Quantifying Protein Mobilization in Transition Dairy Cows

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Abstract

Cows are able to mobilize adipose, protein, and glycogen stores to meet energy and amino acid requirements that increase in late gestation and early lactation. While we are able to use body condition score (**BCS**) change and measurements of non-esterified fatty acids (**NEFA**) and ketones to quantify adipose tissue mobilization, less is understood about protein mobilization. Two primary techniques have been used to quantify protein mobilization: 1. ultrasound images to measure muscle depth at different locations and 2. measuring quantities of waste products from muscle degradation like 3-methylhistidine in blood or urine. These methods combine to give us more insight on when and to what extent animals are mobilizing muscle in order to meet the demands of late gestation and early lactation.

We observed that cows start to mobilize muscle prior to calving and that even 60 days into lactation they are still mobilizing muscle. BCS is not a good indicator of muscle depth or whole body muscle mass; currently there is no visual way to assess for muscle quantity in animals. However, muscle depth was a predictor of muscle mobilization. Cows that had more muscle depth prior to calving ended up mobilizing more muscle through the transition period. On average, cows mobilized 19% of their muscle depth at the longissimus dorsi from before calving to 60 days post calving. However, the amount of muscle that a cow had prior to calving was a strong indicator of how much muscle would be mobilized through transition (R^2 = 0.68; $P < 0.0001$); cows with more muscle prior to calving, mobilize more muscle through early lactation. Amino acids derived from muscle can be used to support milk production through the production of milk protein and lactose; therefore, optimizing muscle mobilization may be beneficial to support milk production in early lactation as long as doing so does not negatively impact health.

Introduction

During late gestation, the cow is mobilizing skeletal muscle in order to meet amino acid and potentially glucose requirements of the fetus in addition to provide amino acids for body maintenance and colostrogenesis. Komaragiri and Erdman (1997) estimated that empty body protein prior to calving represented between 12 and 13% of body weight (**BW**) for an animal at optimal body condition. In their study, cows prior to calving had approximately 95 kg of protein and mobilized in excess of 20 kg of protein from prior to calving to 5 weeks postpartum. At the same time, empty body fat represented 19 to 24% of BW and was dependent on BCS. Cows were capable of mobilizing in excess of 80 kg of adipose from prior to calving to 12 weeks postpartum. Although adipose

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tissue mobilization represents a considerably larger pool, protein mobilization from primarily skeletal muscle represents a considerable pool of amino acids for the transition dairy cow to utilize. Understanding the extent and implications of protein mobilization around parturition will allow us to develop feeding strategies to optimize tissue mobilization in order to maintain both health and production.

Amino Acids Mobilized from Skeletal Muscle

Skeletal muscle that is mobilized prior to parturition can be used for a number of outcomes, including milk protein synthesis, direct oxidation, or gluconeogenesis (Kuhla et al., 2011). Because of the mismatch between amino acids in milk and amino acids in muscle, as well as preferential use of specific amino acids for gluconeogenesis, there may be an imbalance of amino acids in circulation. Alanine and glutamine are the amino acids used in the greatest quantity for gluconeogenesis in the dairy cow (Drackley et al., 2001). Moreover, compared to milk, skeletal muscle is lower in branch chain amino acids; therefore, skeletal muscle may be mobilized in excess in order to meet the amino acid requirements for milk protein synthesis in early lactation. Evidence of an imbalance in plasma amino acid profile in early lactation is observed by a decrease in essential amino acids in plasma and an increase in non-essential amino acids in plasma around parturition (Kuhla et al., 2011).

Individual amino acids have shown to impact feed intake and impact insulin signaling. In early lactation, dairy cattle tissues that is insulin sensitive are more insulin resistant and there are relatively low levels of circulating insulin (De Koster and Opsomer, 2013). However, leucine has been shown to increase insulin secretion in laboratory animals and increase α-amylase production by the pancreas in dairy cattle (Liu et al., 2015; Sadri et al., 2017), showing that individual amino acids likely have an impact on feed regulation and also tissue mobilization due to their effects on insulin. While it is not clearly defined, it is reasonable to believe that amino acids, indirectly through their conversion to ketones and glucose or directly through amino acid signaling, impact feed intake.

