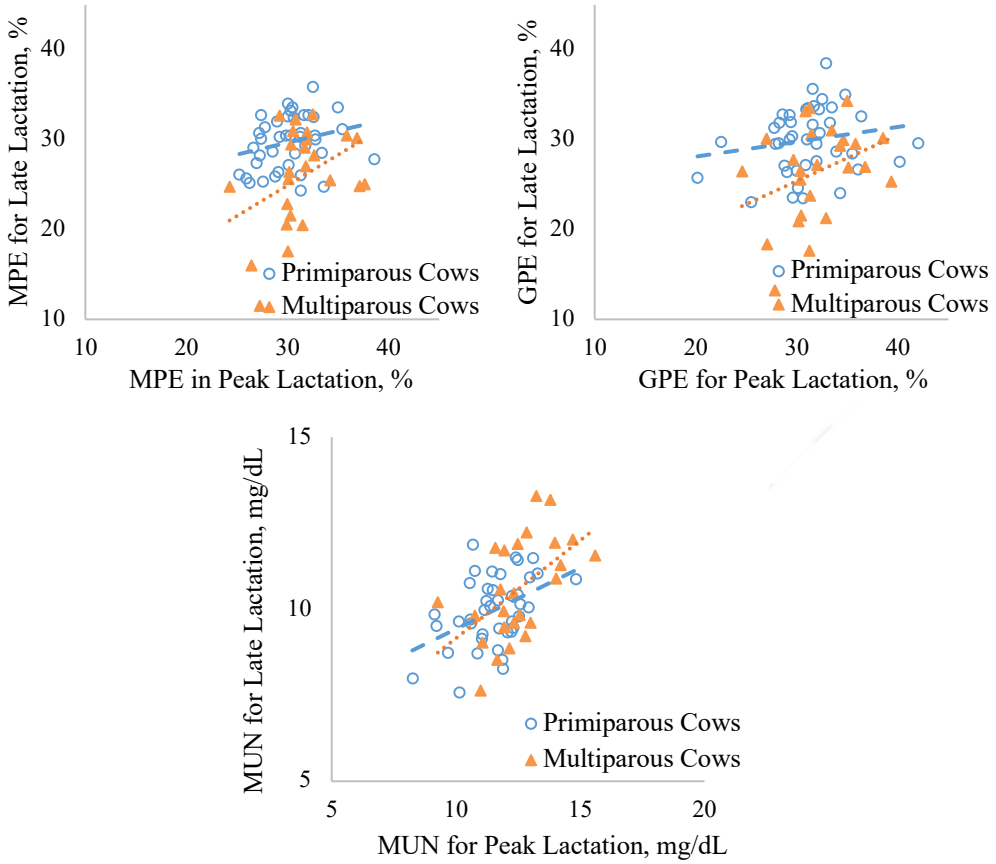

Figure 3.5 Repeatability of protein efficiency (MPE, GPE and MUN) across lactation stage based on average values across diets (n=69). Repeatability of protein efficiency across lactation stage were 0.19 for MPE (Y = 0.307 (± 0.184; $P = 0.10$) × X + 18.82 (± 5.66; $P < 0.01$)), 0.15 for GPE (Y = 0.224 (± 0.149; $P = 0.14$) × X + 21.56 (± 4.73; $P < 0.01$)), and 0.53 for MUN (Y = 0.467 (± 0.095; $P < 0.01$) × X + 4.63 (± 1.14; $P < 0.01$)). Each data point represents one cow's protein efficiency value for each diet (n=69). Open circles indicate primiparous cows (n=42), and solid triangles indicate multiparous cows (n=27). Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

MUN and Protein Efficiency

In peak-lactation cows, GPE was highly correlated with MPE in both HP (r = 0.86, $P < 0.05$) and LP diets (r = 0.91, $P < 0.05$); MUN was not correlated with MPE or GPE in neither HP nor LP diet (Figure 3.6). In late-lactation cows, GPE was highly correlated with MPE in both HP (r = 0.86, $P < 0.05$) and LP diets (r = 0.91, $P < 0.05$); MUN was not correlated with MPE or GPE in neither HP nor LP diet (Figure 3.7).

Based on the average value across dietary protein contents and lactation stages, there was no correlation between MUN and MPE regardless of dietary protein contents and lactation stages. Further details are shown in Figure 3.8. Similar trends were found between MUN and GPE.

Figure 3.6 Relationship between MUN and MPE/GPE across dietary protein contents (HP vs. LP) in peak lactation cows

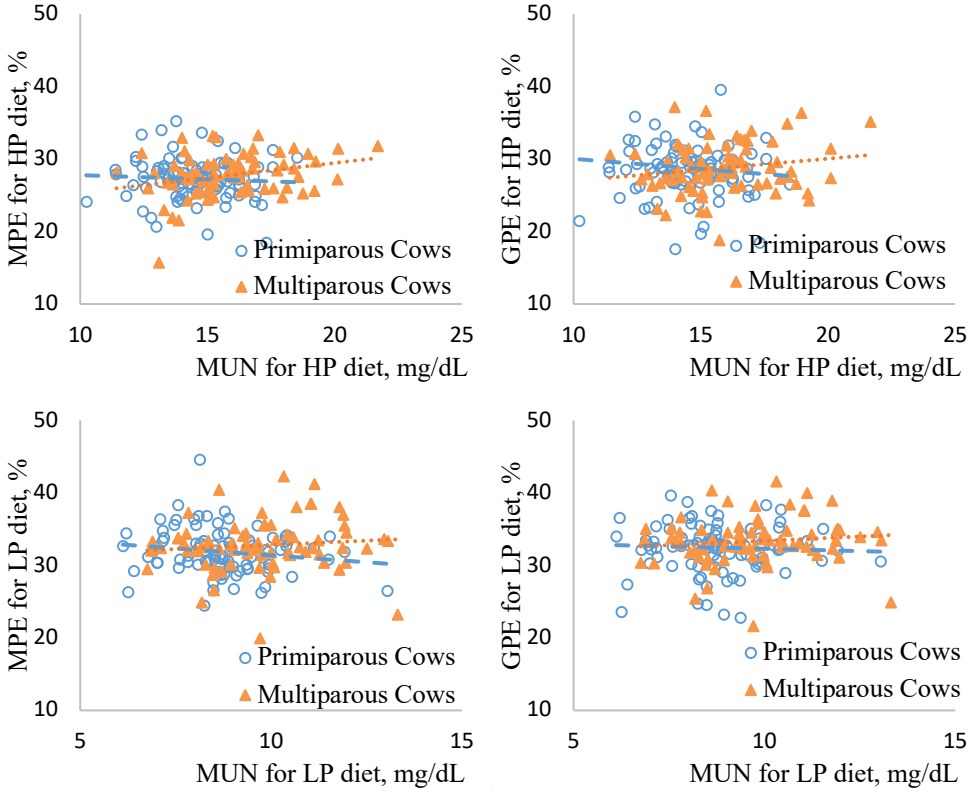

Figure 3.6 Relationship between MUN and MPE/GPE across dietary protein contents (HP vs. LP) in peak lactation cows (n=166). The correlation coefficient between MUN and MPE was -0.05 in HP_{peak} ($Y = 0.204$ ($\pm$ 0.119; $P = 0.10$) $\times X + 24.39$ ($\pm$ 1.80; $P < 0.01$)), -0.12 in LP_{peak} ($Y = 0.055$ ($\pm$ 0.063; $P = 0.75$) $\times X + 31.77$ ($\pm$ 1.63; $P < 0.01$)). The correlation coefficients between MUN and GPE was -0.15 in HP_{peak} ($Y = 0.105$ ($\pm$ 0.123; $P = 0.39$) $\times X + 26.58$ ($\pm$ 1.88; $P < 0.01$)), -0.13 in LP_{peak} ($Y = -0.092$ ($\pm$ 0.193; $P = 0.63$) $\times X + 33.55$ ($\pm$ 1.77; $P < 0.01$)). Each data point represents one value (n=166). Open circles indicate primiparous cows (n= 92), and solid triangles indicate multiparous cows (n= 74). Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

Figure 3.7 Relationship between MUN and MPE/GPE across dietary protein contents (HP vs. LP) in late lactation cows

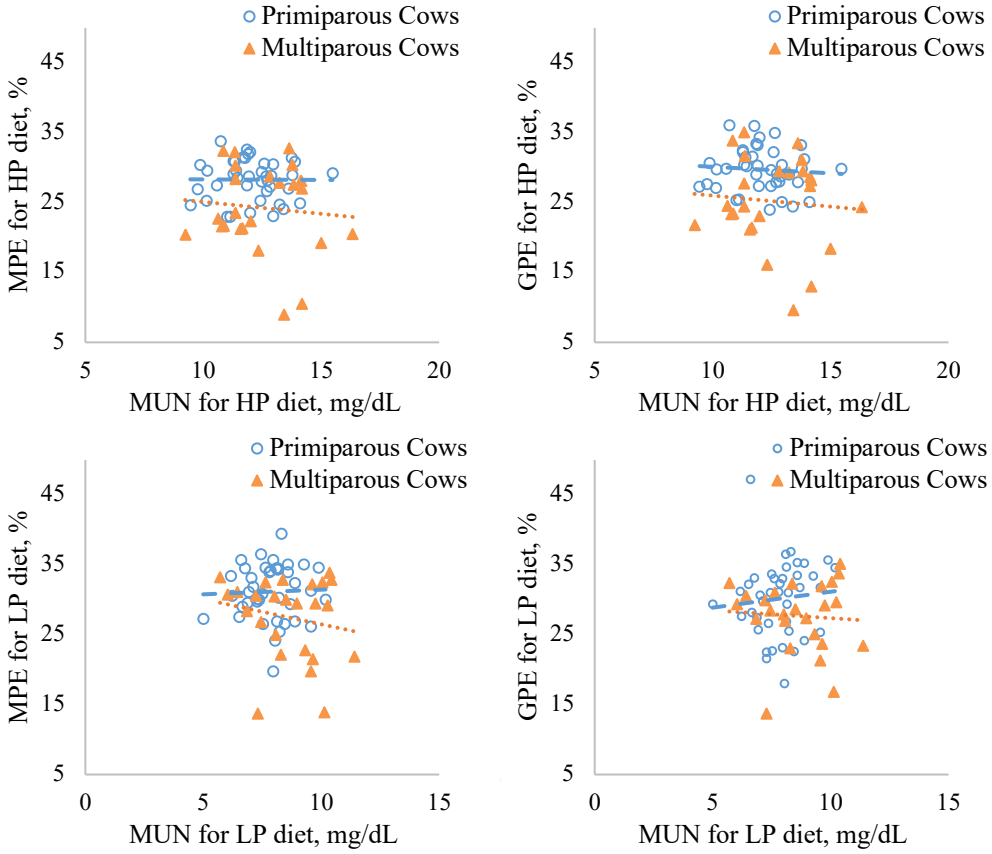

Figure 3.7 Relationship between MUN and MPE/GPE across dietary protein contents (HP vs. LP) in late lactation cows (n=69). The correlation coefficient between MUN and MPE was -0.06 in HP_{late} (Y = - 0.194 (± 0.419; $P = 0.64$) × X + 28.90 (± 5.16; $P < 0.01$)), -0.13 in LP_{late} (Y = -0.581 (± 0.538; $P = 0.29$) × X + 34.05 (± 4.44; $P < 0.01$)). The correlation coefficients between MUN and GPE was -0.14 in HP_{late} (Y = -0.581 (± 0.462; $P = 0.21$) × X + 34.63 (± 5.68; $P < 0.01$)), -0.13 in LP_{late} (Y = -0.521 (± 0.493; $P = 0.29$) × X + 34.34 (± 4.06; $P < 0.01$)). Each data point represents one value (n=69). Open circles indicate primiparous cows (n=42), and solid triangles indicate multiparous cows (n=27). Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

Figure 3.8 Relationship between MUN and MPE across dietary protein contents (HP vs. LP) and lactation stage

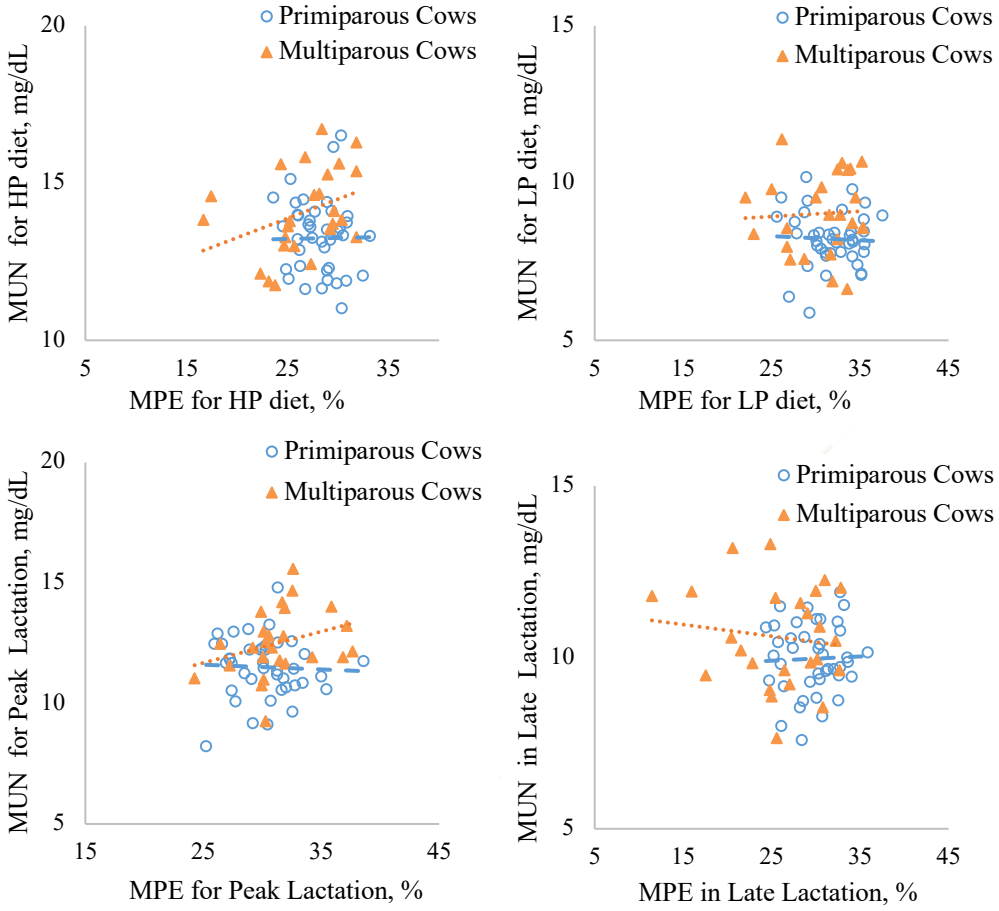

Figure 3.8 Relationship between MUN and MPE across dietary protein contents (HP vs. LP) and lactation stage (n=69). The correlation coefficient between MUN and MPE was 0.10 for the HP diet (Y = 0.046 (± 0.055; $P = 0.41$) × X + 12.29 (± 1.52; $P < 0.01$)), 0.06 for the LP diet (Y = 0.018 (± 0.041; $P = 0.66$) × X + 9.09 (± 1.29; $P < 0.01$)), 0.12 in peak lactation (Y = 0.075 (± 0.056; $P = 0.19$) × X + 9.57 (± 1.75; $P < 0.01$)), and -0.11 in late lactation (Y = -0.044 (± 0.033; $P = 0.19$) × X + 11.45 (± 0.97; $P < 0.01$)). Each data point represents one value (n= 69). Open circles indicate primiparous cows (n= 42), and solid triangles indicate multiparous cows (n= 27). Milk protein efficiency (MPE), milk protein per unit of dietary protein intake. Gross protein efficiency (GPE), milk protein and body protein capture per unit of dietary protein intake.

Variation of Low Protein Resilience 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 standard deviation being - 0.13 kg/d. The final model for LPR in peak-lactation cows was:

$$dCapP = \beta_0 + \beta_1 \times CapP\_MBW_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e.$$

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 standard deviation being - 0.14 kg/d. The final model for LPR in late-lactation cows was:

$$dCapP = \beta_0 + \beta_1 \times CapP\_MBW_{HP} + \beta_2 \times MBW\_HH_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e.$$

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 3.2 and 3.3, among peak-lactation cows, when fed the HP diet, high-LPR cows had similar DMI, MY, ECM, MBW, BCS, protein intake, milk protein yield, CapP, MPE, and MUN, with lower BodyP and GPE, compared to low-LPR cows. When switched to LP diet, high-LPR cows had higher DMI, MY, ECM, protein intake, milk protein yield, BodyP, CapP, and GPE, compared to low-LPR cows. Among late-lactation cows, when fed the HP diet, high-LPR cows had similar DMI, MY, ECM, BCS, protein intake, milk protein yield, CapP, MPE, GPE, and MUN, with lower dEBW and BodyP, compared to low-LPR cows. When

switched to the LP diet, high-LPR cows had higher DMI, MY, ECM, dEBW, protein intake, milk protein yield, BodyP, CapP, MPE, and GPE.

Table 3.2 Comparisons of production parameters of high-, medium- and low- LPR cows in peak and late lactation[1,2]

Item[3]	Peak-lactation Cows N= 166			Late-lactation Cows N= 69		
	HLPR	**MLPR**	**LLPR**	**HLPR**	**MLPR**	**LLPR**
LPR value, kg/d	0.096[a]	0.004[b]	-0.103[c]	0.082[a]	0.001[b]	-0.096[c]
	0.006	0.004	0.006	0.008	0.006	0.009
DMI_HP, kg/d	24.8[a]	23.8[a]	24.1[a]	19.9[a]	19.8[a]	19.4[a]
	0.59	0.64	0.44	0.61	0.43	0.67
DMI_LP, kg/d	24.5[a]	22.7[b]	22.4[b]	19.2[a]	18.0[ab]	17.8[ab]
	0.58	0.62	0.43	0.57	0.41	0.63
dDMI, kg/d	-0.48[a]	-1.21[b]	-2.19[c]	-0.72[a]	-1.80[ab]	-1.60[ab]
	0.25	0.26	0.18	0.41	0.29	0.45
MY_HP, kg/d	42.3[a]	40.9[a]	41.6[a]	26.1[a]	26.0[a]	26.3[a]
	1.18	0.87	1.26	1.35	0.96	1.48
MY_LP, kg/d	39.8[a]	35.3[b]	35.7[b]	23.1[a]	23.5[a]	18.4[b]
	1.32	0.98	1.42	1.23	0.88	1.34
dMY, kg/d	-2.47[a]	-5.67[b]	-5.44[b]	-2.99[a]	-2.38[a]	-7.88[b]
	0.50	0.37	0.54	0.91	0.65	0.98
ECM[4]_HP, kg/d	41.7[a]	40.6[a]	40.9[a]	26.9[a]	27.2[a]	26.2[a]
	1.17	0.87	1.26	1.51	1.07	1.66
ECM_LP, kg/d	38.6[a]	35.8[b]	34.9[b]	24.4[a]	24.6[a]	20.7[b]
	1.03	0.76	1.11	1.34	0.95	1.41
dECM, kg/d	-2.89[a]	-3.87[b]	-5.18[c]	-2.53[a]	-2.38[a]	-5.46[b]
	0.45	0.34	0.49	0.86	0.61	0.94
MBW[5]_HP, kg	129[a]	128[a]	130[a]	138[a]	133[a]	136[a]
	1.83	1.35	1.97	3.37	2.39	3.71
MBW_LP, kg	129[a]	128[a]	129[a]	137[a]	132[a]	136[a]
	1.79	1.32	1.92	3.35	2.39	3.67
dMBW, kg	-0.48[a]	-0.25[a]	-0.91[a]	-0.74[a]	-0.98[a]	-0.54[a]
	0.56	0.42	0.61	0.74	0.41	0.63
BCS_HP	3.12[a]	3.29[a]	3.26[a]	3.78[a]	3.62[a]	3.73[a]
	0.05	0.04	0.05	0.10	0.08	0.10
BCS_LP	3.10[a]	3.27[a]	3.23[a]	3.62[a]	3.46[a]	3.77[ab]
	0.05	0.04	0.05	0.10	0.08	0.10
dBCS	-0.02[a]	-0.02[a]	-0.02[a]	-0.17[a]	-0.16[a]	0.04[b]
	0.03	0.02	0.03	0.05	0.04	0.05
dEBW[6] _HP, kg/d	0.16[a]	0.43[ab]	0.61[b]	0.41[a]	0.54[a]	0.80[ab]
	0.11	0.09	0.11	0.16	0.12	0.17
dEBW _LP, kg/d	0.49[a]	0.18[b]	-0.32[c]	0.73[a]	0.11[b]	0.09[b]
	0.12	0.09	0.12	0.13	0.10	0.15
ddEBW[7], kg/d	0.33[a]	-0.27[b]	-0.92[c]	0.32[a]	-0.43[b]	-0.72[c]
	0.16	0.12	0.12	0.22	0.16	0.24

Table 3.2 (cont'd)

Item[3]	Peak-lactation Cows N= 166			Late-lactation Cows N= 69		
	HLPR	**MLPR**	**LLPR**	**HLPR**	**MLPR**	**LLPR**
CapPMBW[8]_HP	0.0095[a]	0.0095[a]	0.0095[a]	0.0063[a]	0.0064[a]	0.0063[a]
	0.0002	0.0002	0.0002	0.0004	0.0003	0.0004
CapPMBW_LP	0.0092[a]	0.0077[b]	0.0082[c]	0.0058[a]	0.0055[b]	0.0046[b]
	0.0002	0.0002	0.0002	0.0002	0.0002	0.0003
dCapPMBW	-0.0002[a]	-0.0011[b]	-0.0019[c]	-0.0005[a]	-0.0010[b]	-0.0017[b]
	0.0001	0.0001	0.0001	0.0002	0.0001	0.0002

[1] Cows were grouped into high-, medium-, and low- LPR (low protein resilience) groups. Cows > 0.5 SD of the mean LPR were classified as high LPR, cows < -0.5 SD were classified as low LPR, and those ± 0.5 SD were classified as medium LPR. 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 CapP per kg metabolic BW when fed the HP; *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 body weight to height ratio when fed the HP.

