

# Protein During the Dairy Cow Transition Period: What We Feed and What the Cows Lose

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## ■ Take Home Messages

In late gestation and early lactation there are drastic increases in the amino acid (AA) requirements of dairy cows to meet increasing fetal and milk production requirements. Extensive research has shown potential benefits to supplementing additional metabolizable protein (MP) and specific rumen-protected AA in late gestation including improved production, immune status, and reproductive success. Variation exists for supplementing rumen-protected AA in research trials and on commercial herds likely because of interactions between AA and other nutrients supplied. To meet requirements in early lactation, cows mobilize both adipose tissue (fat) and muscle to meet energy and AA requirements. The extent that dairy cattle lose adipose tissue is well documented with negative health outcomes being associated with excessive adipose tissue mobilization; however, protein mobilization is less well understood. In high producing dairy cows, there is a considerable negative MP balance in early lactation that has to be met by mobilized skeletal muscle. A portion of the skeletal muscle can be mobilized but there appears to be a minimum amount of empty body protein that must be maintained. Considerable variation exists between the amount of muscle depth at specific locations of the body that is representative of whole body muscle. There is a moderate negative relationship between the amount of muscle depth prepartum and the extent of muscle that is mobilized through early lactation. On average, cows mobilize approximately 20% of their muscle depth from calving through 30 days in milk (DIM). However, there is considerable variation among cows; these differences in metabolic efficiencies need to be explored. Nutritional considerations for the extent of tissue that is mobilized need to be considered in order to truly maximize production, reproduction, and health outcomes.

## ■ Introduction

In the transition from late gestation to early lactation cows undergo considerable metabolic adaptation to support demands for fetal growth, parturition, and upcoming milk production (Bauman and Currie, 1980). The dairy cow has three main stores of tissue she can mobilize to meet increased requirements: glycogen, adipose tissue, and muscle. Considerable metabolic adaptation occurs in muscle; increased protein catabolism and reduced protein anabolism occur during the transition period. Increased proteolysis (muscle breakdown) contributes AA to the developing fetus in late gestation and provides AA to support colostrum and milk production in early lactation. This increase in proteolysis is a normal homeostatic response of animals adjusting to a new normal where their demands have changed without a sufficient increase in intake to support the increased requirements. Supplementing rumen-protected AA during the transition period has the potential benefit of increasing MP and individual AA that may be limiting in the transition cow.

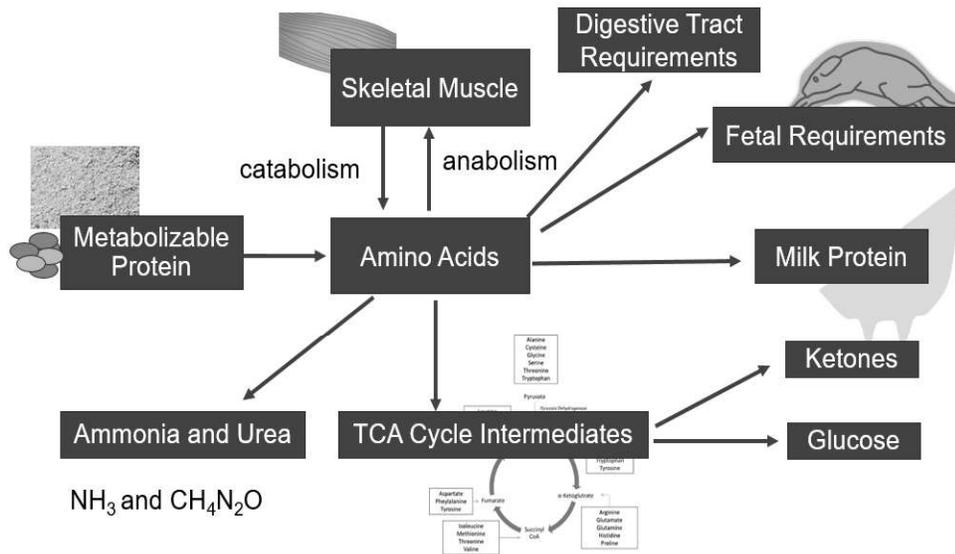
The dairy industry is more familiar with measuring the amount of adipose tissue than the amount of muscle that cows mobilize around the transition period. By using body condition score (BCS) we can visualize changes in subcutaneous fat thickness that occur around calving. We can also measure metabolites related to adipose tissue mobilization such as non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB). While change in BCS is generally associated with changes in adipose tissue, it is moderately correlated to changes in empty body protein as well (NRC, 2001). For the transition dairy cow, depending on BCS, adipose tissue may contribute to over 25% of the empty body weight (BW) of the cow, with protein