Ultrasound Imaging to Quantify Tissue Mobilization

Tissue mobilization during the transition period traditionally equates to mobilization of adipose tissue. Both commercial farms and research trials routinely measure NEFA and β-hydroxybutyrate (**BHBA**) as indicators of how much adipose tissue is being mobilized. Additionally, change in BCS has been used a proxy for subcutaneous adipose tissue mobilization with recommendations to minimize the extent of body condition loss through calving (Garnsworthy, 2006; Roche et al., 2009). It is well established that excess NEFA leads to accumulation of adipose in the liver and reduces the capacity of the liver to synthesize glucose (Drackley, 1999). However, less is known about the extent and implications of muscle mobilization during the transition period.

Researchers are using ultrasound scans of the longissimus dorsi muscle and back fat thickness to quantify muscle depth and back fat thickness prior to calving through peak lactation (van der Drift et al., 2012; Boerman, unpublished). van der Drift (2012) found that even at the same BCS, cows had differences in muscle depth and back fat thickness and speculated that maybe more important was the ratio of fat to muscle to determine which tissue would be mobilized. In research conducted in our lab, ultrasound images were taken from at 7 time points from 35 days before expected calving to 60 DIM. We observed that cows started mobilizing muscle and adipose prior to calving and that 60 DIM represented the smallest quantity of both muscle depth and back fat thickness. At approximately 21 days prior to calving, the muscle depth of cows averaged 4.5 cm, with a range of 2 to 6.5 cm. By 60 DIM, average muscle depth was 3.4 cm with a range of 1.9 to 4.7 cm. Muscle depth was not related to BCS and therefore, cannot be predicted by visual observation of the cow.

Cows with the most muscle depth prior to calving mobilized the most muscle when calculating muscle depth mobilization from 21 days prior to expected calving to 60 DIM (R^2 = 0.68; $P < 0.0001$; Figure 1). On average, cows mobilized 19% of their muscle depth from before calving to 60 days of lactation; however, some cows actually gained muscle depth during early lactation, whereas other cows mobilized nearly 50% of their muscle depth. There is considerable variation in protein mobilization among cows, and the extent of mobilization appears to be related to the amount of muscle depth.

Back fat thickness depth has previously been used to estimate total amount of subcutaneous fat. Although, we know that livestock do not uniformly deposit subcutaneous fat uniformly, Schroder and Staufenbiel (2006) estimated that 1 mm of back fat thickness equates to approximately 5 kg of adipose tissue. Cows with more back fat prior to calving mobilized more back fat (from 21 days prior to expected calving to 60 DIM; R^2 = 0.86; $P <$ 0.0001; Figure 2). Interestingly, animals were all fed the same diet during late lactation and the dry period that met or exceeded both protein and energy requirements; however, there was not a strong relationship between muscle depth and back fat thickness (Figure 3). Together, these data show that cows mobilize the tissue that they have excess of and that there is little relationship between muscle depth and back fat thickness.

Metabolites that Predict Muscle Mobilization

Creatinine and 3-methylhistidine can be used to determine proteolysis around parturition. Creatinine is a waste product produced by muscle at a relatively constant rate and can be used as an indicator of total muscle mass. 3-methylhistidine is used to estimate protein mobilization because it is a product of actin and myosin degradation and is not used for protein synthesis (Chibisa et al., 2008). Concentrations of 3-methylhistidine are excreted at a rate relative to muscle breakdown. At times when muscle catabolism exceeds anabolism, increases in 3-methylhistidine are observed. When analyzing for 3-methylhistidine, it is important to be able to separate out 1-methylhistidine from 3-methylhistidine (Houweling et al., 2012). The former may be difficult to separate out from 3-methylhistidine, but it is not thought to be related to protein mobilization. Being unable to separate out methylhistidine products would result in elevated and inaccurate numbers for 3-methyl histidine and would not accurately represent protein mobilization.

In order to correct for differences in muscle mass between cows and between stage of gestation or lactation, using the ratio of 3-methylhistidine to creatinine allows the comparison of protein mobilization per amount of muscle mass. Muscle mobilization measured as 3-methylhistidine and the ratio of 3-methylhistidine:creatinine were both elevated in the first 2 weeks prior to calving compared to 4 weeks prior to calving and 7 weeks post calving (Pires et al., 2013). Work done in our laboratory observed that cows have elevated 3-methylhistidine concentrations in the week prior to calving and continue to have elevated 3-methylhistidine and 3-methylhistidine:creatinine concentrations in the 3 weeks post calving relative to day of parturition. Indicating that mobilization of protein occurs prior to calving and continues for at least 3 weeks post calving.