[2] Different superscripts indicate significant differences in means ($P < 0.05$). Superscripts intend to compare variables within lactation stage, not between lactation stages.

[3] Upper values within a row = least squares mean (LSM) of the group. Lower values within a row = standard error of the LSM.

[4] Energy-corrected milk; ECM = [(0.327 × kg milk) + (12.95 × kg milk fat) + (7.20 × kg milk protein)] (Tyrrell and Reid, 1965).

[5] Metabolic BW = $BW^{0.75}$.

[6] Change of EBW(empty BW) 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

[7] $ddEBW = dEBW_{LP} - dEBW_{HP}$

[8] Captured protein per unit of MBW, where captured protein= milk protein + protein captured in body tissues

Table 3.3 Comparisons of protein output and protein efficiency parameters of high-, medium- and low- LPR cows in peak and late lactation[1,2]

Item[3]	Peak-lactation Cows N= 166			Late-lactation Cows N= 69		
	HLPR	**MLPR**	**LLPR**	**HLPR**	**MLPR**	**LLPR**
LPR value, kg/d	0.096[a]	0.004[b]	-0.103[c]	0.082[a]	0.001[b]	-0.096[c]
	0.006	0.004	0.006	0.008	0.006	0.009
CPp[4]_HP, %	17.9[a]	17.9[a]	17.9[a]	15.8[a]	15.8[a]	15.8[a]
	0.03	0.03	0.02	0.003	0.003	0.004
CPp_LP, %	14.2[a]	14.2[a]	14.2[a]	12.9[a]	12.9[a]	12.9[a]
	0.05	0.04	0.05	0.001	0.001	0.001
dCPp, %	-3.71[a]	-3.74[a]	-3.73[a]	-2.93[a]	-2.93[a]	-3.00[a]
	0.06	0.05	0.06	0.001	0.001	0.001
CPIntake_HP, kg/d	4.45[a]	4.28[a]	4.38[a]	3.15[a]	3.14[a]	3.06[a]
	0.11	0.08	0.12	0.09	0.06	0.10
CPIntake_LP, kg/d	3.48[a]	3.23[b]	3.18[b]	2.50[a]	2.32[a]	2.30[ab]
	0.08	0.07	0.08	0.08	0.06	0.09
dCPIntake, kg/d	-0.97[a]	-1.05[a]	-1.20[b]	-0.65[a]	-0.82[a]	-0.77[ab]
	0.047	0.035	0.051	0.067	0.047	0.073
MilkP[5]_HP, kg/d	1.23[a]	1.19[a]	1.23[a]	0.829[a]	0.849[a]	0.832[a]
	0.035	0.026	0.038	0.045	0.032	0.049
MilkP_LP, kg/d	1.14[a]	1.03[b]	1.02[b]	0.731[a]	0.712[a]	0.606[b]
	0.031	0.023	0.033	0.039	0.028	0.043
dMilkP, kg/d	-0.083[a]	-0.130[b]	-0.209[c]	-0.098[a]	-0.136[a]	-0.237[b]
	0.013	0.011	0.015	0.026	0.019	0.029
BodyP[6]_HP, kg/d	0.014[a]	0.031[b]	0.040[bc]	0.027[a]	0.037[a]	0.061[b]
	0.007	0.005	0.007	0.012	0.008	0.013
BodyP_LP, kg/d	0.035[a]	0.016[b]	-0.017[c]	0.059[a]	-0.001[b]	0.007[b]
	0.008	0.006	0.008	0.012	0.008	0.013
dBodyP, kg/d	0.021[a]	-0.014[b]	-0.058[c]	0.032[a]	-0.038[b]	-0.055[b]
	0.010	0.008	0.010	0.018	0.019	0.012
PregP[7]_HP, kg/d				0.014[a]	0.009[a]	0.013[a]
				0.005	0.004	0.005
PregP_LP, kg/d				0.011[a]	0.009[a]	0.005[a]
				0.005	0.005	0.005
dPregP, kg/d				-0.002[a]	-0.001[a]	-0.008[a]
				0.007	0.005	0.007
CapP[8]_HP, kg/d	1.24[a]	1.20[a]	1.27[a]	0.823[a]	0.886[a]	0.844[a]
	0.035	0.026	0.038	0.048	0.034	0.053
CapP_LP, kg/d	1.18[a]	1.05[b]	1.00[c]	0.790[a]	0.712[ab]	0.613[c]
	0.037	0.023	0.033	0.035	0.025	0.039
dCapP, kg/d	-0.062[a]	-0.145[b]	-0.266[c]	-0.033[a]	-0.174[b]	-0.230[b]
	0.016	0.012	0.017	0.027	0.019	0.029

Table 3.3 (cont'd)

Item[3]	Peak-lactation Cows N= 166			Late-lactation Cows N= 69		
	HLPR	**MLPR**	**LLPR**	**HLPR**	**MLPR**	**LLPR**
MPE[9]_HP, %	27.6[a]	27.2[a]	27.9[a]	26.4[a]	26.9[a]	26.6[a]
	0.43	0.34	0.46	1.22	0.87	1.34
MPE_LP, %	32.8[a]	32.0[a]	32.1[a]	29.4[a]	30.7[a]	26.1[b]
	0.50	0.37	0.54	1.29	0.92	1.31
dMPE, %	5.23[a]	4.88[a]	4.05[b]	2.98[a]	3.76[a]	-0.49[b]
	0.35	0.26	0.36	0.98	0.68	1.05
MUN_HP	14.9[a]	15.2[a]	14.9[a]	12.52[a]	11.92[a]	12.41[a]
	0.28	0.21	0.30	0.35	0.25	0.38
MUN_LP	9.15[a]	9.31[a]	8.97[a]	8.80[a]	8.67[a]	8.44[a]
	0.22	0.17	0.24	0.29	0.20	0.30
dMUN	-5.84[a]	-5.91[a]	-6.00[a]	-3.72[a]	-3.27[a]	-3.97[a]
	0.25	0.20	0.27	0.31	0.22	0.33
GPE[10]_HP, %	27.9[a]	27.9[a]	29.0[ab]	26.1[a]	28.2[a]	27.6[a]
	0.44	0.32	0.47	1.35	0.96	1.48
GPE_LP, %	34.0[a]	32.6[b]	31.5[c]	32.0[a]	30.7[a]	26.5[c]
	0.52	0.39	0.57	1.16	0.92	1.26
dGPE, %	6.11[a]	4.65[ab]	2.52[c]	5.87[a]	2.47[b]	-1.14[c]
	0.43	0.32	0.47	0.86	0.61	0.93

[1] Cows were grouped into high, medium, and low LPR (low protein resilience) groups. Cows > 0.5 SD of the mean LPR were classified as high LPR, cows < -0.5 SD were classified as low LPR, and those ± 0.5 SD were classified as medium LPR. In peak lactation, LPR was defined as the residual term in the model: d$CapPr\ o = \beta_0 + \beta_1 \times CapP_MBW_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$ where $CapP\_MBW_{HP}$ was the CapP per kg 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: d$CapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + \beta_2 \times MBW_HH_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$ *where* MBW_HH_{HP} was the metabolic body weight to height ratio when fed HP.

[2] Different superscripts indicate significant differences in means ($P<0.05$). Superscripts intend to compare variables within lactation stage, not between lactation stages.

[3] Upper values within a row = least squares mean (LSM) of the group. Lower values within a row = standard error of the LSM.

[4] CP% of the diet, kg CP per kg DM

[5] Milk protein, kg/d

[6] Protein captured in body tissue gain: BodyP = (0.151 – 0.0268 × BCS) × dEBW, when Parity >1; BodyP = 0.132 × 0.14 + ((0.151- 0.0268 × BCS) ×(dEBW – 0.14)), when Parity= 1; dEBW= Change of EBW(empty BW) 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.

Table 3.3 (cont'd)

[7] Protein captured for pregnancy: PregP= 0.00069 × D – 0. 0692 × (CBW/45), where D was the day of gestation between 190 and 279, and CBW was the calf birth weight (NRC 2001).

[8] Total protein captured: Cows in the peak lactation: CapP= Milk Protein+ BodyP; Cows in the late lactation: CapP = Milk Protein+ BodyP+ PregP.

[9] Milk protein efficiency, milk protein per unit of dietary protein intake.

[10] Gross protein efficiency, milk protein and body protein capture per unit of dietary protein intake.

Low Protein Resilience and Protein Efficiency

As shown in Table 3. 4, LPR in peak- lactation cows was correlated with MPE_{LP} (r = 0.23, $P = 0.09$), dMPE (r = 0.13, $P = 0.09$), GPE_{LP} (r = 0.37, $P < 0.05$), and dGPE (r = 0.51, $P < 0.05$); LPR was not correlated with MPE_{HP}, $GPE_{HP,}$ $MUN_{HP,}$ MUN_{LP}, or dMUN.

As shown in Table 3. 4, LPR in late- lactation cows was correlated with MPE_{LP} (r = 0.27, $P < 0.05$), dMPE (r = 0.35, $P < 0.05$), GPE_{LP} (r = 0.48, $P < 0.05$), and dGPE (r = 0.65, $P < 0.05$); LPR was not correlated with MPE_{HP}, $MUN_{HP,}$ GPE_{HP}, $MUN_{LP,}$ or dMUN.

Table 3.4 Pearson Correlation coefficients between LPR (low protein resilience) and various protein efficiency terms

Item[1]	LPR_Mid		LPR_Late	
	r	P Value	r	P Value
MPE_{HP}[2]	0.00	0.97	0.01	0.91
MPE_{LP}	0.23	0.09	0.27	< 0.05
dMPE	0.13	0.09	0.35	< 0.05
GPE_{HP}[3]	0.09	0.24	0.04	0.70
GPE_{LP}	0.37	< 0.05	0.48	< 0.05
dGPE	0.51	< 0.05	0.65	< 0.05
MUN_{HP}[4]	0.04	0.57	0.02	0.88
MUN_{LP}	0.03	0.66	0.01	0.91
dMUN	-0.01	0.81	-0.01	0.95

[1] r stands for the Pearson correlation coefficient of the linear relationship between two variables (MPE vs. LPR, GPE vs. LPR, MUN vs. LPR); correlation was considered significant when $P < 0.05$.

[2] MPE (milk protein efficiency) is milk protein per unit of dietary protein intake. MPE_{HP} is the MPE for the HP diet; MPE_{LP} is the MPE for the LP diet; dMPE is the difference between MPE_{HP} and MPE_{LP}.

[3] GPE (gross protein efficiency) is milk protein and body protein captured per unit of dietary protein intake. GPE_{HP} is the gross protein efficiency (GPE) for the HP diet; GPE_{LP} is the GPE for the LP diet; dGPE is the difference between GPE_{HP} and GPE_{LP}.

[4] MUN, milk urea nitrogen. MUN_{HP} is the MUN for the HP diet; MUN_{LP} is the MUN for the LP diet; dMUN is the difference between MUN_{HP} and MUN_{LP}.

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 3.1). 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 MBW); these factors are shown in Table 3. 2 and Table 3. 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, etc.) that may impact 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, in order to facilitate potential genetic selection.

Low Protein Resilience, 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 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 high-LPR cows, compared to low-LPR 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 milk yield was similar between high- and low- LPR cows (Table 3.2), LPR might be useful to be included in the selection index. In the classic dairy breeding program, milk yield and milk protein yield are the major focuses (Egger-Danner et al., 2015). As a result, modern dairy cows have higher productivity than the cows hundreds of years ago (Rauw and

Gomez-Raya, 2015). The tradeoff between maintaining production and improving resilience is being discussed. LPR provides a potential solution to incorporate both production traits and resilience traits into one genetic selection program, and select more resilient ones among high-producing cows.

Repeatability of Protein Efficiency across Dietary Protein Contents and Lactation Stage

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 instead of milk protein synthesis (Veerkamp, 1998; Dillon et., 2003; Friggens et al., 2013). Thus, cows producing more milk protein in peak lactation might not be the ones in late lactation.

MUN and Protein Efficiency

We did not find any 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 in the same lactation stage, those with lower MUN values do not necessarily utilize protein more efficiently. Although Nousiainen et al. (2004) demonstrated negative links between MUN concentration and protein efficiency, they did not remove the influence of dietary protein levels in the model. As dietary protein content has significant influence on MUN and protein efficiency simultaneously (Huhtanen and Hristov, 2009), it is not surprising that MUN could be negatively linked with protein efficiency if diet effect was not removed. Indeed, in the current

study, we also found that 1) cows on the HP diet had higher MUN and lower protein efficiency (shown in Table 3.1), and 2) MUN was negatively associated with MPE (r_{peak} = -0.49, $P < 0.05$; r_{late} = -0.27, $P < 0.05$) and GPE (r_{peak} = -0.48, $P < 0.05$; r_{late} = -0.27, $P < 0.05$), if the effect of diets was not removed. Thus, the negative link between protein efficiency and MUN in Nousiainen et al. (2004) may be an artifact as a result of the manipulation of dietary protein contents. When accounting for the diet effect, Huhtanen et al. (2015) found a much lower change of protein efficiency per unit change of MUN, compared to Nousiainen et al. (2004).

Even when cows were fed the same protein content, MUN concentration can also be affected by many other factors: dehydration (Weeth and Lesperance, 1965; Steiger Burgos et al., 2001), the season of the year, time of sampling (Depeters and Cant, 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, thus more urea circulating in the body and excreting in the 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 protein feeding 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 to the HP diet, production decreased and efficiency of protein use increased, and cows maintained their protein efficiency ranking across HP and LP diets within lactation stage. Based on the results that protein efficiency was poorly associated with MUN, using MUN to rank cows for protein efficiency is misleading. Significant variation in response to protein reduction existed among cows with some cows

experiencing little drop 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 low protein resilience, or LPR. High-LPR cows had similar protein efficiency, as low-LPR cows, when fed the HP diet but higher protein efficiency when fed the LP diet. Given the existing variation among cows, LPR can potentially have a genetic basis. However, more work is needed to examine whether LPR is repeatable across other types of diet changes (for example, 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 wks., etc.). If LPR is repeatable across diets and time, and it is indeed an individual cow trait, further work on a potential genetic basis for LPR would be warranted and would require collaboration among research institutes to collect adequate data.

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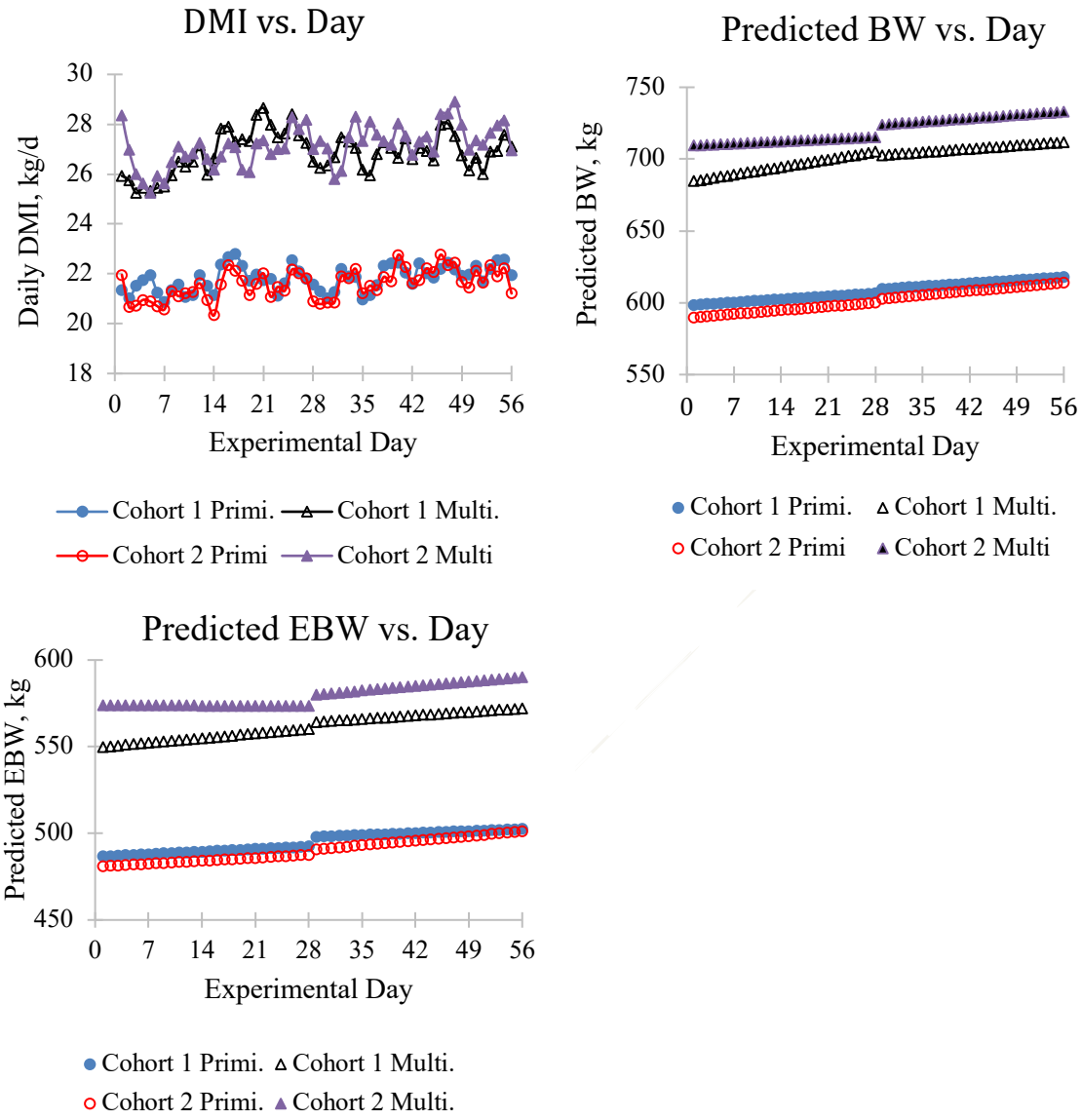
APPENDIX

Supplementary Figure 3.1 The relationship between DMI, predicted BW, predicted EBW and day in experiment in peak lactation cows



Supplementary Figure 3.1 The relationship between DMI, predicted BW, predicted EBW and day in experiment in peak lactation cows (n=166). Solid circles indicate primiparous cows in cohort 1, open circles indicate multiparous cows in cohort 1, solid triangles indicate primiparous cows in cohort 2, and open triangles indicate multiparous cows in cohort 2, where cows in cohort 1 were assigned the treatment sequence from HP to LP while cows in cohort 2 were assigned the treatment sequence from LP to HP. Upton plotting the data of DMI and predicted BW, we could not determine whether the BW change was due to the change of DMI; thus we corrected BW for EBW (empty BW; EBW = BW- 5.2 × DMI). In multiparous cows, the EBW change (kg/d) was different from BW change (kg/d).