representing approximately 13% of empty BW (Komaragiri and Erdman, 1997). Metabolic adaptation to early lactation may result in mobilization of > 80 kg of adipose tissue and 20 kg of protein. From a research perspective we can evaluate muscle mobilization by using ultrasounds and assessing metabolites including creatinine and 3-methylhistidine; however, these are not routinely done on commercial farms. Farms that are implementing several BW measurements during the transition period are assessing whole body changes that include both adipose and muscle tissue changes. Commercial farms may use these changes in BW to determine the relative extent of negative energy and MP balance and may make different breeding strategies based on the extent of tissue lost, with cows that lose more weight having extended voluntary waiting periods compared with cows that lose modest amounts of weight. While BW is not specific for tissue type it can help producers identify individual animals with more severe negative energy and MP balance during the transition period.

### ■ **Amino Acid Requirements for Dairy Cows During the Transition Period**

Because of the inability of dairy cows to consume sufficient dry matter to meet nutrient requirements during the transition period, cattle are more susceptible to metabolic diseases during this time. The ability of the dairy cow to adapt to changing demands of late gestation into early lactation dictates her ability to have a successful lactation for milk production, reproduction, and health outcomes. Changes in insulin sensitivity in early lactation allow for an increased amount of nutrients to be partitioned to the mammary gland, potentially exacerbating the negative effects of negative energy and MP balances.

Cows will meet their AA requirements through MP from feed and microbial protein and from catabolism of skeletal muscle (Figure 1). These AA are then used for several outcomes including fetal AA requirements, milk protein synthesis, and the production of ketones or glucose depending on stage of gestation and lactation. The current NRC (2001) model suggests that in late gestation, Holstein dry cows should receive 850 g/d of MP whereas most nutritional models would suggest MP supply in excess of 1,200 g/d may be more appropriate (recommendations will vary depending on nutritional model used). During the close-up dry period, the MP requirements for the uterus and fetus increase considerably (Bell et al., 1995). Additionally, there are considerable increases in MP requirements for mammary gland development and colostrum synthesis in the last weeks of gestation (Capuco et al., 1997; Bell et al., 2000). As BW and milk production for dairy cattle have increased and our understanding of the role of individual AA has increased, most nutritionists would recommend providing MP in late gestation compared with that recommended by NRC (2001).



**Figure 1. Sources and uses of amino acids in the transition dairy cow.**

In early lactation, the requirement for MP significantly increases as milk production and milk protein yield increase. Additionally, MP is used for increases in the mass of the digestive tract and liver, which increase in size to increase absorption of nutrients and production of glucose required in early lactation (Reynolds et al., 2004; Aschenbach et al., 2010). Bell et al. (2000) suggested that cows are in negative MP balance until approximately 21 days postpartum because of increased requirements in early lactation relative to insufficient dry matter intake. At its nadir, MP balance may be in excess of -600 g/d, with the balance needing to come from skeletal muscle mobilization. The severity and duration of negative MP balance is based upon milk and milk protein production compared to feed intake in early lactation.

Postpartum dairy cows that were abomasally infused with AA with a similar profile to casein had increased milk production and milk protein yields compared with cows infused with water (Larsen et al., 2015). The uptake of essential AA by the mammary gland increased when cows were infused with AA, indicating that supply of AA is the driving factor for uptake by the mammary gland. Although the supplemented cows were supplied with more essential AA, the output of these AA was also increased; therefore, the protein deficiency in early lactation was maintained. The increase in MP as a result of the AA infusions positively impacted production parameters without any effect on MP balance. While this study matched the AA profile of milk protein and had positive effects on milk protein production, intake of this amount of MP (700 g at 5 DIM and 500 g at 15 DIM) may be infeasible in early lactation when dry matter intake is limited. Therefore, several researchers have considered the effects of supplementing individual rumen-protected AA on production and health parameters.