What are the Implications for Mobilized Tissue?

While most cows mobilize more adipose tissue to meet energy requirements around calving, previous research and preliminary data from our lab shows that muscle is mobilized in large and varying quantities. Unlike adipose tissue that can accumulate in the liver, reduce gluconeogenesis, and reduce feed intake, less is known about the potential negative health effects of mobilizing large amounts of muscle. In order to maintain structural soundness, animals will have to maintain a certain amount of skeletal muscle. Although, it is energetically expensive to mobilize and then re-accrete tissue (NRC, 2001), utilizing muscle as an energy source in early lactation is an adaptation to the onset of lactation. Studies have shown that animals that lose more weight in early lactation have reduced reproductive performance (Buckley et al., 2003; Zachut and Moallem, 2017. However, these studies did not try to quantify weight loss as either muscle or adipose. Comparing high weight loss and low weight loss groups of cows, cows that mobilized more body tissue resulted in more days open and lower conception rates (Zachut and Moallem, 2017). More mechanistic approaches are being used to determine if there are relationships between fertility and the gene expression of enzymes related to the conversion of amino acids to glucose (Moran et al., 2016). Data suggest that cows with lower fertility began using body reserves for glucose production earlier than cows with higher fertility, potentially indicating that cows that mobilize more muscle will have reduced fertility. Before making recommendations to try to increase muscle mobilization, we need to have a better understanding of the potential negative impacts on reproduction.

Conclusions

Protein is mobilized from dairy cows prior to parturition to meet fetal amino acid requirements and for milk protein synthesis during colostrogenesis. Post-calving, protein continues to be mobilized for milk protein synthesis and for the production of ketones and glucose. While less is known about skeletal muscle mobilization than adipose tissue mobilization, ultrasound imaging and measuring proteolysis products (i.e., 3-methylhistidine) provides an indication of the extent and timing of protein mobilization. Preliminary work done in our lab indicates that there is a strong relationship between the amount of muscle and the extent that an animal will mobilize muscle. Similarly, there is also a strong relationship between the amount of back fat and the extent of back fat that will be mobilized through late gestation and early lactation. There is not a strong relationship between muscle depth and back fat thickness, indicating that cows mobilize the tissue that they have in excess. Although there are energetic considerations for muscle accretion, there may also be benefits to mobilizing muscle to meet glucose demands in early lactation rather than relying on adipose tissue mobilization. Certainly more work to better understand mechanisms that regulate tissue mobilization, as well as understanding of nutritional strategies that will influence muscle mobilization, are needed.