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CHAPTER 4

ASSOCIATION AMONG DIGESTIBILITY, RESIDUAL FEED INTAKE AND LOW PROTEIN RESILIENCE IN LACTATING DAIRY COWS FED HIGH AND LOW PROTEIN DIETS

ABSTRACT

Our objective was to determine whether variation in total tract digestibility could account for the variation in residual feed intake (RFI) and low protein resilience (LPR) in lactating dairy cows. Lactating Holstein cows (n = 166; 92 primiparous, 77 multiparous) with initial milk yield (MY) 41.3 ± 9.8 kg/d were included in the crossover experiments with two treatments (high-protein diets, HP; low-protein diets, LP) and two periods of 28- 35 d each. Experiments were repeated in 69 of the 166 cows (42 primiparous, 27 multiparous) in late lactation. Low-protein diets were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function. Expeller soybean meal was added in place of corn and soyhulls to create high-protein diets, which were 18% CP in peak lactation and 16% CP in late lactation. 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. Samples of feed ingredients, orts and feces were collected in the last 5 days of each treatment period and analyzed to determine the digestibilities of DM, NDF, and CP for each cow on each diet. RFI was calculated for each cow on each diet based on the actual intake, milk energy output, metabolic BW, and retained body energy (calculated from BW change and BCS over the treatment period). LPR was calculated for each cow in each lactation stage based on the captured protein (milk protein + body protein gain) and feed intake. Neither RFI nor LPR was correlated with digestibilities of DM, NDF, or CP in either diet or lactation stage. The changes in

digestibilities of DM, NDF, and CP from the HP to LP diet did not account for LPR. In conclusion, variation in digestibility among cows could not explain the variations in RFI or LPR; we suggest that post-absorptive metabolism explains most of the variation in RFI and LPR when lactating cows are fed diets with minimal NDF in peak lactation and 40% NDF in late lactation.

INTRODUCTION

Variation in residual feed intake (RFI) of lactating cows is well documented (Tempelman et al., 2015; VandeHaar et al., 2016). Variability in digestion is one factor that has been investigated to explain this variation. Richardson and Herd (2004) found that digestive variability accounted for 10% of the RFI variation in finishing beef steers. Consistently, Nkrumah et al. (2006) and Rius et al. (2012) found that growing heifers with low RFI values had better nutrient digestibility than those with high RFI values. In contrast with those results, no relationship was found between RFI and nutrient digestibility by Cruz et al. (2010) and Lawrence et al. (2011). Given different diets being fed across those studies, Rius et al. (2012) raised the possibility that the inconsistent findings among studies perhaps are due to differences in diets of these studies. Following this idea, Potts et al. (2017) examined the association of RFI and nutrient digestibility in lactating dairy cows across high- and low-starch diets. In their study, RFI was only correlated with DM digestibility in the low-starch diet but not in the high-starch diet. Whether differences in other nutrients (e.g., CP) also influence the possible correlation of digestibility with RFI is not clear.

Some cows are more resilient to low-protein diets, in other words, they are able to continue to produce normal quantities of milk per unit of BW when fed diets that have insufficient protein for the average cow, even after adjusting for all factors that would be expected to alter protein requirements. The underlying mechanisms for low protein resilience (LPR) are not clear, but the resilience could be due to a better ability to efficiently digest foods in the face of a low-protein diet or to have more efficient post-absorptive metabolisms. Thus, the objective of the current study was to determine whether variation in total tract digestibility could account for the variation in RFI and LPR in lactating dairy cows. We hypothesized that 1)

digestive efficiency would account for some of the variation in RFI and this relationship would be altered by dietary protein content, and 2) cows with higher LPR value would have greater digestibility when fed low-protein diets.

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 lactating Holstein dairy cows were used in this study. Data of MY, milk components, BW, and hip height were collected in the experiments. These are the same animal as in Liu and VandeHaar (2020). In brief, lactating Holstein cows (n= 166; 92 primiparous, 77 multiparous) with initial milk yield (MY) 41.3 ± 9.8 kg/d were included in the crossover experiments with two treatments (high-protein diets, HP; low-protein diets, LP) and two periods of 28-35 d each. Experiments were repeated in 69 of the 166 cows (42 primiparous, 27 multiparous) in late lactation. Low-protein diets were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function. Expeller soybean meal was added in place of corn and soyhulls to create high-protein diets, which were 18% CP in peak lactation and 16% CP in late lactation. 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.

Sample Collection and Analyses

Collection and analyses of diet ingredients, orts, and fecal samples followed similar procedures in all the experiments. During the last 5 days of experimental periods, samples of feed ingredients (0.5 kg) and orts (12.5% of the amount) were obtained daily to determine the nutrient profile of the diets. Samples of feces were collected every 15 h in the last 5 d to obtain 8 samples per cow to represent every 3 h of a day to account for variations. All samples were frozen after collection until analysis.

The reported nutrient and ingredient composition of diets were calculated by averaging across both periods for each experiment. Samples of feed ingredients, orts and feces were analyzed for CP, NDF, indigestible NDF, and ash. Crude protein was determined according to Hach et al. (1987). Neutral detergent fiber was determined according to Mertens (2002). Indigestible NDF, which was used as an internal marker to estimate fecal output and nutrient digestibility (Cochran et al., 1986), was estimated as NDF residue after 240 h of in vitro fermentation (Goering and VanSoest, 1970); flasks were re-inoculated at 120 h to ensure a viable microbial population. Rumen fluid for the in vitro incubations was collected from a cow fed only dry hay. Ash was determined after 5 h combustion at 500°C.

Calculations and Statistical Analyses

Milk energy output (**MilkE**; Mcal/d) for individual cows was estimated by 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.

For cows > 190 d pregnant, BW was corrected for conceptus weight (**CW**) for use in the RFI equation and to calculate energy and protein change of body tissues. Conceptus weight was calculated using the 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.

Empty BW (**EBW**) was calculated for each cow to adjust BW for the gut fill (Gibbs et al., 1992; Andrew et al., 1994),

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

where DMI was the daily DMI when BW was measured.

Mean daily EBW change (**dEBW**; kg/d) was calculated for each cow within the treatment period by linear regression after two rounds of removing outliers in the data; an outlier was any BW > 3.5 SD from the regression line.

For multiparous cows, EBW change was considered to be all body condition, and the body energy gained or lost with changes in EBW (**BodyE**; Mcal/d) was estimated by the equation as:

$$BodyE = RE\ (Mcal/kg) \times dEBW,$$

where $RE = 3.52 + 1.27 \times BCS$ (derived from NRC 2001, Table 2-4).

For primiparous cows, we assumed their mature BW would be 700 kg and that they had to gain 0.14 kg EBW/d of true growth across the first lactation to reach 92% of mature BW by their second calving (NRC, 2001). Based on the NRC (2001) equations (11-1 and 11-2), the RE content of true growth is 4.4 Mcal/kg dEBW. Any deviation in dEBW from 0.14 kg/d was

considered to be body condition gain or loss, and the dEBW associated with body condition change was the same as for multiparous cows (3.52 + 1.27 × BCS) Mcal/kg dEBW.

Thus, the equation to calculate BodyE was:

$$\text{BodyE} = \begin{cases} (3.52 + 1.27 \times \text{BCS}) \times \text{dEBW}, & \text{Parity} > 1 \\ 4.4 \times 0.14 + [(3.52 + 1.27 \times \text{BCS}) \times (\text{dEBW} - 0.14)], & \text{Parity} = 1 \end{cases}$$

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

PregE = [(0.00318 × D - 0.0352) × (CBW/45)] / 0.218,

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

Apparent diet net energy content (**DietNE_L**; Mcal/kg) was calculated for each diet as the average NE_L required by each cow for maintenance, milk, pregnancy, and body gain divided by her average daily intake for the diet:

DietNE_L = Average [(MilkE + 0.08 × MBW + BodyE + PregE) / DMI],

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

Under the similar assumptions and information used in NRC energy calculation (2001; Table 2-4, Equations 11-4 and 11-5), protein captured for body tissue gain (**BodyP**; kg/d) was calculated from dEBW and BCS as:

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

where (0.151- 0.0268 × BCS) kg protein per kg dEBW was assumed when dEBW was considered as body condition gain or loss, and 0.132 kg protein per kg dEBW was considered for the 0.14 kg/d growth.

Protein captured for pregnancy (PregP; kg/d) was calculated using the equation from NRC (2001):

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

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 estimated for each cow in each treatment (HP and LP) as:

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

Residual feed intake was calculated similar to the residual term in the prediction of DMI, as previously described in Liu and VandeHaar (2020). Intake for an individual cow during each period was regressed as a function of major energy sinks using GLM procedure in SAS (9.4):

$$DMI = \beta_0 + \beta_1 \times MilkE + \beta_2 \times MBW + \beta_3 \times BodyE + \beta_4 \times DIM + \beta_5 \times DIM \times DIM + Parity + Experiment + Cohort\ (Experiment) + Diet\ (Cohort \times Experiment) + e,$$

where DMI was the observed DMI, MilkE was the observed milk energy output, MBW was the average $BW^{0.75}$, BodyE was the predicted change in body energy based on dEBW 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 (1-7), 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. RFI was defined as the residual term (e) in the model. For cows in the late lactation, a term for pregnancy energy was included.

Low protein resilience was calculated as described by Liu and VandeHaar (2019, submitted) as:

$$dCapP = \beta_0 + \beta_1 \times CapP\_MBW_{HP} + \beta_2 \times BCS_{HP} + \beta_3 \times MBW\_HH_{HP} + \beta_4 \times dCP + \beta_5 \times CP_{HP} + \beta_6 \times DIM_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$$

where dCapP was the change of captured protein (milk protein, body protein and pregnancy protein) when switched from the HP to LP diet, $CapP\_MBW_{HP}$ was the CapP per kg metabolic BW when fed the HP diet; BCS_{HP} was the BCS when fed the HP diet; MBW_HH_{HP} was the metabolic body weight 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 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 defined as the residual term.

Cows were grouped into high (**HRFI**), medium (**MRFI**), and low (**LRFI**) RFI groups. Cows > 0.5 SD of the mean RFI were classified as HRFI group, cows < -0.5 SD were classified as LRFI, and those ± 0.5 SD were classified as MRFI. Cows were also grouped into high (**HLPR**), medium (**MLPR**), and low (**LLPR**) LPR groups based on similar criteria. Comparison among different groups was performed. The effect of efficiency group (RFI or LPR) was determined using the GLM Procedure of SAS according to the model $Yi = \mu + R_i + e$, where μ was the overall mean, R_i was the fixed effect of efficiency group, and *e* was the residual error.

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**).

To quantify the association among RFI, LPR, MPE and nutrient (CP, NDF and DM) digestibility within each diet, Pearson correlations were obtained using the GLM procedure in SAS (9.4) after accounting for effects of parity, treatment sequence, and experiment.

Production responses to diets and digestibility difference 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 $P \leqslant 0.05$ and tendency at $P \leqslant 0.10$. Interactions were considered significant at $P \leqslant 0.10$ and trends at $P \leqslant 0.15$.

RESULTS

Cow Performance for High- and Low- Protein Diets

As shown in Table 4.1, in peak-lactation cows, compared to the LP diet, the HP diet increased apparent NE_L intake, BodyE, and MilkE; HP also increased milk protein yield and MUN concentration, and decreased MPE. Compared to the LP diet, the HP diet increased digestibilities of DM, NDF, and CP by 2.8, 2.8, and 6.2 percentage units ($P < 0.05$), respectively.

As shown in Table 4.2, in late-lactation cows, compared to the LP diet, the HP diet increased apparent NE_L intake, BodyE, and MilkE; HP also increased milk protein yield and MUN concentration, and decreased MPE. Compared to the LP diet, the HP diet increased digestibilities of DM, NDF, and CP by 2.0, 1.8, and 7.4 percentage units ($P < 0.05$), respectively.

Table 4.1 Energy output, protein efficiency and digestibility for cows fed treatment diets in peak lactation[1,2]

	Treatments[3]			Parity			P-value[4]		
	HP_{peak} n=166	LP_{peak} n=166	SEM	Primi. n=184	Multi. n=148	SEM	TRT	Parity	TRT × Parity
Calculated Energy Values									
Apparent NE_L[5], Mcal/d	42.3	36.7	0.03	34.6	44.4	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[6], Mcal/d	3.81	1.34	0.71	1.66	3.49	0.75	<0.01	<0.01	0.30
Maintenance[7], Mcal/d	10.4	10.3	0.01	9.6	11.0	0.10	<0.01	<0.01	0.10
N Metabolism									
Milk Protein, kg/d	1.21	1.07	0.01	1.01	1.28	0.02	<0.01	<0.01	0.18
Body Protein[8], kg/d	0.03	0.01	0.01	0.03	0.01	0.01	<0.01	0.04	0.36
MUN, mg/dL	15.1	9.2	0.13	11.6	12.7	0.20	<0.01	<0.01	0.16
MPE[9], %	27.6	32.4	0.18	29.6	30.3	0.46	<0.01	0.11	0.31
Nutrient Digestibilities									
DM, %	65.7	62.9	0.42	64.5	64.2	0.43	<0.01	0.54	0.23
NDF, %	48.3	45.5	0.58	47.5	46.3	0.60	<0.01	0.06	0.70
CP, %	69.4	63.2	0.49	66.2	66.4	0.50	<0.01	0.78	0.09

[1]Average DIM was 125 for primiparous cows in HP_{peak} diet, 126 for primiparous cows in LP_{peak} diet, 122 for multiparous cows in HP_{peak} diet and 121 for multiparous cows in LP_{peak} diet.

[2]Average parity for multiparous cows was 2.94 in peak lactation

[3] Treatments contained 18% and 14% crude protein on a DM basis for peak lactation cows

[4] *P*-value associated with treatment differences (HP_{peak} vs. LP_{peak}; TRT) and parity differences (Primi vs. Multi.; Parity) in peak lactation cows. Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

[5] NE_L= MilkE + 0.08 × MBW + BodyE, where MilkE is net energy utilized for milk synthesis, MBW is metabolic body weight, and BodyE is estimated energy captured in body tissue.

[6] BodyE = (3.52 + 1.27 × BCS) × dEBW, when Parity >1; BodyE = 4.4 × 0.14 + ((3.52 + 1.27 × BCS) × (dEBW – 0.14)), when Parity= 1. dEBW is the estimated change of EBW(empty BW) 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.

[7] Maintenance energy = 0.08 × MBW, where MBW= $BW^{0.75}$

Table 4.1 (cont'd)

[8] Protein captured in body tissue gain: BodyP = (0.151 – 0.0268 × BCS) × dEBW, when Parity >1; BodyP = 0.132 × 0.14 + ((0.151-0.0268 × BCS) ×(dEBW – 0.14)), when Parity = 1.

[9] MPE, milk protein efficiency, defined as the dietary protein captured in milk.

Table 4.2 Energy output, protein efficiency and digestibility for cows fed treatment diets in late lactation[1,2]

	Treatments[3]			Parity			P-value[4]		
	HP_{late} n=69	LP_{late} n=69	SEM	Primi. n=84	Multi. n=54	SEM	TRT	Parity	TRT × Parity
Calculated energy values									
Apparent NE_L[5], Mcal/d	33.3	28.3	0.77	29.9	31.8	1.01	<0.01	<0.01	0.12
Milk, Mcal/d	18.4	16.0	0.31	18.1	16.3	1.04	<0.01	0.09	0.02
Body Tissue Gain[6], Mcal/d	3.81	1.34	0.71	1.66	3.50	0.75	<0.01	<0.01	0.32
Maintenance[7], Mcal/d	10.8	10.6	0.06	9.9	11.5	0.18	<0.01	<0.01	0.09
Pregnancy[8], Mcal/d	0.34	0.37	0.11	0.27	0.45	0.13	0.77	0.17	0.72
N Metabolism									
Milk Protein, kg/d	0.80	0.58	0.01	0.77	0.72	0.04	<0.01	0.25	<0.01
Body Protein[9], kg/d	0.04	0.02	0.01	0.03	0.03	0.01	0.03	0.71	0.21
MUN, mg/dL	12.1	8.1	0.16	9.9	10.2	0.27	<0.01	0.28	0.25
MPE[10], %	26.0	28.8	0.43	29.8	25.0	1.10	<0.01	<0.01	0.84
Nutrient Digestibilities									
DM, %	69.8	67.8	0.48	69.2	68.5	0.50	<0.01	0.12	0.91
NDF, %	57.6	55.8	0.90	57.0	55.4	0.81	0.09	0.06	0.33
CP, %	74.7	67.3	0.59	70.9	71.0	0.62	<0.01	0.88	0.37

[1]Average DIM was 258 for primiparous cows in HP_{late} diet, 257 for primiparous cows in LP_{late} diet, 263 for multiparous cows in HP_{late} diet, and 264 for multiparous cows in LP_{late} diet.

[2]Average parity for multiparous cows was 3.12 in late lactation

[3] Treatments contained 16% and 13% crude protein on a DM basis for late lactation cows

[4] *P*-value associated with treatment differences (HP_{late} vs. LP_{late}; TRT) and parity differences (Primi vs. Multi.; Parity) in late lactation cows. Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

[5] NE_L= MilkE + 0.08 × MBW + BodyE, where MilkE is net energy utilized for milk synthesis, MBW is metabolic body weight, and BodyE is estimated energy captured in body tissue.

[6] BodyE = (3.52 + 1.27 × BCS) × dEBW, when Parity >1; BodyE = 4.4 × 0.14 + ((3.52 + 1.27 × BCS) × (dEBW – 0.14)), when Parity= 1. dEBW is the estimated change of EBW(empty BW) 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.

[7] Maintenance energy = 0.08 × MBW, where MBW = $BW^{0.75}$

Table 4.2 (cont'd)

[8] Energy expended for pregnancy = [(0.00318 × D - 0.0352) × (CBW/45)] / 0.218, where D was the day of gestation between 190 and 279, and CBW was the calf birth weight.

[9] Protein captured in body tissue gain: BodyP = (0.151 – 0.0268 × BCS) × dEBW, when Parity >1; BodyP = 0.132 × 0.14 + ((0.151-0.0268 × BCS) ×(dEBW – 0.14)), when Parity = 1.

[10] MPE, milk protein efficiency, defined as the dietary protein captured in milk

Residual Feed Intake and Digestibility

The relationships between RFI and digestibilities of DM, NDF, and CP are illustrated in Table 4.3 and Table 4.4. For both peak- and late- lactation cows, RFI was not correlated with digestibilities of DM, NDF, or CP, regardless of diets. Based on the comparisons between LRFI cows and HRFI cows in Table 4.5, regardless of diets and lactation stages, cows with lower RFI values did not have greater digestibilities of DM, NDF, or CP, except that LRFI cows tended to have a higher DM digestibility in peak lactation.

Table 4.3. Correlation coefficients of RFI, LPR with digestibilities of DM, CP, and NDF in peak- lactation cows (n=166)

Item[1]	DMd_{HP}[2]	$NDFd_{HP}$[3]	CPd_{HP}[4]	DMd_{LP}[5]	$NDFd_{LP}$[6]	CPd_{LP}[7]	dDMd[8]	dNDFd[9]	dCPd[10]
RFI_{HP}[11]	-0.10 0.19	-0.07 0.36	-0.14 0.11						
RFI_{LP}[12]				-0.06 0.46	-0.07 0.38	-0.05 0.56			
LPR[13]	0.02 0.85	-0.01 0.88	0.00 0.96	0.14 0.11	0.06 0.42	0.13 0.12	-0.08 0.30	-0.06 0.49	-0.08 0.30
MPE_{HP}[14]	0.01 0.86	-0.08 0.30	0.15 0.08						
MPE_{LP}[15]				-0.13 0.12	-0.12 0.14	-0.14 0.11			

[1] Upper 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. LP, low- protein diets; HP: high-protein diets

[2] DM digestibility for HP

[3] NDF digestibility for HP

[4] CP digestibility for HP

[5] DM digestibility for LP

[6] NDF digestibility for LP

[7] CP digestibility for LP

[8] Change of DM digestibility from HP to LP

[9] Change of NDF digestibility from HP to LP

[10] Change of CP digestibility from HP to LP

Table 4.3 (cont'd)

[11] Residual feed intake (RFI) in cows fed the HP diet, where RFI was defined as the residual term in the model: $DMI = \beta_0 + \beta_1 \times MilkE + \beta_2 \times MBW + \beta_3 \times BodyE + \beta_4 \times PregE + \beta_5 \times DIM + \beta_6 \times$ BCS$+ Parity + Experiment + Cohort(Experiment) + Diet(Cohort \times Experiment) + e$. DMI was the observed DMI, MilkE was the observed milk energy output, MBW was the average $BW^{0.75}$, BodyE 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 (1-7), 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.