### ■ Supplemental Amino Acids Fed to Dairy Cows During the Transition Period

While there are clear increases in the MP requirements of close-up dry cows and even more in early lactation compared with the far-off dry period, a considerable amount of research has shown the benefits of supplementing specific AA during the transition period. Methionine is generally considered the first-limiting AA for dairy cows (NRC, 2001) and is used for milk protein synthesis, lipoprotein synthesis in the liver, and antioxidant synthesis. Lipoprotein synthesis is especially important in the transition dairy cow because lipoproteins are required for fat transport and may be critical as cows are accumulating fat in their liver as a result of adipose tissue mobilization due to negative energy balance. Supplemental methionine during the dry period had positive effects on milk yield and milk protein concentration with a corresponding increase in dry matter intake in early lactation (Osorio et al., 2013; Zhou et al., 2016; Batistel et al., 2017).

Supplemental methionine during early lactation increased milk protein concentration (e.g. Casper and Schingoethe, 1988) and a meta-analysis conducted by Zanton et al. (2014) demonstrated that protected methionine fed to lactating cows, including early lactation, consistently increased milk protein yield and concentration compared with control diets. However, others have reported supplementation of rumen-protected methionine had no effect on milk protein or any other production parameters (e.g., Chen et al., 2011; Lee et al., 2019).

Supplemental rumen-protected lysine in the prepartum period resulted in increased milk and component yields (Fehlberg et al., 2020). In this study, feeding lysine prepartum had a greater effect on early lactation than supplementing postpartum, with minimal effects when animals were only supplemented with additional lysine postpartum. Supplementing rumen-protected lysine to close-up and early lactation cows increased dry matter intake both pre- and postpartum. Postpartum, free fatty acid (FA) and BHB concentrations were reduced when rumen-protected lysine was supplemented with no differences in milk production parameters (Girma et al., 2019). The reduced free FA and BHB concentrations may be a result of carnitine synthesis, the methylated form of lysine, because carnitine aids in oxidation of FA in the liver and may therefore reduce fatty liver and ketone body formation (Carlson et al., 2006). These differences in ketone bodies postpartum were more evident with a high energy prepartum diet, indicating that supplementation of rumen-protected lysine pre and postpartum may have variable effects depending on other nutrients in the diet.

Mammary uptake of branched-chain AA (BCAA; leucine, isoleucine, and valine) and arginine from circulation greater than needed for milk protein synthesis in the lactating cow indicates increased catabolism of these AA in the mammary gland to produce glutamine, alanine, aspartate, asparagine and proline (Rezaei et al., 2016). However, the limited research on supplementing rumen-protected BCAA in the postpartum period has demonstrated moderate effects on reducing hyperketonemia events with insignificant changes in milk or milk protein yield (Leal Yepes et al., 2019). Additionally, muscle protein is lower in several AA, including BCAA, relative to milk protein, so skeletal muscle, although an important contributor, is not an ideal match for the milk AA profile (Table 1). We must rely on dietary and microbial AA sources to meet the AA deficits from mobilized skeletal muscle.

**Table 1. Ratio of essential AA in skeletal muscle and milk protein in dairy cattle.**

Amino Acid	Ratio of muscle AA <sup>1</sup> / milk AA <sup>2</sup>
Arginine	2.15
Histidine	0.99
Isoleucine	0.54
Leucine	0.81
Lysine	0.92
Methionine	0.81
Phenylalanine	0.82
Threonine	1.13
Tryptophan	0.53
Valine	0.75

<sup>1</sup>Muscle AA reported by Doepel et al., (2004).

<sup>2</sup>Milk AA reported by Waghorn and Baldwin, (1984)

The variable responses from supplementing rumen-protected AA indicate there are interactions between the supply of AA and other nutrients. Additionally, some of the variation may be caused by our inability to accurately model MP in the transition dairy cow and the discrepancies between models for calculating MP. Cows have considerable variation in their metabolic efficiencies that likely contribute to the inconsistency in response with any supplemented nutrient, including AA. However, supplementing MP above the recommendation from NRC (2001) or some rumen-protected AA have shown the potential to improve both health and production outcomes in the transition dairy cow.