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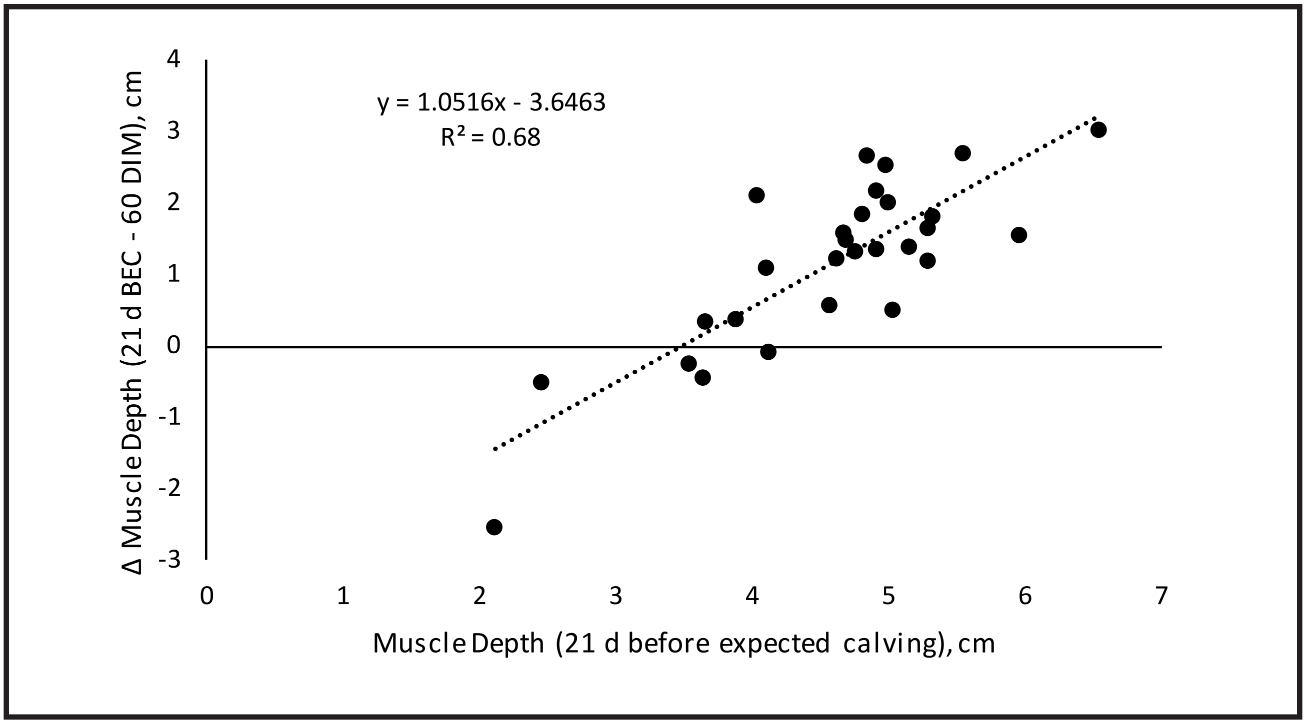

Figure 1. Muscle depth measured at the longismuss dorsi muscle at 21 days before expected calving by muscle mobilization measured from 21 days before expected calving (BEC) to 60 DIM. If values on the y axis are positive, it indicates that cows mobilized muscle from 21 d before expected calving to 60 days in milk.