[12] Residual feed intake in cows fed LP

[13] Low protein resilience (LPR) was defined as the residual term in the model: d$CapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$ where $CapP\_MBW_{HP}$ was the CapP per kg metabolic BW when fed HP; *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.

[14] Milk protein efficiency in cows fed HP, defined as the dietary protein captured in milk

[15] Milk protein efficiency in cows fed LP, defined as the dietary protein captured in milk

Table 4.4. Correlation coefficients of RFI, LPR with digestibilities of DM, CP, and NDF in late- lactation cows (n= 69)

Item[1]	DMd_{HP}[2]	$NDFd_{HP}$[3]	CPd_{HP}[4]	DMd_{LP}[5]	$NDFd_{LP}$[6]	CPd_{LP}[7]	dDMd[8]	dNDFd[9]	dCPd[10]
RFI_{HP}[11]	-0.11 0.38	-0.17 0.18	-0.07 0.54						
RFI_{LP}[12]				0.05 0.66	0.06 0.63	0.15 0.25			
LPR[13]	0.01 0.91	0.08 0.57	0.03 0.78	0.08 0.56	0.00 0.98	0.11 0.41	0.07 0.60	0.05 0.72	0.06 0.63
MPE_{HP}[14]	-0.13 0.37	0.09 0.50	-0.14 0.35						
MPE_{LP}[15]				-0.04 0.71	-0.02 0.88	-0.03 0.77			

[1] Upper 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. LP, low- protein diets; HP: high-protein diets.

[2] DM digestibility for HP

[3] NDF digestibility for HP

[4] CP digestibility for HP

[5] DM digestibility for LP

[6] NDF digestibility for LP

[7] CP digestibility for LP

[8] Change of DM digestibility from HP to LP

[9] Change of NDF digestibility from HP to LP

[10] Change of CP digestibility from HP to LP

Table 4.4 (cont'd)

[11] Residual feed intake (RFI) in cows fed HP, where RFI was defined as the residual term in the model: $DMI = \beta_0 + \beta_1 \times MilkE + \beta_2 \times MBW + \beta_3 \times BodyE + \beta_4 \times PregE + \beta_5 \times DIM + \beta_6 \times$ BCS+ *Parity* + *Experiment* + *Cohort(Experiment)* + *Diet(Cohort* × *Experiment)*+*e*. DMI was the observed DMI, MilkE was the observed milk energy output, MBW was the average $BW^{0.75}$, BodyE 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 (1-7), 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.

[12] Residual feed intake in cows fed LP

[13] Low protein resilience (LPR) was defined as the residual term in the model: d$CapP=\beta_0 + \beta_1 \times CapP_MBW_{HP} + \beta_2 \times MBW_HH_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$ where $CapP\_MBW_{HP}$ was the CapP per kg metabolic BW when fed HP; MBW_Ht_{HP} was the metabolic body weight to height ratio when fed HP; *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.

[14] Milk protein efficiency in cows fed HP, defined as the dietary protein captured in milk

[15] Milk protein efficiency in cows fed LP, defined as the dietary protein captured in milk

Table 4.5 Nutrient digestibility for high-, medium- and low-RFI cows fed high- and low- protein diets in peak and late lactation[1,2,3,4]

	High-protein diets			Low Protein diets		
Variable	HRFI	MRFI	LRFI	HRFI	MRFI	LRFI
Peak lactation	n_{peak}= 47	n_{peak}= 80	n_{peak}= 39	n_{peak}= 49	n_{peak}= 73	n_{peak}= 44
DM, %	64.5[a]	65.2[a]	65.2[a]	60.8[a]	63.1[ab]	63.0[ab]
CP, %	67.8[a]	68.8[a]	69.3[a]	61.5[a]	63.4[a]	62.9[a]
NDF, %	48.4[a]	48.9[a]	48.5[a]	44.2[a]	46.9[a]	46.1[a]
Late lactation	n_{late}= 19	n_{late}= 28	n_{late}= 22	n_{late}= 22	n_{late}= 26	n_{late}= 21
DM, %	70.1[a]	71.1[a]	70.0[a]	69.5[a]	67.6[ab]	68.2[a]
CP, %	74.8[a]	76.1[ab]	74.5[a]	69.2[a]	66.7[ab]	67.3[a]
NDF, %	56.6[a]	58.7[a]	57.2[a]	57.8[a]	55.5[a]	56.3[a]

[1] In peak lactation cows, residual feed intake (RFI) was defined as the residual term in the model: $DMI = \beta_0 + \beta_1 \times MilkE + \beta_2 \times MBW + \beta_3 \times BodyE + \beta_4 \times DIM + \beta_5 \times DIM \times DIM + \beta_6 \times$ BCS + *Parity* + *Experiment* + *Cohort(Experiment)* + *Diet(Cohort* × *Experiment)* + *e*, where DMI was the observed DMI, MilkE was the observed milk energy output, MBW was the average $BW^{0.75}$, BodyE 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 (1-7), 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.

[2] In late lactation cows, RFI was defined as the residual term in the model: $DMI = \beta_0 + \beta_1 \times MilkE + \beta_2 \times MBW + \beta_3 \times BodyE + \beta_4 \times PregE + \beta_5 \times DIM + \beta_6 \times$ BCS + *Parity* + *Experiment* + *Cohort(Experiment)* + *Diet(Cohort* × *Experiment)* + *e*.

[3] 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.

[4] *P*-values associated with group difference were all >0.05.

Low Protein Resilience and Digestibility

The relationships between LPR and digestibilities of DM, NDF, and CP are illustrated in Table 4.3 and Table 4.4. For both peak- and late- lactation cows, LPR was not correlated with digestibilities of DM, NDF, or CP. In addition, LPR was also not correlated with the change in digestibilities of DM, NDF, or CP from HP to LP diet. Based on the comparisons between LLPR cows and HLPR cows in Table 4.6, regardless of diets and lactation stages, cows with higher LPR values did not have greater digestibilities of DM, NDF, or CP.

Table 4.6 Nutrient digestibility for high-, medium- and low-LPR cows fed high- and low- protein diets in peak and late lactation[1,2,3,4]

	High-protein diets (HP)			Low-Protein diets (HP)			Change from HP to LP		
Variable	HLPR	MLPR	LLPR	HLPR	MLPR	LLPR	HLPR	MLPR	LLPR
Peak									
DM, %	65.2[a]	65.1[a]	64.6[a]	62.0[a]	62.2[a]	63.4[a]	3.16[a]	2.98[a]	1.65[a]
CP, %	68.9[a]	69.0[a]	68.2[a]	62.9[a]	62.2[a]	63.7[a]	5.88[a]	6.81[a]	4.39[a]
NDF[6], %	48.8[a]	48.5[a]	48.6[a]	45.7[a]	44.9[a]	47.3[a]	3.01[a]	3.64[a]	1.21[a]
Late									
DM, %	71.3[a]	70.0[a]	70.8[a]	70.2[a]	68.0[a]	68.4[a]	1.09[a]	2.09[a]	2.34[a]
CP, %	75.2[a]	75.7[a]	75.2[a]	69.4[a]	67.8[a]	66.8[a]	6.03[a]	7.39[a]	8.64[a]
NDF, %	58.1[a]	57.1[a]	59.0[a]	58.1[a]	56.4[a]	57.0[a]	0.15[a]	0.90[a]	2.04[a]

[1] In peak lactation cows, low protein resilience (LPR) was defined as the residual term in the model: d$CapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e.,$ where $CapP\_MBW_{HP}$ was the CapP per kg metabolic BW when fed HP; *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.

[2] In late lactation cows, low protein resilience (LPR) was defined as the residual term in the model: d$CapP = \beta_0 + \beta_1 \times CapP_MBW_{HP} + \beta_2 \times MBW_HH_{HP} + Par\ (Seq \times Exp) + Seq\ (Exp) + Exp + e,$ where $CapP\_MBW_{HP}$ was the CapP per kg metabolic BW when fed HP; MBW_Ht_{HP} was the metabolic body weight to height ratio when fed HP; *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.

[3] Cows were grouped into high (HLPR), medium (MLPR), and low (LLPR) RFI groups. Cows > 0.5 SD of the mean LPR for a cohort were classified as HLPR, cows < -0.5 SD were classified as LLPR, and those ± 0.5 SD were classified as MLPR.

[4] *P*-values associated with group difference were all > 0.05.

Protein Efficiency and Digestibility

The relationships between MPE and digestibilities of DM, NDF, and CP are illustrated in Table 4.3 and Table 4.4. For peak-lactation cows fed the HP diet, MPE was positively correlated with CP digestibility ($r = 0.15$; $P = 0.08$), but not correlated with DM and NDF digestibility; when fed the LP diet, MPE was not correlated with digestibilities of DM, CP, or NDF digestibility. For late-lactation cows, MPE was not correlated with digestibilities of DM, NDF, or CP in either HP or LP diet.

DISCUSSION

Cow Performance and Digestibilities of DM, NDF and CP in High- and Low- Protein Diets

Along with increasing milk production, the HP diet increased digestibilities of DM, NDF and CP in both stages of lactation. The effect of protein on digestibility of NDF (and thus DM digestibility) has been shown previously by Broderick and Reynal (2009), with the possible mechanism being that supplementing nitrogen supported growth of rumen microbes (Russell et al., 1992; Allen, 2000). However, NDF digestibility was not expected to be different in the current study, as both diets (HP and LP) were calculated to contain at least 9.8% RDP for peak-lactation cows and 9.0% for late-lactation cows. According to NRC (2001), these are expected to be adequate to support maximal ruminal microbial function. Thus, our results suggest that supplementing extra RDP that exceeds NRC 2001 recommendation can still improve NDF digestibility. The greater CP digestibility for the HP diet is likely due to the higher digestibility of protein from expeller soybean meal, compared to the LP-diet protein where 44% of the protein was from forage. The major difference between our treatment diets was that the HP diet contained 4% expeller soybean meal in addition to the protein in the LP diet. Lee et al. (2012) showed that supplementing expeller soybean meal increased digestibilities of DM, NDF, and CP. Although the expeller soybean meal provides primarily RUP, it also contains RDP. This RDP along with the N recycled back to the rumen from extra RUP provides extra rumen available nitrogen to improve digestibilities of DM and NDF (NRC, 2001). Another potential mechanism to explain the difference in NDF digestibility between HP and LP diets is the extra 2% starch in the LP diet. When formulating the LP diet, we added starch and fiber in place of protein. Based on de Souza et al. (2018), increasing dietary starch by 2% would decrease NDF digestibility by 1.2%; this is 43% of the 2.8% drop in NDF digestibility of peak-lactation cows and 67% of the 1.8% drop in NDF digestibility of late-lactation cows, when switched from the HP diet to the LP

diet. When comparing the results between lactation stages, digestibilities of DM, NDF, and CP in peak-lactation cows were lower than those in late-lactation cows. The difference between the two lactation stages is likely due to 1) faster passage rates for peak-lactation cows with higher intake levels, and 2) higher starch content in the diets fed to peak-lactation cows. Peak-lactation cows in the current study consumed 25% more DM than late-lactation cows. Given that digestibilities of DM and fiber are negatively correlated to intake (de Souza et al., 2018), it was expected that digestibilities of DM and NDF were lower in peak-lactation cows. However, 25% more DMI might not be able to lead to the 4%-unit difference in NDF digestibility in the current study; thus, the significant difference in NDF digestibility between peak- and late- lactation cows was expected to be mostly due to the higher starch content in peak-lactation diets. Based on de Souza et al. (2018), increasing dietary starch by 4.5%, as it was from peak to late lactation in the current study, would decrease NDF digestibility by 2.7%, which was 68% of the drop of NDF digestibility in the current study when comparing peak- and late- lactations.

Residual Feed Intake and Digestibility

Based on our previous work, we realized that some, if not all, of the BW change in the current study could be attributed to change in gut fill, and BodyE in primiparous cows should be different from that in multiparous cows. In order to calculate BodyE more accurately, BW was adjusted based on DMI (Liu and VandeHaar, submitted 2019), and BodyE was calculated based on new equations. RFI values based on the new method in the current study were strongly correlated with the RFI values calculated from unadjusted BW (Liu and VandeHaar, 2020), with the correlation coefficients being 0.98 in peak-lactation cows on the HP diet, 0.99 in peak-lactation cows on the LP diet, 0.99 in late-lactation cows on the HP diet, and 0.98 in late-lactation cows on the LP diet.

In the current study, no association was observed between RFI and digestibilities of DM, CP, or NDF, regardless of dietary protein contents and lactation stages. Several previous studies have shown similar results; for example, no correlation existed between RFI and nutrient digestibility in studies with steers (Cruz et al., 2010), dairy heifers (Lawrence et al., 2011; 2013), beef cattle (Fitzsimons et al., 2014), and lactating cows (Thornhill et al., 2014; Olijhoek et al., 2018). However, some studies have demonstrated correlations between RFI and nutrient digestibility. Specifically, Nkrumah et al. (2006) found RFI to be negatively correlated with digestibilities of DM and CP in steers fed diets containing 18-21% NDF and 12-13% CP. Rius et al. (2012) showed that RFI and nitrogen digestibility were negatively correlated in lactating dairy cattle fed diets with 36% NDF and 23% CP. In McDonnell et al. (2016), low-RFI heifers had higher digestibilities of CP and DM than high-RFI heifers in a nutrient-limiting diet but not in nutrient-adequate diets. Potts et al. (2017) found that DM digestibility explained 9-31% of the variation in RFI in cows fed low-starch diets (less than 17% starch), but 0% in cows fed diets containing ~30% starch. Based on the results from prior work, we suggest that perhaps nutrient digestibility only accounts for the difference in RFI among individual animals when they are fed nutrient-deficient diets. In Nkrumah et al. (2006), steers were fed at 2.5 maintenance level, which is considered as restricted feeding (Olijhoek et al., 2018). Cows in Rius et al. (2012) were fed on pasture, where nutrient deficiency could also be a potential issue for certain animals. In McDonnell et al. (2016), the association of RFI with nutrient digestibility was only present in grass-silage fed animals but not in pasture or TMR-fed animals. However, the idea of nutrient availability was not able to explain the results in Potts et al. (2017). In Potts et al. (2017), cows in both treatment groups were fed with adequate nutrients. Although the dietary starch content was 12-16% in low-starch diets, dietary NE was still ~ 42 Mcal/d, which was similar to the high-

starch diets in their study. The relationship between RFI and nutrient digestibility in low-starch diets could be because low-starch (or high-fiber) diets can allow low-RFI cows to express their superior digestive ability, while high-starch diets are already highly digestible and thus barely allow low-RFI cows to express their superior digestibility. In one study of broiler chicken, Rougière et al. (2009) found that the digestive ability of chicken with lower efficiency was improved when fed coarse-particle diet, while the digestive efficiency in chicken with higher-efficiency could not. Rougière et al. (2009) concluded that chicken with lower efficiency would need the stimulation of coarse particles to achieve greater digestive efficiency, while chicken with higher efficiency that have already achieved superior digestibility did not respond to the stimuli. This result suggests that animals with different efficiency status would respond differently to diets. Taken together, both ideas, nutrient deficiency and high-fiber stimuli, can potentially explain the varying relationships between RFI and nutrient digestibility among diets. However, limitations still exist; further examination is in need.

Interestingly, in the current study, there was no association observed between RFI and nutrient digestibility in cows fed the LP diet. The seemingly contradictory finding to the idea of nutrient availability indeed suggests that it is the nutrient availability specifically to ruminal microbes that influence the association between RFI and nutrient digestibility. In the current study, the rumen is expected to be fully functional in both HP and LP diets, as both diets contained adequate RDP and energy (starch), with the LP diet only deficient in RUP. Taken together, when RDP and starch are adequate to allow rumen to function to the fullest extent, RFI is not related to digestion regardless of species, growth stages, or physiological states, given that no association was detected in steers or beef (Cruz et al., 2010; Fitzsimons et al., 2014), heifers or cows (Lawrence et al., 2011; McDonnel et al., 2016; Potts et al., 2017), Holstein cows or

Jersey cows (Olijhoek et al., 2018), peak-lactation cows or late lactation cows (current study). Thus, when fed nutrient-sufficient diets with low fiber contents, cows' variability in RFI should be largely attributed to post-digestive metabolisms.

Low Protein Resilience, Protein Efficiency, and Digestibility

In the current study, the results suggested that cows with better resilience do not necessarily have better digestibility in low-protein diets, nor better ability to maintain their digestibility when switching from the HP to LP diet. That is, post-absorptive mechanisms should contribute more to cows' resilience to low protein.

In the current study, no association was observed between protein efficiency and nutrient digestibility in both peak-lactation and late-lactation cows, except for a low-moderate association between CP digestibility and MPE in peak-lactation cows fed the HP diet. This is consistent with findings in Huhtanen and Hristov (2009), where protein efficiency was poorly associated with rumen protein degradation. Given that protein degradation significantly impacts nutrient digestibility (NRC, 2001), the findings in Huhtanen and Hristov (2009) indicated that protein efficiency might not be associated with digestibility in dairy cows. Indeed, according to Apelo et al. (2014), compared to the digestion ability, post-absorption metabolisms play more important roles in regulating protein efficiency. About 60% of the nitrogen lost occurs in amino acid (AA) catabolism after absorption, especially in portal-drained viscera (PDV) and liver (Hanigan et al., 2004). The maximal theoretical efficiency to convert an ideal absorbed essential amino acids (EAA) profile into milk protein in dairy cows is 75- 85% (AFRC, 1992). Baker (1996) demonstrated that when each EAA supply matched exactly with tissue needs, a similar maximal post-absorptive nitrogen efficiency can be achieved in pigs. If this maximal efficiency is achieved, the theoretical protein efficiency for dairy cows can be as high as 0.49- 0.60, assuming

that 65-70% of the dietary protein can be digested and absorbed (NRC, 2001). However, the protein efficiency in the modern dairy cows averages as 0.25 and ranges between 0.15 and 0.40 (Huhtanen and Hristov, 2009). Thus, there is a great potential for the protein efficiency in dairy cow to improve. In order to improve protein efficiency, future nutrition studies should focus more on identifying ways to minimize post-absorptive AA catabolism.

CONCLUSIONS

High-protein diets significantly increased energy output, MUN, and digestibilities of DM, NDF, and CP in both peak- and late- lactation cows. RFI was not associated with digestibilities of DM, CP, or NDF in neither HP nor LP diet. In other words, we expect that cows' energy efficiency is more related to post-absorptive metabolisms when fed diets with adequate ruminal N and energy. Lastly, cows with higher LPR values did not have higher digestibilities of DM, NDF, and CP in the LP diet, nor better ability to maintain their digestibility. Work on post-absorptive metabolisms should be performed to further explore the mechanisms of this resilience ability.

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CHAPTER 5

IMPORTANCE OF CONSIDERING BODY WEIGHT CHANGE IN RESPONSE TO DIETARY PROTEIN REDUCTION IN LACTATING DAIRY COWS

ABSTRACT

Our objectives were to 1) determine the importance of including body weight (BW) change in the response of lactating dairy cows to a shortage of dietary protein, and 2) develop models to predict BW change when cows are fed low-protein diets. Cows in peak-lactation (n=166) were fed high and low protein diets in a cross-over design of two periods; the study was repeated with 69 of these cows in late-lactation. BW change was used to predict energy and protein changes. Feed intake, milk protein yield, BW change, total protein capture (CapP, calculated as the sum of milk protein and body protein gain), and total energy capture (CapE, calculated as the sum of milk energy and retained body energy) were calculated for each cow on each diet. Income over feed cost (IOFC) for each cow on each diet and the decrease of IOFC in response to protein reduction were calculated. Fixed effects of diet, parity, treatment sequence nested in experiment, treatment period nested in experiment, interaction of diet and parity, and the random effects of experiment and cow nested within experiment were included in the model to compare production performance and IOFC between diets. A sensitivity analysis was performed to examine the significance of different factors influencing IOFC. Prediction models of BW change from high-protein to low- protein diets included fixed (e.g., CP%, BW, and DIM when cows were on high-protein diets) and random (e.g., experiment) effects. In peak lactation, reducing protein from 18% to 14% saved $1.06 per cow in daily feed cost but resulted in estimated daily losses of: 1) 2.9 Mcal milk energy and 2.2 Mcal body energy, 2) 0.13 kg milk protein and 0.02 kg body protein, 3) $1.80 milk income and $0.36 body salvage value.