## ■ **How Much Protein do Cows Lose During the Transition Period?**

Dairy cows lose an estimated -3 to 17% of their BW from one to five weeks postpartum (Zachut and Moallem, 2017). This would indicate that a 700 kg dairy cow may lose > 100 kg through peak milk production. Some cows through early lactation actually increase their BW. There is considerable variation in BW change in early lactation, so a genetic component that is unaccounted for on commercial dairy farms likely exists (Friggens et al., 2007). Cows that gained weight had improved conception rate; however, they had reduced milk yield through the first 30 DIM (Zachut and Moallem, 2017). Variation in BW loss exists for animals consuming the same diet because of differences in intake and milk yield resulting in changes in energy balance. However, in individual cows, there are marked differences in efficiency of metabolic pathways that allow two cows consuming the same dry matter and producing the same milk output to have differences in BW change.

The main tissues mobilized are adipose tissue and skeletal muscle (protein). Adipose tissue is mobilized to meet energy requirements, whereas protein can be used to meet AA requirements and to meet energy requirements because AA can be converted to glucose and/or ketone bodies depending on the specific AA. A study that fed a protein-restricted diet to achieve a nitrogen balance of zero estimated that the labile supply of protein that can be mobilized is approximately 27% of empty body protein (Botts et al., 1979). However, studies that fed diets that supplied more protein estimated lower amounts of protein mobilized from calving through early lactation, with only 20% of empty body protein mobilized (Komaragiri and Erdman, 1997; van der Drift et al., 2012). Much like the variability in BW loss, the extent of protein mobilized is highly variable in transition dairy cows, with some cows losing considerably more than 20% and others gaining muscle depth during this period.

## ■ **Measuring Protein Mobilization**

The amount of protein stored in skeletal muscle has been documented best in studies that have evaluated carcass characteristics of dairy cattle at different stages of lactation. Commercial dairies and most researchers will not have access to these data on individual dairy farms; therefore, alternatives to assess the amount of empty body protein at specific time points is a way to estimate body reserves and therefore tissue mobilization. In beef cattle, ultrasound measurements of the longissimus dorsi muscle depth have been highly correlated to whole body protein (Greiner et al., 2003). Ultrasounds of dairy cattle at this location as well as other locations have been used to assess the amount of muscle depth with extrapolation to whole body protein (Schroder and Staufenbiel, 2006; van der Drift et al., 2012).

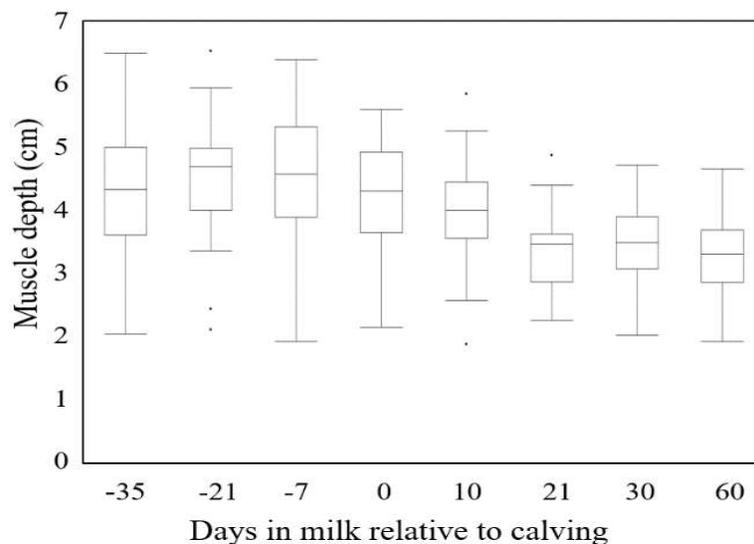
Metabolites related to AA metabolism can be measured in transition dairy cows to estimate the amount of muscle mass and proteolysis that is occurring. Muscle mass can be estimated from creatinine, the waste product produced by muscle from the breakdown of creatine and phosphocreatine (Wyss and Kaddurah-Daouk, 2000). This step is nonreversible and nonenzymatic, with relative increases in concentration indicating muscle accretion and relative decreases in concentration indicating muscle mobilization between two time points. During muscle degradation, 3-methylhistidine (3-MH) is produced and can be quantified to indicate muscle mobilization. Because muscle is constantly being degraded and accreted there will always be 3-MH present; however, relative differences between time points indicates more or less protein mobilization. To standardize across animals of different sizes, 3-MH relative to creatinine concentrations would indicate relative differences in protein mobilization per unit of muscle mass; the higher the ratio the more protein being mobilized. Feeding additional MP for the three weeks postpartum resulted in reduced 3-MH, indicating less muscle degradation in the period of time when MP balance is most negative (Carder and Weiss, 2017).