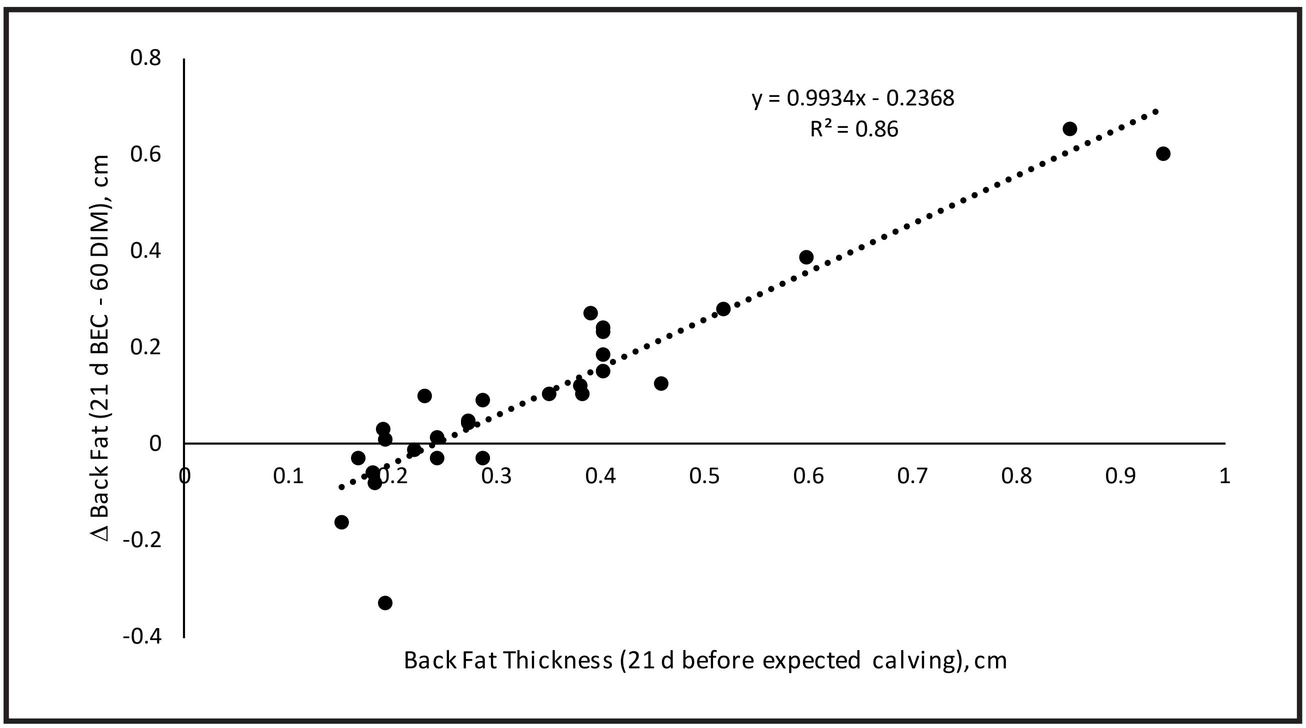

Figure 2. Back fat thickness measured above the longismuss dorsi muscle at 21 days before expected calving (BEC) by back fat mobilization measured from 21 days before expected calving to 60 DIM. If values on the y axis are positive, it indicates that cows mobilized back fat from 21 days before expected calving to 60 days in milk.

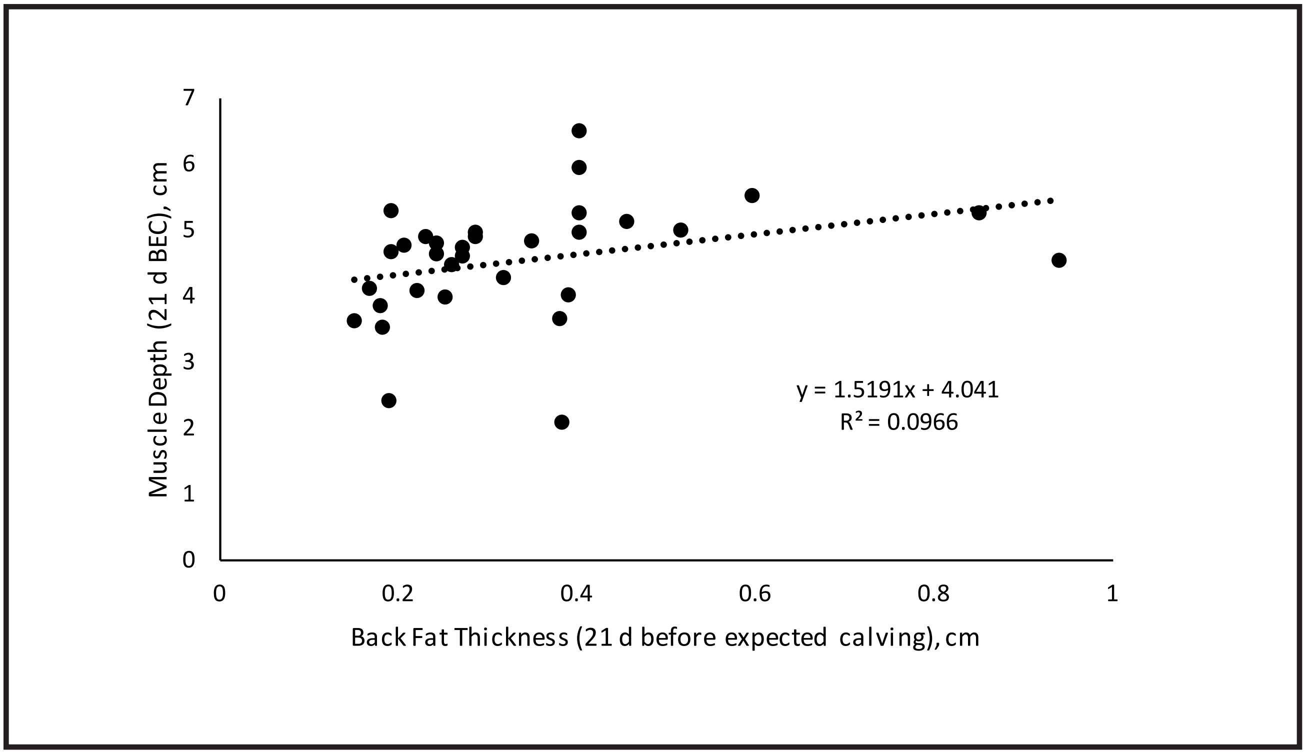

Figure 3. Relationship between muscle depth and back fat thickness measured from ultrasound images taken 21 days before expected calving (BEC) at the longissimus dorsi muscle.