Therefore, BW loss accounted for 43% of the estimated energy loss, 11% of estimated protein loss, and 17% of total income loss. In late-lactation, body tissue loss resulting from feeding less CP (13% vs. 16%) accounted for 1) 51% of estimated energy loss, 2) 14% of estimated protein loss, and 3) 25% of total income loss. In the sensitivity analysis, when calculating IOFC, milk fat price was the most influential factor when cows were fed specific diets, regardless of lactation stages. When calculating the decrease of IOFC from high-protein to low-protein diets, feed cost was the most influential factor. In conclusion, body reserve change should be considered when assessing the cow response to changes in dietary protein.

INTRODUCTION

Given that protein accounts for 40% of the total feed cost for lactating cows (St-Pierre, 2012), feeding diets with less protein would reduce feed cost and in turn improve farm profitability. However, studies examining the effect of reducing dietary protein on farm profitability were inconclusive. For example, Fadul-Pacheco et al. (2017) found that reducing dietary protein from 16.5 to 15.0% increased income over feed cost (IOFC); whereas Stewart et al. (2012) observed no change in IOFC when dietary CP was reduced from 18.0 to 16.5%. These inconsistencies could be due to different economic conditions, different base diets or protein sources, different animals, or different environments. It is also likely that 16.5% CP in Stewart et al. (2012) has already met or nearly met cows' genetic capacity to synthesize milk protein. As demonstrated by the Law of dimishing returns, milk response to each unit of successive increase of protein becomes smaller as consumption of dietary protein increases (VandeHaar and St-Pierre; 2006).

To maximize profit, we should minimize the protein feeding, while at the same time meeting or nearly meeting the metabolizable protein requirements for a cow's genetic potential. A deficiency of protein would diminish milk income and excessive protein feeding would increase feed costs with no production benefit. Researchers have examined this tradeoff for many years. Wu and Satter (2000) measured the response of lactating dairy cows to different amounts of dietary protein content during a complete lactation and suggested that feeding cows 17-19% CP before week 30 and 16% CP after week 30 maximized milk production. Hundreds of studies in the last 50 years have examined effects of dietary protein content and source on milk production. Dietary protein is commonly assumed to be sufficient or deficient based on milk production responses. However, dietary protein should not be considered sufficient if body

protein is lost to make up for a shortage of dietary protein. This is an important aspect that is commonly overlooked.

The importance of body reserve mobilization in supporting milk production in early lactation is widely accepted (NRC, 2001). According to Chilliard and Robelin (1983), when dietary protein is limited, cows might mobilize body protein to support milk production. Milk response to dietary protein reduction has been modeled by Moraes et al. (2018). According to Morae's et al. (2018), body protein mobilization should be considered when assessing cow response; however, due to the limitation of data, they did not examine this idea. In nutrition trials of lactating cows, responses in BW gain or loss have not been extensively examined in the response to dietary protein reduction. If milk loss is consistently accompanied with BW loss when cows are fed low protein, then failing to account for BW change in individual cows can greatly underestimate the loss. Thus, the objectives of the current study were to 1) determine the importance of including BW change in cow response to dietary protein reduction, and 2) develop models to predict BW change when reducing dietary protein contents.

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 MY, milk components (milk protein and MUN), BW, and hip height were collected in the experiments. These are the same animals described by Liu and VandeHaar (2020). In brief, lactating Holstein cows (n= 166; 92 primiparous, 77 multiparous) with initial milk yield (MY)

41.3 ± 9.8 kg/d were included in the crossover experiments with two treatments (high-protein diets, HP; low-protein diets, LP) and two periods of 28-35 d each. Experiments were repeated in 69 of the 166 cows (42 primiparous, 27 multiparous) in late lactation. Low-protein diets were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function. Expeller soybean meal was added in place of corn and soyhulls to create high-protein diets, which were 18% CP in peak lactation and 16% CP in late lactation. 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.

Calculations

Data from peak lactation and late lactation was analyzed separately. All the data used in the analyses below were for cows in the peak lactation, and the methods were applied to late-lactation cows as well.

Energy-corrected milk (**ECM**; kg/d) was calculated for each cow on each diet based on the equation in NRC 2001:

$$ECM = 0.327 \times \text{milk yield (kg/d)} + 12.95 \times \text{milk fat (kg/d)} + 7.2 \times \text{milk protein (kg/d)}$$

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

Empty BW (**EBW**) was calculated for each cow to adjust BW (after being corrected for CW) for gut fill (Gibbs et al., 1992; Andrew et al., 1994),

EBW = BW – 5.2 × DMI - CW,

where DMI was the daily DMI when BW was measured.

For multiparous cows, EBW change was considered to be all body condition, and the body energy gained or lost with changes in EBW (**BodyE**; Mcal/d) was estimated by the equation as:

BodyE = RE (Mcal/kg) × dEBW,

Where RE = 3.52 + 1.27 × BCS (derived from NRC 2001, Table 2-4).

For primiparous cows, we assumed their mature BW would be 700 kg and that they had to gain 0.14 kg EBW/d of true growth across the first lactation to reach 92% of mature BW by their second calving (NRC, 2001). Based on the NRC (2001) equations (11-1 and 11-2), the RE content of true growth is 4.4 Mcal/kg dEBW. Any deviation in dEBW from 0.14 kg/d was considered to be body condition gain or loss, and the dEBW associated with body condition change was the same as for multiparous cows (3.52 + 1.27 × BCS) Mcal/kg dEBW.

Thus, the equation to calculate BodyE was:

$$\text{BodyE} = \begin{cases} (3.52 + 1.27 \times \text{BCS}) \times \text{dEBW}, & \text{Parity} > 1 \\ 4.4 \times 0.14 + [(3.52 + 1.27 \times \text{BCS}) \times (\text{dEBW} - 0.14)], & \text{Parity} = 1 \end{cases}$$

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

MilkE = 9.29 × fat (kg) + 5.63 × true protein (kg) + 3.95 × lactose (kg), where each component was based on the period average output of a cow.

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

PregE = (0.00318 × D - 0.0352) × (CBW / 45) / 0.218,

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

Total energy capture (**CapE,** Mcal/d) was estimated as:

$$\text{CapE} = \begin{cases} \text{MilkE} + \text{BodyE}, & \text{DIM} < 200 \\ \text{MilkE} + \text{BodyE} + \text{PregE}, & \text{DIM} \geq 200 \end{cases}$$

Under the similar assumptions and information used in NRC energy calculation (2001; Table 2-4, Equations 11-4 and 11-5), protein captured for body tissue gain (**BodyP**; kg/d) was calculated from dEBW and BCS as,

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

where (0.151- 0.0268 × BCS) kg protein per kg dEBW was assumed when dEBW was considered as body condition gain or loss, and 0.132 kg protein per kg dEBW was considered for the 0.14 kg/d growth.

Protein captured for pregnancy (**PregP**; kg/d) was calculated using the equation from NRC (2001):

PregP = 0.00069 × D – 0. 0692 × (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 estimated for each cow in each treatment (HP and LP) as:

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

Feed cost was calculated as the average feed cost from 2016 to 2018. Historical feed prices in the Midwestern area of the U.S. from 2016 to 2018 were used to calculate the average price for each ingredient (Ishler, 2020). The prices ($/kg DM) used in the current study were: $0.13/kg corn silage, $0.19/kg legume silage, $0.18/kg soybean hulls, $0.17/kg ground corn, $0.40/kg solvent extracted soybean meal, $0.47/kg expeller soybean meal, $1.37/kg mix of urea, vitamins, and minerals. Milk income was determined based on individual production of fat ($5.48/kg), protein ($4.13/kg), and lactose ($1.44/kg), then adjusted for the premium including volume ($0.04/kg) and somatic cell count ($0.00080/kg; if SCC < 350,000). Milk component prices were determined based on the 2016- 2018 Class and Components Prices for Federal Milk Marketing Order 33 (Mideast Marketing Area). BW gain was assigned a value of $ 1.36/kg, calculated as the average value of a cull cow ($/kg) from 2016 to 2018. Income over feed cost was calculated as:

IOFC = milk price ($/kg) × milk yield (kg/d) + gain value ($/kg) × BW gain (kg/d) – feed cost ($/kg) × feed intake (kg/d).

The difference of IOFC (**dIOFC**) between HP and LP diets was calculated for each cow.

A sensitivity analysis was performed on critical financial factors (e.g., feed cost, milk price, and cull cow value), with an assumption of ± 30% variation applied to each of the factors.

The change in EBW gain associated with the diet change (**dEBWg**; kg/d) was calculated as:

$$dEBWg = dEBW_{LP} - dEBW_{HP}.$$

The following model was used to examine the extent to which dEBWg can be predicted by the factors listed on the right side of the equation.

$$\text{dEBWg} = \beta_0 + \beta_1 \times dEBW_{HP} + \beta_2 \times ECM_{HP} + \beta_3 \times MBW_{HP} + \beta_4 \times BCS_{HP} + \beta_5 \times HH_{HP} + \beta_6 \times DIM_{HP} + Par + Seq\ (Exp) + Exp + e,$$

where $dEBW_{HP}$ was the dEBW when fed HP; ECM_{HP} was the ECM when fed HP; MBW_{HP} was the MBW when fed HP; HH_{HP} was the hip height when fed HP; BCS_{HP} was the BCS when fed HP; *Par* was parity (primiparous or multiparous); DIM_{HP} was the starting days in milk when fed HP; *Seq* was sequence (HP-LP or LP-HP); *Exp* was experiment; and *e* was the residual term in the model.

In the model, all covariates were jointly checked for multicollinearity through variance inflation factors (VIF) analysis (SAS, 9.4). When covariates had VIF greater than 10, the covariate with lesser interest was removed from the analysis. The final model was selected based on backward and stepwise selection criteria (SAS, 9.4).

Production responses to diets with each lactation stage were analyzed analyzed using the HPMIXED procedure in SAS (9.4), with fixed effects of diet, parity, treatment sequence nested in experiment, treatment period within experiment, interaction of parity and diet, and the random effects of experiment and cow nested within experiment. Significance was considered at $P \leqslant 0.05$ and tendency at $P \leqslant 0.10$. Interactions were considered significant at $P \leqslant 0.10$ and trends at $P \leqslant 0.15$.

As mentioned before, all the analyses above were for cows in the peak lactation. The same analyses were performed for the late- lactation cows as well.

RESULTS

Animal Performance

Cows fed the LP diet ate less, produced less milk, and gained less BW than cows fed the HP diet, in both peak and late lactations. Primiparous cows in general ate less, and produced less milk, but with greater milk component concentration, in both peak and late lactations. Further details are shown in Table 5.1 and Table 5.2. Further descriptive information regarding BW change is shown in Table 5.3. Time series of cow response (DMI, milk production, and body weight) to the dietary protein reduction are shown in Supplementary Figure 5.1 and Supplementary Figure 5.2.

Table 5.1 Dry matter intake, milk production, and body reserve change for cows fed treatment diets in peak lactation[1,2]

	Treatments[3]			Parity[4]			P-value[5]		
	HP_{peak} n=166	LP_{peak} n=166	SEM	Primi. n=184	Multi. n=148	SEM	TRT	Parity	TRT × Parity
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	41.2	37.3	0.23	33.7	44.7	0.96	<0.01	<0.01	0.01
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
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, kg/ d[6]	0.57	0.20	0.10	0.38	0.40	0.10	<0.01	0.88	0.23
Changes in EBW, kg/ d[7]	0.39	0.12	0.07	0.27	0.25	0.07	<0.01	0.83	0.45
Change in BCS, unit/28 d	0.071	0.017	0.03	0.044	0.045	0.03	0.06	0.83	0.10

[1]Average DIM was 125 for primiparous cows in HP_{peak} diet, 126 for primiparous cows in LP_{peak} diet, 122 for multiparous cows in HP_{peak} diet, and 121 for multiparous cows in LP_{peak} diet.

[2]Average parity for multiparous cows was 2.94 in peak lactation.

[3] Treatments contained 18% (HP_{peak}) and 14% (LP_{peak}) crude protein on a DM basis for peak lactation cows.

[4] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[5] *P*-value associated with treatment differences (HP_{peak} vs. LP_{peak}; TRT) and parity differences (Primi vs. Multi.; Parity) in peak lactation cows. Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

[6] Determined by linear regression using BW measurements throughout the period.

[7] Determined by linear regression using EBW (empty BW= BW- 5.2× DMI) throughout the period.

Table 5.2 Dry matter intake, milk production, and body reserve change for cows fed treatment diets in late lactation[1,2]

	Treatments[3]			Parity[4]			P-value[5]		
	HP_{late} n=69	LP_{late} n=69	SEM	Primi. n=84	Multi. n=54	SEM	TRT	Parity	TRT × Parity
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	25.1	22.2	0.42	24.2	23.1	1.37	<0.01	0.42	0.06
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	0.68	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
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, kg/ d[6]	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[7]	0.43	-0.05	0.09	0.14	0.23	0.09	<0.01	0.33	0.09
Change in EBW, kg/ d[8]	0.62	0.23	0.14	0.30	0.56	0.13	<0.01	0.01	0.34
Change in BCS, unit/28 d	0.067	-0.005	0.37	-0.020	0.082	0.37	0.16	0.34	0.14

[1]Average DIM was 258 for primiparous cows in HP_{late} diet, 257 for primiparous cows in LP_{late} diet, 263 for multiparous cows in HP_{late} diet, and 264 for multiparous cows in LP_{late} diet. LP diet were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function but deficient to support milk production. The HP diet were 18% CP in peak lactation and 16% CP in late lactation and contained extra expeller soybean meal to increase RUP.

[2]Average parity for multiparous cows was 3.12 in late lactation.

[3] Treatments contained 16% (HP_{late}) and 13% (LP_{late}) crude protein on a DM basis for late lactation cows.

Table 5.2 (cont'd)

[4] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[5] *P*-value associated with treatment differences (HP_{late} vs. LP_{late}; TRT) and parity differences (Primi vs. Multi.; Parity) in late lactation cows. Values within each TRT × Parity interaction are shown in Supplementary Table 2.1

[6] Determined by linear regression using BW measurements throughout the period.

[7] Determined by linear regression using adjusted BW measurements (subtracting conceptus weight) throughout the period.

[8] Determined by linear regression using EBW (empty BW= BW- 5.2× DMI) throughout the period.

Table 5.3 Mean, standard deviation, minimal and maximal values for body tissue change in peak and late lactation cows across diets[1,2]

	HP								LP							
	Primi				Multi				Primi				Multi			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
Peak Lactation																
BW[3]	600	50	471	791	718	65	583	908	593	51	448	798	714	64	597	915
dBW[4]	0.62	1.13	-1.70	6.49	0.51	1.16	-4.55	4.71	0.14	0.59	-1.69	2.66	0.29	0.65	-1.15	3.57
dEBW[5]	0.44	0.69	-1.15	2.06	0.36	0.82	-1.54	3.61	0.11	0.95	-1.57	7.25	0.17	0.72	-1.62	1.87
BCS[6]	3.26	0.29	2.58	4.08	3.22	0.44	2.42	4.58	3.22	0.32	2.42	4.17	3.19	0.44	2.42	4.50
dBCS[7]	0.093	0.25	-0.47	0.92	0.050	0.29	-1.00	0.75	0.002	0.22	-0.50	0.67	0.038	0.28	-0.75	0.83
Late Lactation																
BW	628	50	524	734	788	73	651	904	619	50	527	713	779	74	649	930
dBW	0.64	0.48	-0.21	1.64	0.70	0.54	-0.39	2.06	-0.06	0.57	-1.03	1.17	0.23	0.67	-1.48	1.33
dEBW	0.53	0.72	-0.96	2.36	0.62	0.62	-0.41	1.87	0.04	0.58	-1.14	1.10	0.33	0.76	-0.97	2.60
BCS	3.48	0.37	2.83	4.42	3.83	0.46	3.25	4.83	3.42	0.34	2.92	4.08	3.78	0.45	3.25	4.75
dBCS	0.035	0.38	-2.07	0.35	0.169	0.37	-0.74	1.31	-0.005	0.43	-1.06	2.04	-0.005	0.62	-2.04	1.52

[1] HP stands for high-protein diets and LP stands for low-protein diets. The LP diet were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function but deficient to support milk production. The HP diet were 18% CP in peak lactation and 16% CP in late lactation and contained extra expeller soybean meal to increase RUP.

[2] Primi. stands for primiparous cows and Multi. stands for multiparous cows.

[3] Average BW (kg) measured throughout the treatment period.

[4] Change of BW (kg/d), determined by linear regression using BW measurements throughout the period.

[5] Change of EBW (kg/d), determined by linear regression using EBW (empty BW= BW- 5.2× DMI) throughout the period.

[6] Average BCS (unit), determined by three investigators at the beginning and end of each period, and averaged for the period.

[7] Change of BCS (unit/ 28 day).

Importance of Including BW Change in Cow Response

As shown in Figure 5.1, reducing protein from the HP to LP diet in peak-lactation cows saved $1.06 per cow in daily feed cost but resulted in estimated daily losses of: 1) 2.9 Mcal MilkE and 2.2 Mcal BodyE, 2) 0.13 kg milk protein and 0.02 kg BodyP, 3) $1.80 milk income and $0.36 body salvage value. Therefore, body tissue loss resulting from the 4% CP reduction in peak lactation cows contributed to 1) 42% of estimated energy loss, 2) 11% of estimated protein loss, and 3) 17% of gross income loss. As shown in Table 5.4, when cows in peak lactation were underfed protein, the major saving of feed cost was from decreased DMI (24.3 kg/d vs. 23.3 kg/d), and the major loss of milk income was from decreased milk production (41.2 kg/d vs. 37.3 kg/d). The milk price ($/cwt) and feed price ($ /kg DM) were barely different between HP and LP diets.