Measuring BW and body condition score through the transition period is likely to be used on commercial farms to measure muscle mobilization. While this is not specific to measuring whole body protein, animals in negative energy balance mobilize both adipose and muscle because of insufficient nutrient intake. Some commercial farms already implement reproductive protocols based on BW loss; animals that lose the most BW will have an increased voluntary waiting period to increase conception rates by delaying first

insemination to a time when they are more likely to conceive. As technology increases on farms and the ability to routinely monitor BW and body condition scoring increases, the ability to assign cattle to reproductive protocols depending on inputs like milk production and change in BW and body condition scoring will also increase.

## ■ Muscle Depth Loss from Transition Dairy Cows

Protein mobilization starts before parturition as intake of dietary protein is reduced and fetal and colostrum requirements for AA are increased (van der Drift et al., 2012). Considerable variation in the amount of longissimus dorsi muscle depth has been recorded in transition dairy cattle, with a minimum of approximately 2 cm and a maximum of 6.5 cm (McCabe, 2020). While we generally do not think of muscle as being a storage tissue for AA as adipose is for FA, the variation between cows indicates that some animals store more AA in muscle than others. Dairy cows appear to mobilize muscle between -7 and 30 DIM (Figure 2), with less variation at 30 and 60 DIM compared with prepartum time points and maintain a minimum depth of approximately 2 cm at the longissimus dorsi location. A study was conducted with prepartum cows to evaluate the effects of exercise on muscle and liver composition and plasma concentrations of metabolites and hormones. Exercising dairy cattle during the prepartum period caused physiological adaptation with an increase in muscle and liver triglycerides and plasma NEFA and a reduction in plasma BHB and insulin concentration (Davidson, 2002). Using exercise as an environmental change for the cows, the authors noted differences in metabolic response between animals that were exercised and those that were not exercised and suggested that increasing muscle tone may have benefits for reducing metabolic diseases due to changes in metabolites and insulin concentration. Very little is known about if, and to what extent, we can alter muscle depth in cattle through nutrition and management; however, limited work with exercising cattle has demonstrated that this may positively impact the metabolic status of prepartum dairy cattle.

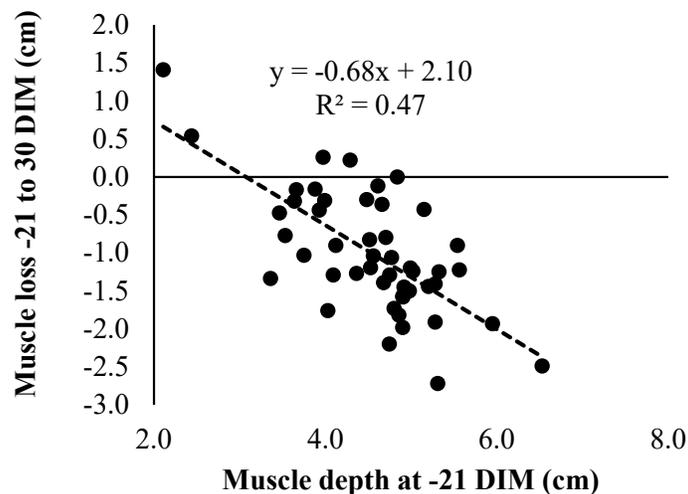


**Figure 2. Box and whisker plot of muscle depth relative to calving for multiparous Holstein cattle (n=48). The horizontal line within the box represents the mean, the upper box represents the 75<sup>th</sup> percentile, the lower box represents the 25<sup>th</sup> percentile. The whiskers represent the minimum and maximum excluding outliers with outliers identified as individual points. Adapted from McCabe, 2020.**

Cows with higher BCS i.e., more adipose tissue, are more likely to have reduced dry matter intake and increased likelihood of developing metabolic diseases such as ketosis and fatty liver. Less is known about the consequences of animals mobilizing excessive muscle during the transition period. In early lactation,

the use of alanine specifically and AA generally for gluconeogenesis is increased because of the increased demand for glucose (Overton et al., 1999). The use of AA for glucose synthesis in early lactation when MP is insufficient from intake suggests some AA are likely mobilized from skeletal muscle.