Figure 5.1 Energy capture, protein capture, and income in milk and body tissue in peak lactation cows

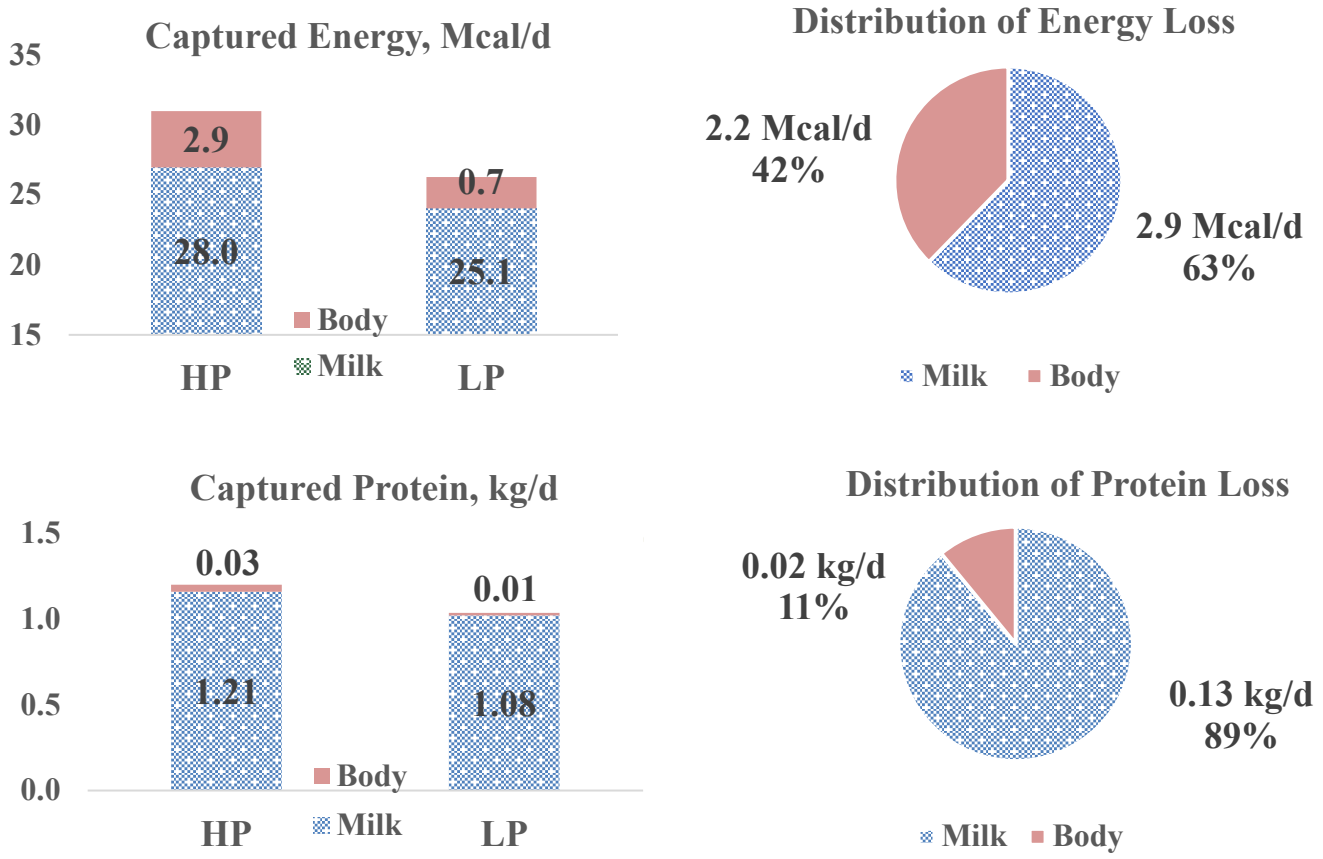


Figure 5.1 (cont'd)

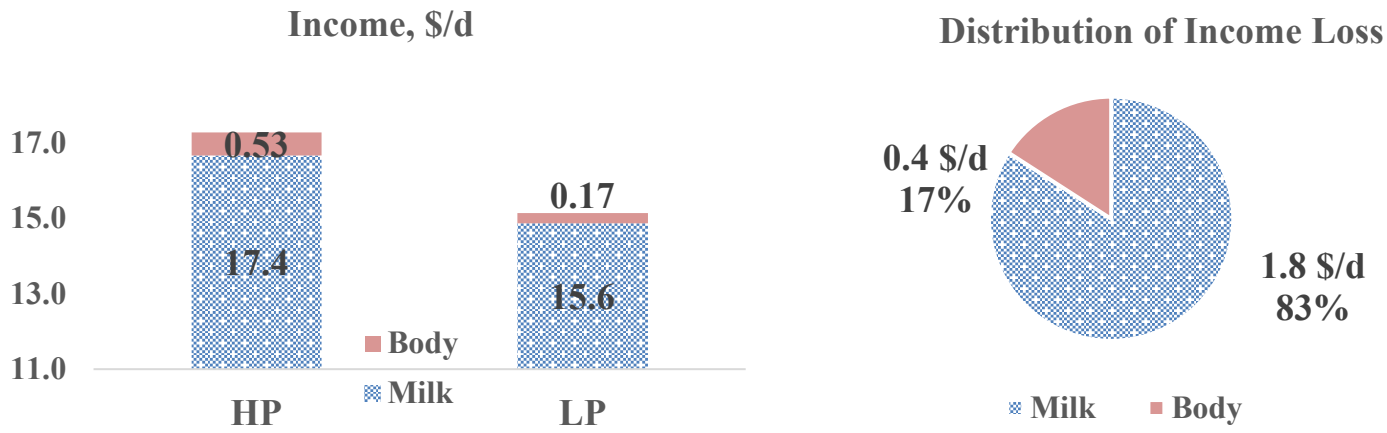


Figure 5.1 Energy capture, protein capture, and income in milk and body tissue in peak lactation cows (n=166). Reducing protein from 18P to 14P in peak-lactation cows resulted in estimated daily losses of: 1) 2.9 Mcal milk energy and 2.2 Mcal body tissue energy, 2) 0.13 kg milk protein and 0.02 kg body protein, 3) $1.80 milk income and $0.36 body salvage value. Body tissue loss resulting from the 4% units CP reduction in peak lactation cows contributed to 1) 42% of the estimated energy loss, 2) 11% of estimated protein loss, and 3) 17% of gross income loss. Milk energy was estimated based on production of milk fat, protein, and lactose. Body energy and body protein were estimated based on change of empty body weight and BCS. Milk income was determined based on individual production of fat ($5.48/kg), protein ($4.13/kg), and lactose ($1.44/kg), then adjusted for the premium including volume ($0.04/kg) and somatic cell count ($0.0008/kg). Milk components price was determined based on the 2016-2018 Class & Components Prices for Federal Milk Marketing Order 33 (Mideast Marketing Area). The profit gain of BW was assigned to $ 1.36/kg, calculated as the average value of a cull cow ($/kg) from 2016 to 2018.

Table 5.4 Income and IOFC in peak and late lactation cows when fed high and low protein diets[1,2]

	Peak Lactation			Late Lactation		
	HP_{Peak}	LP_{Peak}	Delta[3]	HP_{Late}	LP_{late}	Delta
Income_Milk[4], $/d	17.35 ± 0.33	15.56 ± 0.32	1.80	11.45 ± 0.53	9.96 ± 0.52	1.50
Milk Price, $/cwt	19.31 ± 0.16	19.14 ± 0.15	0.17	20.69 ± 0.55	20.45 ± 0.55	0.24
Milk Production, kg/d	41.2 ± 0.85	37.3 ± 0.85	3.89	25.1 ± 0.41	22.2 ± 0.41	2.91
Income_Body Tissue Gain[5], $/d	0.53 ± 0.11	0.17 ± 0.10	0.37	0.84 ± 0.13	0.31 ± 0.13	0.52
Feed Cost[6], $/d	6.06 ± 0.15	5.00 ± 0.14	1.06	4.67 ± 0.12	3.80 ± 0.11	0.87
Feed Cost, $/kg DM	0.25 ± 0.01	0.22 ± 0.01	0.03	0.24 ± 0.01	0.21 ± 0.01	0.03
DMI, kg/d	24.3 ± 0.14	23.3 ± 0.12	1.01	19.8 ± 0.20	18.4 ± 0.20	1.40
IOFC_Milk only[7], $/d	11.27 ± 0.19	10.53 ± 0.18	0.74	6.79 ± 0.17	6.16 ± 0.17	0.63
IOFC_Milk+Body[8], $/d	11.80 ± 0.20	10.80 ± 0.20	1.01	7.61 ± 0.21	6.46 ± 0.20	1.15

[1]Average parity for multiparous cows was 2.94 in peak lactation, and 3.12 in late lactation

[2] Treatments contained 18% and 14% crude protein on a DM basis for peak lactation cows, and 16% and 13% crude protein on a DM basis for late lactation cows

[3] Difference between HP and LP (All *P* values associated with treatments (HP vs. LP) were less than 0.05)

[4] Milk income was determined based on individual production of fat ($5.48/kg), protein ($4.13/kg), and lactose ($1.44/kg), then adjusted for the premium including volume ($0.04/kg) and somatic cell count ($0.0008/kg). Milk components price was determined based on the 2016-2018 Class & Components Prices for Federal Milk Marketing Order 33 (Mideast Marketing Area).

[5] The profit gain of BW was assigned to $ 1.36/kg, calculated as the average value of a cull cow ($/kg) from 2016 to 2018.

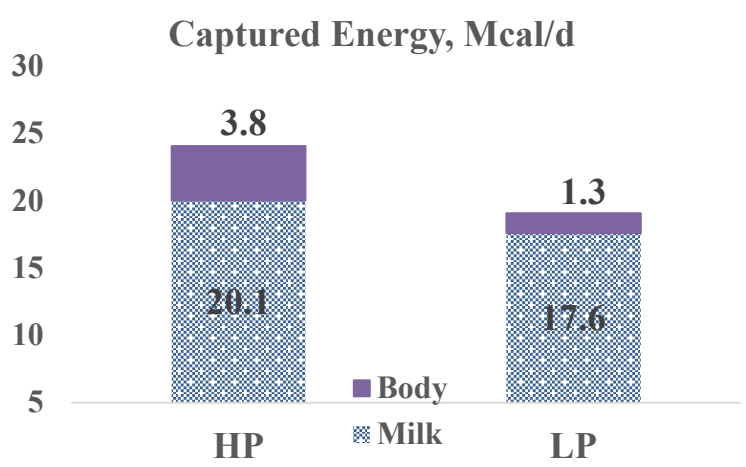
[6] The prices ($/kg DM) used were: $0.13/kg corn silage, $0.19/kg legume silage, $0.18/kg soybean hulls, $0.17/kg ground corn, $0.40/kg solvent extracted soybean meal, $0.47/kg expeller soybean meal, $1.37/kg mix of urea, vitamins, and minerals.

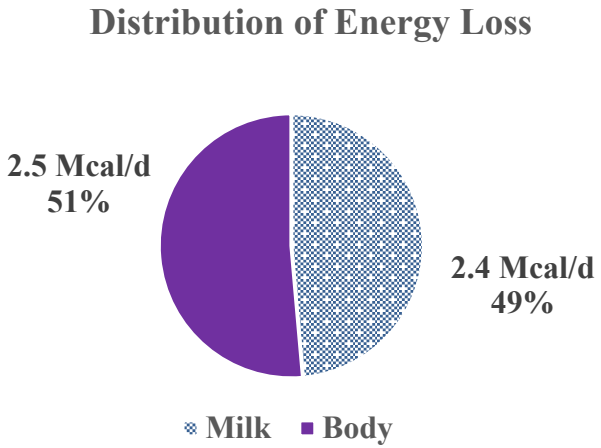
[7] Income over feed cost (**IOFC**) was calculated as milk income ($/d) – feed cost ($/d)

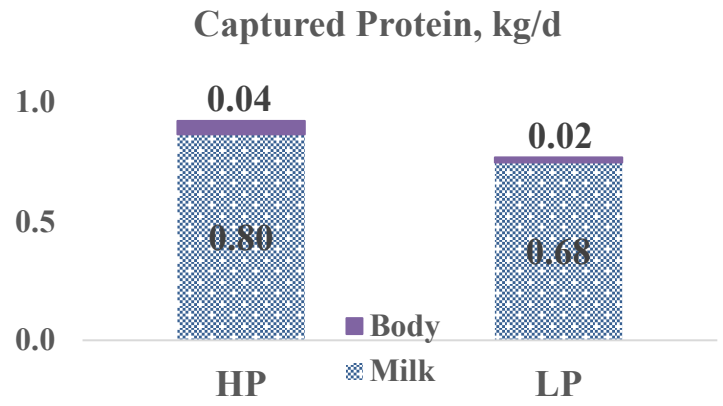
[8] Income over feed cost (**IOFC**) was calculated as milk income ($/d) + body tissue gain ($/d) – feed cost ($/d)

As shown in Figure 5.2, reducing protein from the HP to LP diet in late-lactation cows saved $0.87 per cow in daily feed cost but resulted in estimated daily losses of: 1) 2.4 Mcal MilkE and 2.5 Mcal BodyE, 2) 0.12 kg milk protein and 0.02 kg BodyP, 3) $1.50 milk income and $0.52 body salvage value. Therefore, body tissue loss resulting from the 3% units CP reduction in late lactation cows contributed to 1) 51% of estimated energy loss, 2) 14% of estimated protein loss, and 3) 25% of gross income loss. As shown in Table 5.4, when cows in late lactation were underfed protein, the major saving of feed cost was from decreased DMI (19.8 kg/d vs. 18.4 kg/d), and the major loss of milk income was from depressed milk production (25.1 kg/d vs. 22.2 kg/d). The milk price ($/cwt) and feed price ($ /kg DM) were not different between HP and LP diets.

Figure 5.2 Energy capture, protein capture, and income in milk and body tissue in late lactation cows







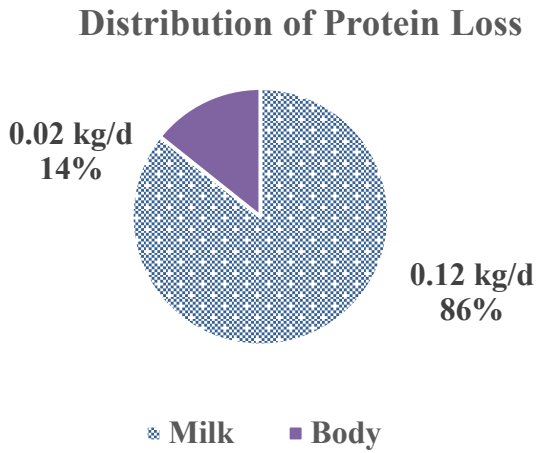


Figure 5.2 (cont'd)

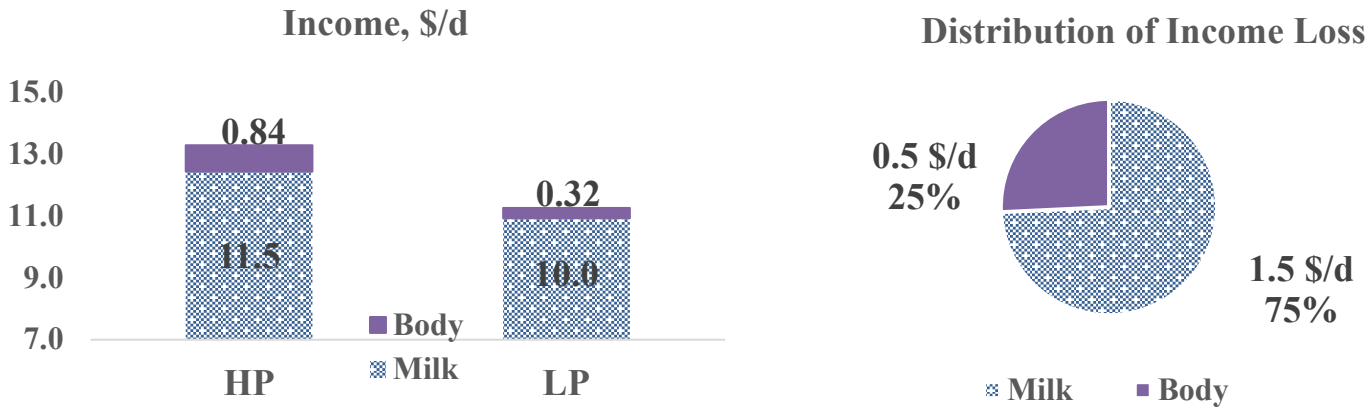


Figure 5.2 Energy capture, protein capture, and income in milk and body tissue in late lactation cows (n=69). Reducing protein from 16P to 13P in late-lactation cows resulted in estimated daily losses of: 1) 2.4 Mcal milk energy and 2.5 Mcal body tissue energy, 2) 0.12 kg milk protein and 0.02 kg body protein, 3) $1.50 milk income and $0.52 body salvage value. Body tissue loss resulting from the 3% units CP reduction in late lactation cows contributed to 1) 51% of estimated energy loss, 2) 14% of estimated protein loss, and 3) 25% of gross income loss. Milk energy was estimated based on production of milk fat, protein, and lactose. Body energy and body protein were estimated based on change of empty body weight and BCS. Milk income was determined based on individual production of fat ($5.48/kg), protein ($4.13/kg), and lactose ($1.44/kg), then adjusted for the premium including volume ($0.04/kg) and somatic cell count ($0.0008/kg). Milk components price was determined based on the 2016-2018 Class & Components Prices for Federal Milk Marketing Order 33 (Mideast Marketing Area). The profit gain of BW was assigned to $ 1.36/kg, calculated as the average value of a cull cow ($/kg) from 2016 to 2018.

Sensitivity Analysis

As the key parameters varied between the range of -30% to +30%, IOFC (calculated as milk income ($/d) + body tissue gain ($/d) – feed cost ($/d)) varied as shown in Figures 5.3 and 5.4. The most influential factor was milk fat price, causing ~20% variation of IOFC regardless of diets and lactation stages. Following that, feed price and milk protein price were the second and third most influential factors on IOFC. Body salvage value was not important in determining IOFC when cows were on specific diet (HP or LP); however, the impact of body salvage value became greater, when estimating change of IOFC from the HP to LP diet.

Figure 5.3 Sensitivity analysis for peak-lactation cows on HP and LP diets

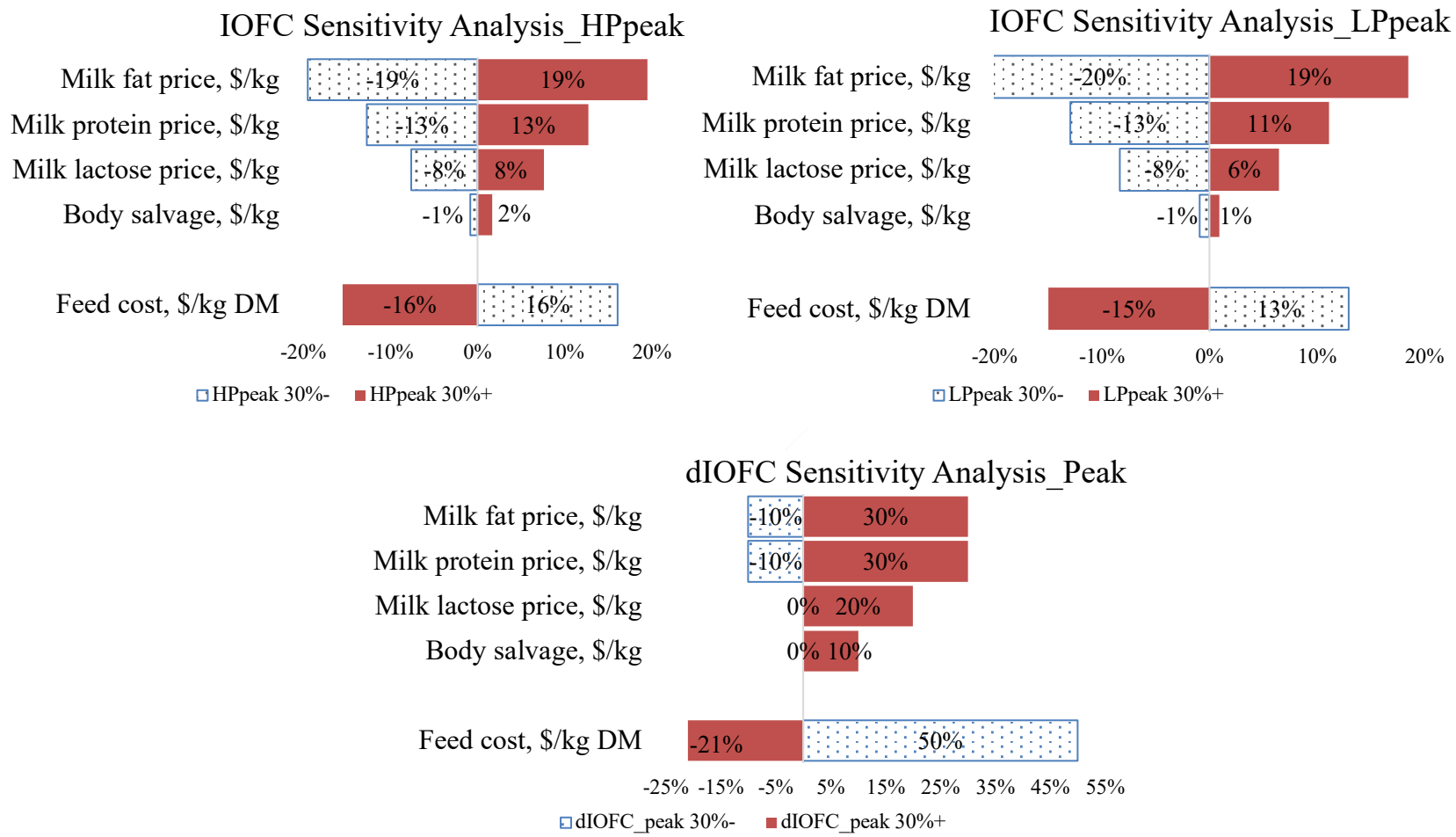


Figure 5.3 Sensitivity analysis for peak-lactation cows on HP and LP diets. With 30% change of each factor (listed on the Y-axis), income over feed cost (IOFC) varied. The solid filled sections are the response of IOFC to +30% change of each factor, and pattern filled sections are the response of IOFC to -30% change of each factor. IOFC (income over feed cost) =milk income ($/d) + gain value of BW gain ($/d) – feed cost ($/d). dIOFC is the difference of IOFC between HP and LP diets.

Figure 5.4 Sensitivity analysis for late-lactation cows on HP and LP diets

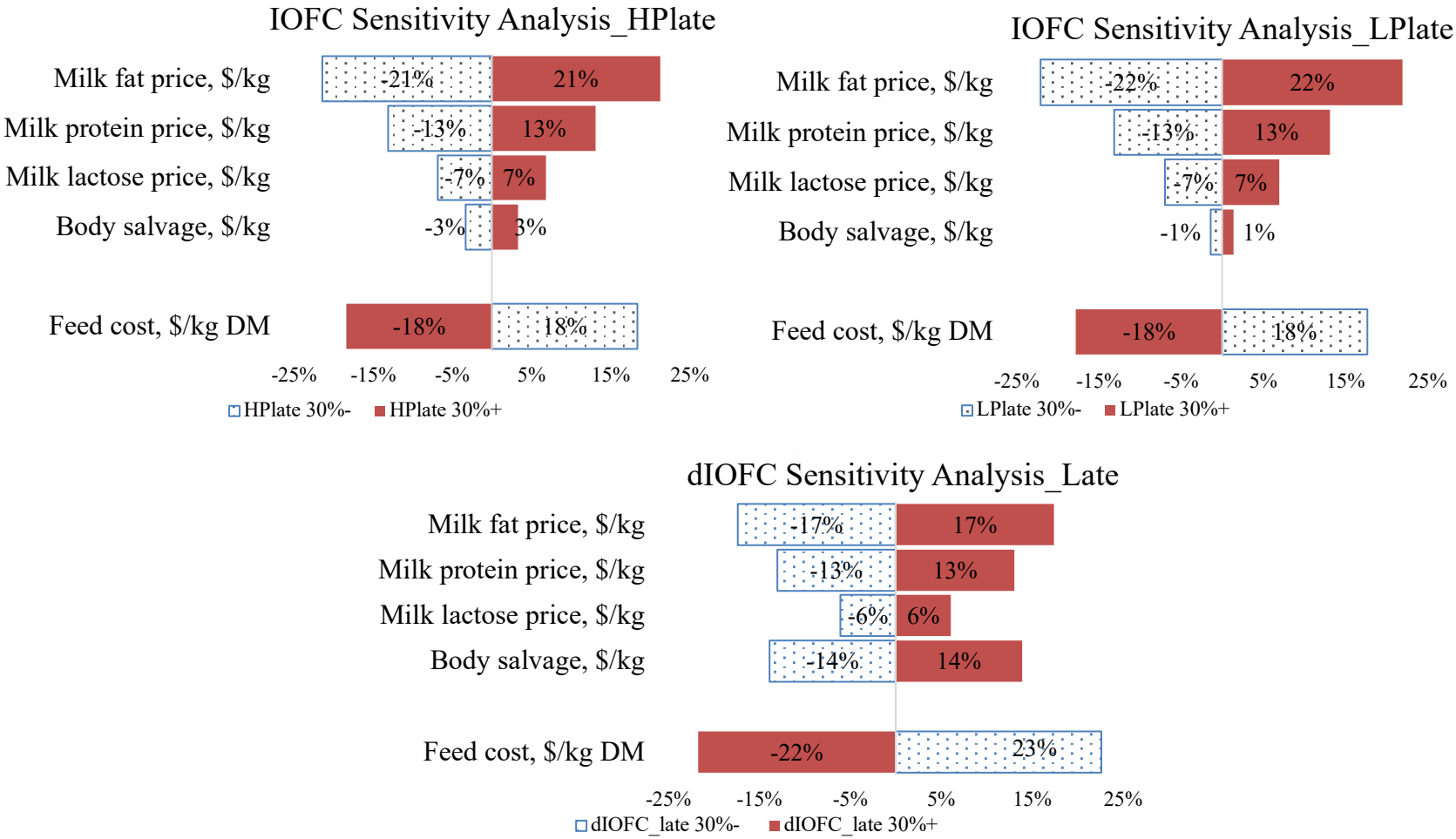


Figure 5.4 Sensitivity analysis for late-lactation cows on HP and LP diets. With 30% change of each factor (listed on the Y-axis), income over feed cost (IOFC) varied. The solid filled sections are the response of IOFC to +30% change of each factor, and pattern filled sections are the response of IOFC to -30% change of each factor. IOFC (income over feed cost) =milk income ($/d) + gain value of BW gain ($/d) – feed cost ($/d). dIOFC is the difference of IOFC between HP and LP diets.

Prediction Model for BW Change Responding to Dietary Protein Reduction

For peak-lactation cows, the dEBWg prediction model was:

$$dEBWg\ (kg/d) = -1.33 + 1.09 \times dEBW_{HP} + 0.02 \times ECM_{HP} + Parity\ (R^2 = 0.49),$$

where $dEBW_{HP}$ was the dEBW when fed the HP diet and ECM_{HP} was the ECM when fed the HP diet.

For late-lactation cows, the dEBWg prediction model was:

$$dEBWg\ (kg/d) = -1.44 + 1.27 \times dEBW_{HP} + 0.03 \times ECM_{HP} + Parity\ (R^2 = 0.58),$$

where $dEBW_{HP}$ was the dEBW when fed the HP diet and ECM_{HP} was the ECM when fed the HP diet.

DISCUSSION

Dietary protein supplements are expensive, but milk loss resulting from inadequate dietary protein can be even more expensive (VandeHaar and St-Pierre, 2006). To minimize the risk of losing milk production, producers usually feed cows with excessive protein (Colmenero and Broderick, 2006; Edouard et al., 2016). However, excessive protein feeding increases feed costs with no production benefit. To maximize profit, efforts have been made to find solutions to minimize protein feeding but still meet or nearly meet metabolizable protein (MP) requirements for lactating dairy cows. Studies examining cow responses to changes in dietary protein have focused on milk production, and generally have not included changes in body tissues (Ipharraguerre and Clark, 2005; Lean et al., 2018; Moraes et al., 2018). Thus, dietary protein was considered to be sufficient or deficient based on milk production alone. However, dietary protein should not be considered sufficient if body protein mass is lost or desired growth or condition gain is decreased to support milk production. This is an important aspect that is commonly overlooked. To our knowledge, the current study is the first to quantify changes in body mass in response to a reduction in dietary protein content. Our low protein diet was designed to be protein deficient, with a goal of meeting RDP requirements to maintain normal rumen function but feeding low RUP so that MP requirements to maintain milk production and body reserve were not met. With this diet, milk production was impaired, and thus our model enabled us to determine how much of the total response to a change in protein was body tissue and how much was milk production.

Importance of Considering BW Change when Evaluating Nutritional Responses.

Energy efficiency in dairy cows is commonly defined as milk energy per unit of dietary energy intake. According to the current study, this simplified calculation can potentially underestimate true energy efficiency by 3-17% units in dairy cows. When assessing energy loss to dietary protein reduction, the proportion of body energy capture in total energy capture was even more significant. Specifically, the proportion of total energy loss that was due to body energy loss was 42% for peak-lactation cows and 51% for late-lactation cows, respectively. Therefore, body energy capture should not be neglected when calculating energy loss resulted from feeding less protein.

The proportion of body protein capture in total protein gain was relatively small for cows fed either diet (HP: 3% and 5%; LP: 1% and 3%). However, when reducing dietary protein, the proportion of total protein loss that can be attributed to body protein loss was significant. Specifically, it was 11% for peak-lactation cows and 14% for late-lactation cows. In the current study, we recognize that we did not measure body protein mass or N balance directly; the body protein loss from dietary protein reduction was estimated from BW change. Accurate measurement of N loss would have precluded our ability to accurately measure milk and BW responses to 2 diets in >160 cows. However, we improved our estimation accuracy by correcting for changes in DMI that might have influenced gut fill and using changes in EBW to predict tissue energy and protein balance. To further examine whether the value assigned to each kg weight change influences the result, we compared the method used in the current study with the

one in our previous study (Liu and VandeHaar, 2020). Using the same cows, Liu and VandeHaar (2020) assumed that all the EBW change in multiparous cows was due to body condition change and all the EBW change in primiparous cows was due to growth; and thus, in Liu and VandeHaar (2020), we assigned 0.07 kg protein per kg dEBW for multiparous cows and 0.12 kg protein per kg dEBW for primiparous cows. We then performed a sensitivity analysis on the original coefficients assumed for protein gain/loss in body tissue change (0.07 kg protein per kg EBW change for multiparous cows, and 0.12 kg protein per kg EBW change for primiparous cows). Scenarios listed in Table 5.5 are different conditions. For example, in scenario 2, we assumed that all the EBW change was due to body condition regardless of parity and contained 7% protein; in scenario 4, we assumed that all the EBW change was from growth and contained 12% protein regardless of parity. These are extreme conditions that can help understand the range for the contribution of BodyP to total protein loss. As shown in Table 5.5, in peak-lactation cows, the contribution was no less than 11%, where larger coefficients led to larger contribution of BodyP to total protein loss. In late-lactation cows, the contribution was no less than 18%. These results clearly suggest that no matter what assumptions we made, body protein change is large enough that it should be considered when estimating cow responses to changes in dietary protein.

To further determine the importance of including body reserve mobilization into cow response to dietary protein reduction, we estimated the NE_L- allowable milk in peak- lactation cows. If no energy was utilized for body tissue gain, 4.4 kg/d milk would be lost when cows

switched from the HP to LP diet. Comparing to the actual loss of 3.9 kg/d milk in the current study, 0.5 kg/d milk loss was compensated by body reserve mobilization. In other words, 11% of milk loss was potentially compensated by body reserve mobilization.

The assumptions that we made in the estimation of NE_L- allowable milk were:

1) $DE_{density}$ (digestible energy density of diet, Mcal/kg) = % NDF_{diet} × $NDF_{digestibility}$ × 4.2 + % NFC_{diet} × 0.90 × 4.2 + % $Lipid_{diet}$ × 0.75 × 9.5 + % CP_{diet} × $CP_{digestibility}$ × 5.65

2) DE (digestible energy, Mcal/d) = $DE_{density}$ (Mcal/kg) × DMI (kg/d)

3) ME (metabolizable energy, Mcal/d) = DE × 0.85

4) total NE_L(net energy of the diet, Mcal/d) = ME × 0.66

5) $NE_{maintenance}$ (maintenance energy, Mcal/d) = 0.08 × $BW^{0.75}$

6) NE_L available for milk (Mcal/d) = total NE_L – $NE_{maintenance}$.

7) NE_L - allowable milk (kg/d) = $\frac{\text{NE allowable for milk}}{NEmilk\ (Mcal/kg)}$

Table 5.5 Protein captured in body tissue gain for HP and LP diets across lactation stages with different assumptions of protein gain per kg body weight change[1,2]

	Coefficients[3,4,5]		HP, kg/d	LP, kg/d	Delta, kg/d	Contribution of BW to Total Protein Loss[6], %
Peak Lactation	**Multi**	**Primi**				
Scenario 1	7	12	0.042	0.017	0.025	15.2
Scenario 2	7	7	0.032	0.014	0.018	11.4
Scenario 3	7 ×1.3	12	0.028	0.008	0.020	12.5
Scenario 4	7 × 0.7	12	0.038	0.014	0.024	14.6
Scenario 5	12	12	0.054	0.024	0.030	17.6
Scenario 6	7	12 × 1.3	0.051	0.019	0.032	18.6
Scenario 7	7	12 × 0.7	0.035	0.015	0.020	12.5
Scenario 8	7 × 1.3	12 × 1.3	0.055	0.022	0.033	19.1
Scenario 9	7 × 0.7	12 × 0.7	0.030	0.012	0.018	11.4
Late Lactation	**Multi**	**Primi**				
Scenario 1	7	12	0.057	0.021	0.036	23.1
Scenario 2	7	7	0.044	0.017	0.027	18.4
Scenario 3	7 × 1.3	12	0.067	0.025	0.042	25.9
Scenario 4	7 × 0.7	12	0.055	0.019	0.036	23.1
Scenario 5	12	12	0.075	0.029	0.046	27.7
Scenario 6	7	12 × 1.3	0.074	0.026	0.048	28.6
Scenario 7	7	12 × 0.7	0.049	0.018	0.031	20.5
Scenario 8	7 × 1.3	12 × 1.3	0.080	0.029	0.051	29.8
Scenario 9	7 × 0.7	12 × 0.7	0.043	0.015	0.028	18.9

[1]Average parity for multiparous cows was 2.94 in peak lactation, and 3.12 in late lactation

[2] The LP diet were 14% CP in peak lactation and 13% CP in late lactation and were formulated to contain adequate RDP to maintain rumen function but deficient to support milk production. The HP diet were 18% CP in peak lactation and 16% CP in late lactation and contained extra expeller soybean meal to increase RUP.

Table 5.5 (cont'd)

[3] Protein gain was assumed to be 0.07 kg per kg BW change for multiparous cows, and 0.12 kg per kg BW change for primiparous cows

[4] An assumption of ± 30% variation was applied to the two coefficients (0.07 and 0.12) to examine the contribution of BodyP in different scenarios

[5] Multi= multiparous cows; Primi= primiparous cows

[6] Milk protein loss was 0.14 kg/d when switching from HP to LP in peak-lactation cows, and 0.12 kg/d when switching from HP to LP in late-lactation cows

Importance of Considering BW Change when Estimating Changes in IOFC

Gain or loss of BW has rarely been taken into account when estimating profitability of diet changes. Based on the current study, the contribution of BW change to calculations of IOFC was small for cows on a specific diet (HP or LP) but was economically significant when calculating the response in IOFC to a reduction in dietary protein. Among peak-lactation cows, when reducing protein from 18% to 14%, the decrease in profit was 27% greater when considering BW loss. The difference was even larger in late-lactation cows. The economic analysis performed in the current study was based on the salvage value of cull cows and thus only the direct cost of BW loss was considered. Loss of BW might also affect health and fertility, but these indirect costs were not considered in the current study. In other words, the difference between IOFC when only considering milk and IOFC when considering milk and body responses would be even greater when including the indirect cost of losing BW. Therefore, at the very least, we suggest that changes in BW must be considered when considering the economic returns to changes in dietary protein; evaluating only milk responses underpredicts the total economic response.

We recognize that there are some pitfalls in the current financial analyses. First, certain lost protein could be replenished later in lactation or during the dry period when feed costs per unit of energy and protein is lower. Given that the value of dietary protein and energy declines as dietary protein content declines, the cost of restoring weight loss from early lactation would be much cheaper in late lactation than in early lactation. Moreover, to maintain an optimal body

condition in late lactation, energy and protein consumption is commonly restricted for dairy cows. Therefore, maintaining or limiting body reserve gain in late lactation would have a positive economic value rather than a negative one. Such an outcome would have to be added to the calculation for late lactation cows, which would certainly reduce the cost of the BW loss. Second, limitations associated with the sensitivity analysis also existed. We found that milk fat price was the most influential factor for profitability when calculating IOFC in specific diet (HP or LP); this was not surprising because milk fat price was higher than the price for all other milk components during the period we sampled. If the milk protein price was higher than milk fat, as it was from August 2016 to December 2016 ($4.97 per kg milk fat vs. $5.71 per kg milk protein), milk protein price would be the most influential factor for profitability. Another limitation in the sensitivity analysis was that all the prices in the analysis were from the Midwestern U.S. and may not be relevant in other areas, although the relative rankings of dIOFC of individual cows would likely not change across regions.

Prediction Model on EBW Change Response to Dietary Protein Deficiency

The response of cows to changes in dietary protein content has been widely studied and modeled (Hristov et al., 2005; Lean et al., 2018; Moraes et al., 2018); however, previous studies mainly focused on milk production. We showed that BW change in response to decreased dietary protein should also be considered, and we attempted to predict the BW response based on information that could easily be measured. Based on the individual data from ~170 cows, factors that were significant in the prediction model included production level, BW, and parity. The

effect of experiment was also important in modeling BW change because it accounted for all environmental and dietary differences among experiments, such as forage quality, temperature, and humidity.

The current study did not propose prediction models to estimate changes in body protein or energy in response to a shortage of dietary protein. However, the reader can use the equations used in the current study to estimate body protein and energy change based on dEBW.

$$\text{BodyE} = \begin{cases} (3.52 + 1.27 \times \text{BCS}) \times \text{dEBW}, & \text{Parity} > 1 \\ 4.4 \times 0.14 + [(3.52 + 1.27 \times \text{BCS}) \times (\text{dEBW} - 0.14)], & \text{Parity} = 1 \end{cases}$$

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

Milk price, feed price, and body salvage value are region specific and vary over time as supply and demand fluctuate. Thus, a prediction model for profitability also was not proposed in the current study. In addition, because we had only 2 levels of protein, previous models based on many protein levels will be more accurate for predicting changes in milk (Hristov et al., 2005; Moraes et al., 2018). However, without additional data on BW change, we suggest that our predictions for change in EBW should be more accurate than assuming no change in EBW. These estimated responses in milk production and BW change could be combined with prices to estimate the economic return to changes in dietary protein for a group of cows.

We also tried to predict EBW change based on MY response. After exploring an exhaustive list of factors to account for (parity, ECM when cows on the HP diet, ECM per kg MBW when cows on the HP diet, DIM, treatment sequence, and etc.), we still could not find any relationship between MY response and EBW change. This result suggests that considerable variation exists in EBW change relative to the response in milk production of individual cows. More specifically, cows losing more milk when fed diets with less protein do not necessarily lose more body reserve. However, interestingly, BW change can be predicted based on the ECM change, after adjusting for several factors (ECM per kg MBW when cows on the HP diet, parity, treatment sequence, and experiment). Based on the model of dBW on dMY, for each kg decrease of ECM, BW would be expected to decrease by 1.89 kg. However, the prediction of BW change is not useful to farmers, because cull cows are generally sold based on carcass weight, not live weight. It drew our attention that no correlation existed between EBW change and ECM change while BW change was correlated with ECM change. As ECM change was highly associated with DMI change ($P < 0.01$) in the current study, it could be validated that part, if not all, of the BW change was due to DMI change. Future work on BW change in lactating dairy cows must adjust BW based on DMI; otherwise, the BW change information might be misleading. Based on all the information above, we suggest that BW change be routinely measured in studies evaluating responses of lactating cows to dietary changes in protein content, protein source, or amino acid supplements.

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, BW change in dietary protein reduction significantly contributed to the total change of energy capture, protein capture, and income. When cows in peak lactation were underfed protein, the loss in net profit was estimated as 27% greater if BW change was included in the response; in late lactation cows, the loss in net profit could be 45% greater. Therefore, BW change should be monitored to fully assess cow response to dietary protein.