In a relatively small dataset, we observed that there was a moderate negative correlation between muscle depth of the longissimus dorsi location three weeks before calving and the amount of muscle depth that is lost from three weeks before calving to one month postpartum (adapted from McCabe, 2020; Figure 3). Cows with lower muscle depth prepartum gained muscle depth during this time while cows that had higher muscle depth tended to lose more muscle. Similar results have been reported by van der Drift et al. (2012) with animals with greater muscle depth prepartum having increased mobilization of muscle postpartum. Although we generally do not see skeletal muscle as an AA store, the variation in the extent that animals mobilize indicates that there are factors that regulate both catabolism and anabolism that can be influenced by environment, diet, and genetics.



**Figure 3. Muscle loss from -21 to 30 DIM vs. muscle depth at -21 days prior to expected calving for multiparous Holstein cattle (n=48). Adapted from McCabe (2020).**

### ■ Energetics Around Losing and Gaining Muscle

The energy requirements for maintenance are based on metabolic BW multiplied by a constant coefficient of 0.080 Mcal/kg BW<sup>0.75</sup>. As an animal increases or decreases in muscle mass, the amount of energy required for maintenance is impacted. The proportion of empty body fat increases as BCS increases whereas the relative proportion of empty body protein is thought to be reduced as BCS increases (NRC, 2001). Energetic inefficiency exists when an animal is converting AA to muscle and mobilizing muscle into AA. Therefore, although animals are able to use skeletal muscle to meet MP requirements in early lactation, this inefficiency needs to be accounted. The MP requirements for increasing muscle synthesis when MP balance is positive also needs to be taken into account. Liu and VandeHaar (2020) suggested that considerable variation exists for a cow's ability to capture protein in both milk and body tissue. They proposed there is likely a genetic component to the ability of cattle to capture dietary protein and convert it into body protein. When formulating diets for transition dairy cattle, the change in BW, and therefore changes in adipose and muscle tissue amounts, need to be accounted for regarding the nutrients supplied by mobilization of tissue (energy and AA in the case of muscle) and the energy and MP required to regain this tissue at other stages of lactation.

## ▪ Relationship Between Muscle Loss and Oxidative Stress

The transition period is marked by a considerable amount of disease because the cow is unable to adapt to the new physiological normal. This period of time is characterized by increases in oxidative stress, further exacerbating the risk for metabolic diseases. As previously mentioned, supplementation of rumen-protected methionine benefits production, and reduces oxidative stress and immunological markers in the transition period (Osorio et al., 2014). As tissue becomes more metabolically active, in late gestation and early lactation, more oxygen is consumed and the mobilization of adipose tissue leads to an increase in reactive oxygen species (Abuelo et al., 2019). Oxidative stress contributes to inflammation, which may increase the requirements of AA to be mobilized from muscle. However, we know less about the ability of protein mobilization to directly contribute to oxidative stress compared with the ability of adipose tissue mobilization. Supplementing rumen-protected methionine has been linked to both reducing oxidative stress and reducing protein degradation. Work done in humans with sarcopenia or muscle wasting (which I admit is not a perfect match for protein mobilization in dairy cows in early lactation but does represent the magnitude of muscle loss that dairy cattle experience) has demonstrated links between oxidative stress, inflammation and muscle atrophy (Meng and Yu, 2010). This work in humans suggests at least that increased oxidative stress and inflammation in maladapted dairy cattle may contribute to more muscle mobilization in early lactation.

## ▪ Conclusions

Because of the increased demands for AA in late gestation and early lactation, there is a relative consensus for increasing MP above NRC (2001) requirements for close-up dry cows. Insufficient intake of MP in early lactation causes cows to mobilize skeletal muscle to meet the AA gap. Increasing the MP available in early lactation will preferentially allocate AA to the mammary gland, with some variation in response evident from supplementing individual rumen-protected AA. Dairy cattle can mobilize a considerable amount of muscle; however, it appears that there is a minimum amount of muscle depth they must maintain. While muscle mobilization may start before calving, there appears to be little difference between 30 and 60 days postpartum indicating cows likely are not using skeletal muscle to a large extent after 30 DIM. Skeletal muscle can be stored and mobilized to meet MP requirements in early lactation. To truly model requirements in a dairy cow, the extent of tissue that is mobilized needs to be accounted for during mobilization and re-accretion as well as the energetic inefficiencies around protein catabolism and anabolism.

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