ACKNOWLEDGEMENTS

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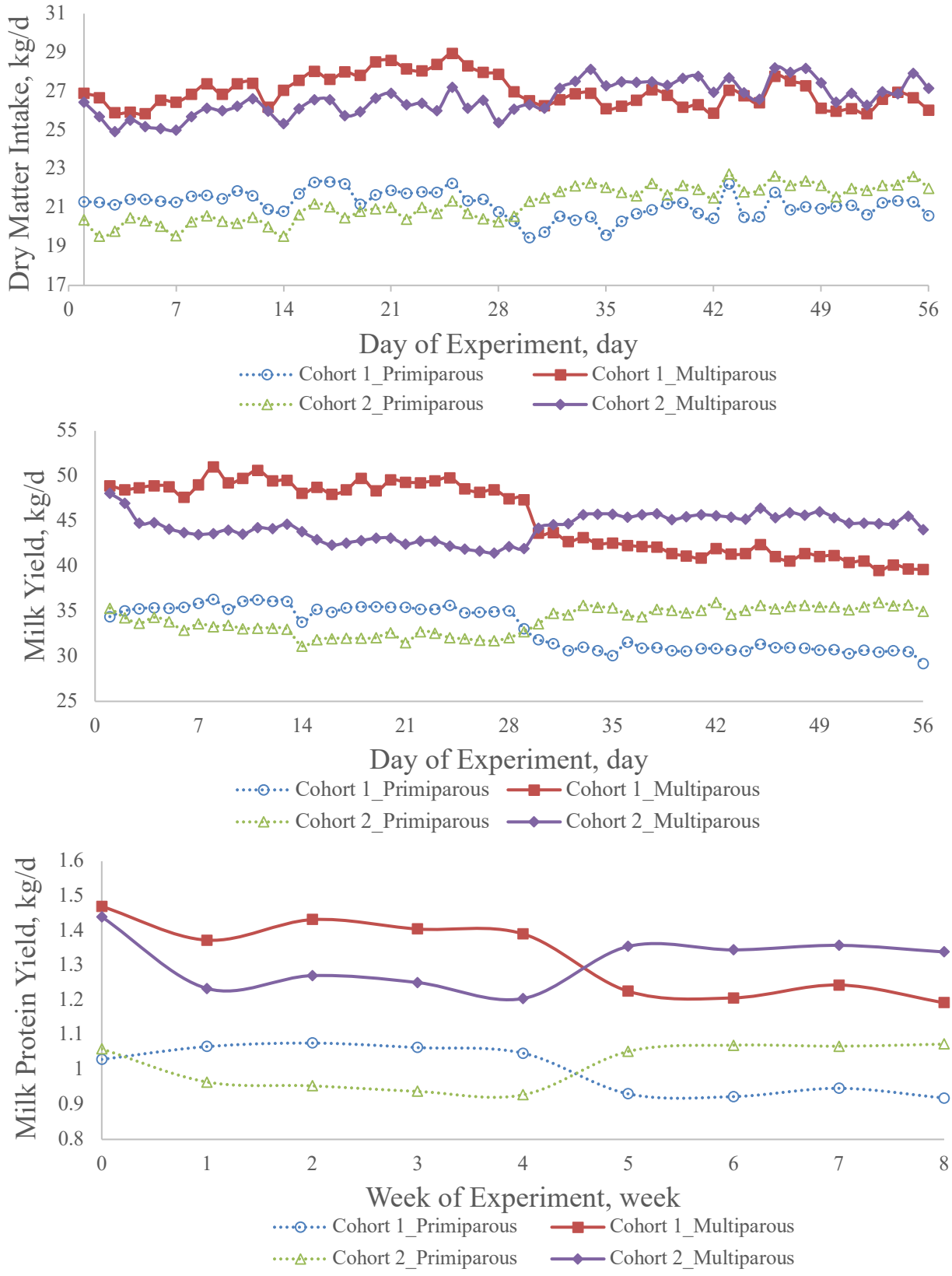
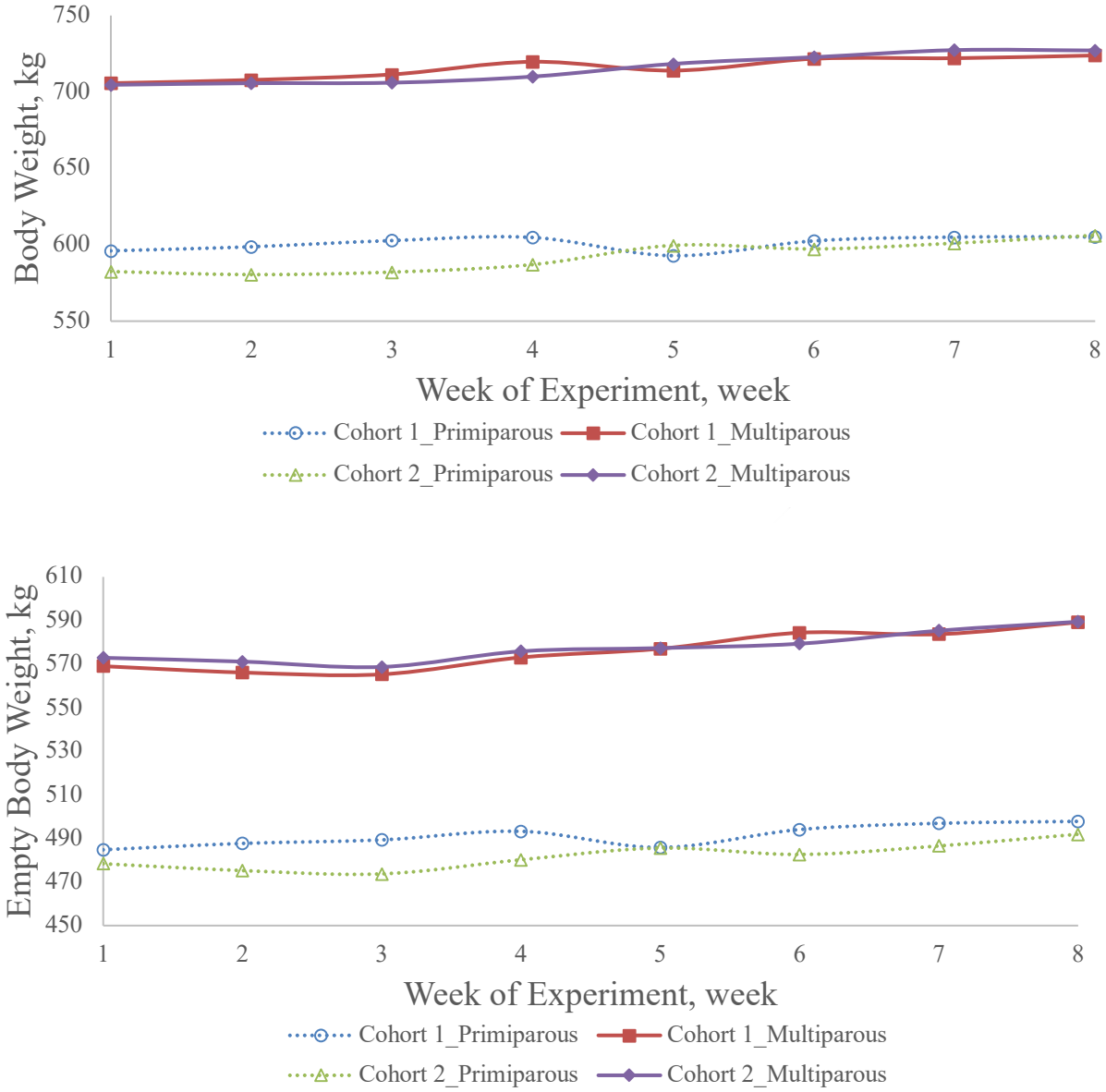
APPENDIX

Supplementary Figure 5.1 Time series of cow response (dry matter intake, milk production, and body weight) to dietary protein reduction in peak lactation

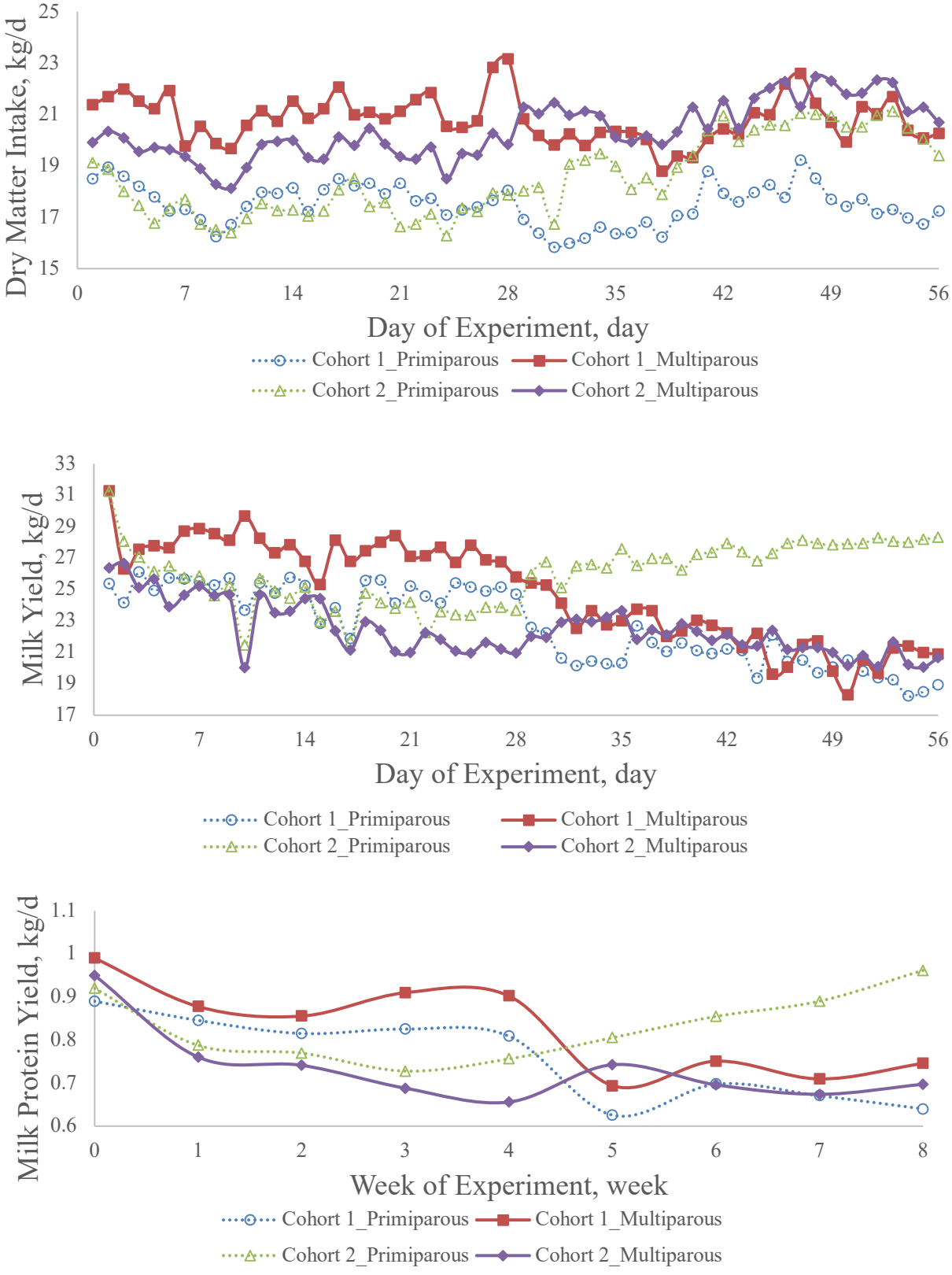


Supplementary Figure 5.1 (cont'd)

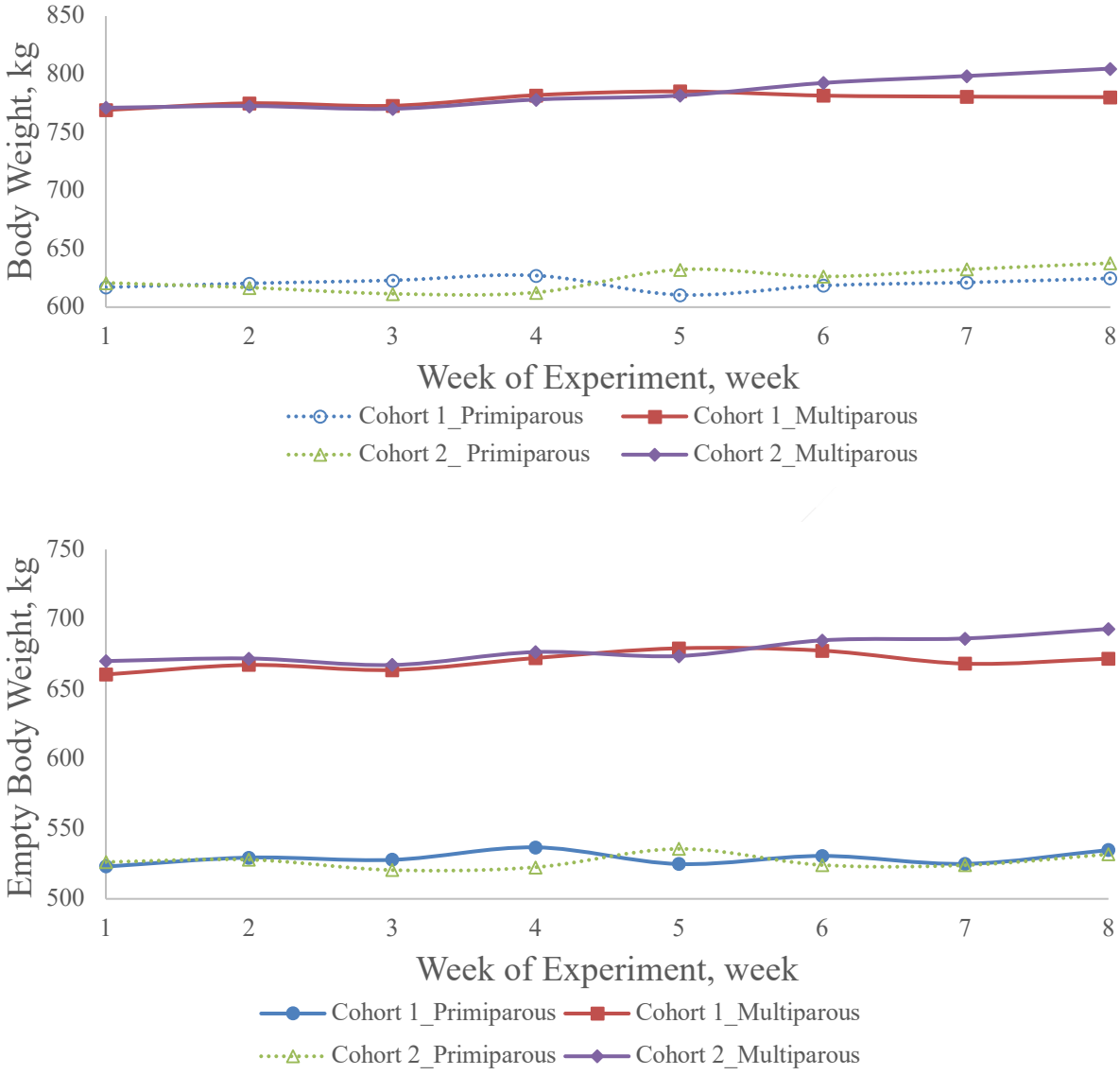


Supplementary Figure 5.1. Dry matter intake and milk yield were averaged across experiments (1,…,7) by day. Milk protein yield, body weight, and empty body weight were averaged across experiments (1,…,7) by week. Empty body weight (kg)= body weight (kg) – 5.2 × DMI (dry matter intake, kg/d). Cohort 1 included the cows fed 18% CP in period 1 and 14% CP in period 2; LP included the cows fed 14% CP in period 1 and 18% CP in period 2. Primiparous= primiparous cows (parity=1); multiparous= multiparous cows (parity > 1).

Supplementary Figure 5.2 Time series of cow response (dry matter intake, milk production, and body weight) to dietary protein reduction in late lactation



Supplementary Figure 5.2 (cont'd)



Supplementary Figure 5.2. Dry matter intake and milk yield were averaged across experiments (1,…,4) by day. Milk protein yield, body weight, and empty body weight were averaged across experiments (1,…,4) by week. Empty body weight (kg)= body weight (kg) – 5.2 × DMI (dry matter intake, kg/d). Cohort 1 included the cows fed 16% CP in period 1 and 13% CP in period 2; LP included the cows fed 13% CP in period 1 and 16% CP in period 2. Primiparous= primiparous cows (parity=1); multiparous= multiparous cows (parity > 1).

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

Residual feed intake (RFI) was repeatable across various dietary protein content within each lactation stage. Among peak-lactation cows, the moderate level of RFI repeatability was in line with previous studies examining repeatability of RFI across diets (Potts et al., 2015; Mangual et al., 2016). Lower RFI repeatability across dietary protein contents in late lactation, compared to peak lactation, might have been due to inaccuracies in measuring tissue gain of pregnant cows. The RFI repeatability across lactation stages was lower than expected, which 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. To generate the most accurate estimates of RFI, RFI estimation should be based on data between DIM 150 and DIM 230 when tissue gain or loss was minimal. Aligning with what Xi et al. (2016) and Mangual et al. (2016) speculated, RFI was associated with protein efficiency in peak-lactation cows and also in late-lactation cows when protein was not limiting. The poor correlation in late-lactation cows fed low-protein diets could be due to the nutrient partitioning to pregnancy; when pregnancy does not take the priority over milk synthesis, cows with lower RFI utilized protein more efficiently. To estimate RFI more accurately in late-lactation cows, better estimates of pregnancy weight gain and pregnancy energy gain are needed.

Despite the high repeatability observed across dietary protein contents, cows did not maintain their protein efficiency rankings across lactation stages. This is consistent with the 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 stage could be due to shifts in nutrient partitioning between production and reproduction. For cows fed the same dietary protein in the same lactation stage, those individual cows with lower MUN values do not necessarily utilize protein more efficiently. Thus, MUN of groups of cows can be a good indicator for protein feeding in daily practice; however, ranking cows for protein efficiency based on MUN may be misleading. To identify the cows that need less feed protein to produce the same amount of milk protein, low protein resilience, or LPR, was proposed. After accounting for all factors that can be measured, LPR accounted for 40% of the overall variation in cow responses when switched from high-protein to low-protein diets. Cows with higher LPR were the more efficient cows when fed low protein diets; thus, LPR could be a useful way to think about the relative protein efficiency of individual dairy cows in the future, especially if protein efficiency ever becomes a target trait for genetic selection. Interestingly, it was also observed that the cows with high LPR, compared to cows with low LPR, captured less body protein when fed high protein diets, but more when fed low protein diets. These results imply that cows with less body protein deposition in high-protein diets were likely more resilient to low protein diets. Given the existing variation among cows, LPR can potentially have a genetic basis. However, more work is needed to examine whether LPR is repeatable across other types of diet changes (for example, other types of base diets and other protein or amino acid supplements) and different lengths of the time period (1 wk. vs. 4 wk. vs. 10 wk., etc.). If LPR is repeatable across diets and time, and it is indeed an individual cow trait, further work on a potential genetic basis for LPR would be warranted and would require collaboration among research institutes to collect adequate data.

To investigate the underlying mechanisms of RFI and LPR, I examined the association of total tract digestibility to RFI and LPR. No association was observed between RFI and digestibilities of DM, CP, or NDF, regardless of dietary protein contents and lactation stages. The findings in the association of RFI to total tract digestibility were inconclusive in the literature, and results from previous studies suggest that the association of RFI and digestibility varies among diets. Potts et al. (2017) found that RFI and digestibility were correlated for low-starch, high-fiber diets but not high-starch diets. Perhaps the high fiber diets allowed low-RFI cows to express their superior digestive ability, while high-starch diets are already highly digestible and thus a more efficient digestive ability had no impact on overall efficiency. It is also likely that the nutrient availability to ruminal microbes influences the association between RFI and nutrient digestibility. Both ideas can potentially explain the findings, and further examination is in need. In any case, both the low and high protein diets in the present study were relatively high in starch and low in fiber, so perhaps differing digestive abilities of cows in this study had little impact on RFI. High-fiber diets containing different sources of NDF (forage vs. non-forage) can help examine the two ideas.

Difference in total tract digestibility also did not contribute to difference in LPR. The results suggested that cows with better resilience do not necessarily have better digestibility in low-protein diets, nor better ability to maintain their digestibility when switching from high-protein to low-protein diets. Thus, post-absorptive mechanisms must contribute more to cows' resilience to low protein. Previous studies investigating underlying physiological mechanisms in animal resilience to various stressors indicate that these mechanisms are very specific to the type of stressor (Doeschl-Wilson et al., 2012; Colditz and Hine, 2016; Elgersma et al., 2018). The only common factor that was considered across all studies on resilience traits is blood cortisol

level. For example, newborn piglet with larger adrenal glands and higher concentration of cortisol in blood were found to be more resilient to disease and had higher survival rates (Leenhouwers et al., 2002). Poultry with higher glucocorticoid levels adapted better to social stress when moved to a new group (Morme` de et al., 2010). In the same study, Morme` et al. also found that birds with a more intense hypothalamic–pituitary–adrenocortical axis stress response had greater immune responses and resistance to disease. Taken together, it seems that cortisol plays an important role in differentiating animals for stress resilience. Indeed, there are reasons to believe that blood cortisol could influence LPR. Cortisol has been shown to increase metabolic rate and catabolic processes such as protein degradation (Knot et al., 2008). Given that protein degradation contributes to protein turnover, a higher level of cortisol may lead to a greater protein turnover rate. Thus, it could be that cows with generally higher levels of blood cortisol already have higher protein turnover rates, and in turn, are less responsive to low-protein diets (as the stressor). Blood cortisol concentrations of the cows should be examined in the future LPR studies.

When adjustments are made in the protein content or source of a diet for lactating dairy cows, the dietary protein is commonly assumed to be sufficient based on responses in milk or milk protein production. However, dietary protein should not be considered sufficient if body protein mass is reduced in the process. When reducing dietary protein, the proportion of total energy loss that was due to body energy loss was estimated as 42% for peak-lactation cows and 51% for late-lactation cows; the proportion of estimated protein loss that was attributed to estimated body protein loss was 11% for peak-lactation cows and 14% for late-lactation cows. In addition, if only milk responses are considered when reducing dietary protein, the loss in profit could be underestimated by 27% and 45%, in peak lactation cows and late lactation cows,

respectively, compared to considering both milk and body changes. The economic analysis performed in the current study was based on the salvage value of cull cows and thus only the direct cost of BW loss was considered. Loss of BW might also affect health and fertility, so the difference between IOFC when only considering milk and IOFC when considering both milk and body responses might be even greater when including these indirect costs of losing BW.

Based on the individual data from ~170 cows in this study, factors that were significant in predicting BW change in response to a reduction in dietary protein included production level of cows when fed high-protein diets, change in BW when fed high-protein diets, and parity. Because there were only 2 levels of protein in the study, previous models based on many protein levels would be more accurate for predicting changes in milk (Hristov et al., 2005; Moraes et al., 2018). However, without additional data on BW change, the predictions for change in EBW should be more accurate than assuming no change in EBW. These estimated responses in milk production and BW change could be combined with prices of feed and milk to estimate the economic return to changes in dietary protein for a group of cows. To improve the BW prediction model, more data from cows fed diets with various protein contents is needed. This will require collaboration among research institutes to collect adequate data on production performance with different feeds specific to different geographic areas.

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