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PHYSIOLOGY: *Invited Review*

Invited Review: Quantifying protein mobilization in dairy cows during the transition period

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ABSTRACT

Purpose: Published literature reporting protein mobilization measurements and potential methods to quantify protein mobilization were reviewed to provide information about the timing and extent of tissue mobilization for dairy cattle.

Sources: The main sources for information and data included in the review were peer-reviewed literature.

Synthesis: During late gestation, the dairy cow undergoes a homeorhetic change for protein metabolism by mobilizing muscle to meet AA and potentially glucose requirements of the fetus and uterus in addition to provide AA for body maintenance and colostrogenesis. The onset of lactation represents a large increase in the demand for AA to support milk production before sufficient dietary protein intake is able to meet those requirements. To meet AA demands in late gestation and early lactation, high-producing dairy cows mobilize muscle. Some studies suggest the extent of muscle mobilization of a cow exceeds 13% of body protein in well-fed transition dairy cattle and 25% in N-limiting diets; however, there appears to be large variation in the extent of protein that is mobilized.

Conclusions and Applications: There are several non-terminal approaches (e.g., ultrasound imaging and measuring metabolites from muscle degradation) to provide information about the extent of mobilization that allow us to evaluate how much protein is being mobilized and when mobilization occurs in a lactating dairy cow. Each of these techniques comes with their own tradeoffs but can ultimately provide information regarding protein mobilization. Further research will need to evaluate how these techniques can be combined to allow for both ease of use and accurate representation of the quantity of muscle present and therefore estimate the extent of mobilization over time.

Key words: muscle, tissue mobilization, ultrasound, 3-methylhistidine

INTRODUCTION

The transition from late gestation to early lactation is one example of a physiological shift known as homeorhesis (Bauman and Currie, 1980). Homeorhesis refers to the coordinated metabolic adaptation that occurs to support the priorities of the physiological state. Although multiple organs are involved in metabolic adaptation to initiate a successful lactation, some of the most significant changes occur in the mammary-gland, liver, adipose, digestive-tract, and muscle tissues. Although the onset of lactation involves coordinated changes in multiple tissues, this review will specifically focus on protein mobilization through the transition period. Specifically, we will discuss our current understanding of the requirements of protein precursors and nonterminal strategies to quantify the amount of protein mobilized during the transition period.

To understand the extent of protein mobilization, we must first quantify the amount of protein present in a dairy cow. Komaragiri and Erdman (1997) estimated that empty body protein, the protein component of the animal's weight minus gastrointestinal weight, before calving averaged between 12 and 13% of empty BW, representing 95 kg of protein in 760-kg cows. They also reported that cows mobilized in excess of 20 kg of protein from before calving to 5 wk postpartum. During late gestation empty body fat, the portion of empty BW constituting fat, represented a mean of 19 to 24% of BW, which was dependent on BCS and, therefore, s.c. fat. Cows were capable of mobilizing in excess of 80 kg of adipose tissue from before calving to 12 wk postpartum. In energy equivalents, the authors found the proportion of change in empty body energy was 0.93 and 0.07 for fat and muscle, respectively, and these proportions were maintained in cows of various body conditions.

Pool of Protein in the Dairy Cow

Muscle can be mobilized during late gestation and early lactation as a mechanism of normal metabolic adaptation for the onset of lactation. Paquay et al. (1972) estimated a 17-kg labile protein reserve in a nonlactating dairy cow when animals weighing 530 to 650 kg were subjected to limit feeding. Due to the metabolic demands of lactation, a lactating dairy cow may be able to mobilize an even

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larger amount of protein. Botts et al. (1979) conducted a trial limiting CP in early lactation until cows reached an N balance of 0 g/d. After depletion, cows were fed diets with varying levels of CP to measure the quantity of protein accreted. They found lactating dairy cows could mobilize approximately 25% of their total body protein. However, as will be discussed later, we expect cows fed higher CP diets would mobilize a smaller percentage of body protein. The BW of cows has increased over time, and Gibb and Ivings (1993) reported a positive linear relationship between body CP content and live weight of a dairy cow. An increased emphasis on frame size has increased CP content of the animal. Therefore, there are increasing MP requirements, especially in early lactation, to support increased body CP content and high levels of milk production in the modern dairy cow.

AA Metabolism

Mobilized muscle mass contributes free AA to the plasma pool, which can be used for several biological processes including milk protein synthesis, direct oxidation, or gluconeogenesis, among others. Evidence of metabolic adaptation to the onset of lactation is observed by an increased rate of proteolysis during the transition period. Greenwood et al. (2009) reported an increase in mRNA expression of ubiquitin in skeletal muscle as part of the ubiquitin-mediated proteolytic pathway after calving compared with before calving. This increase shows regulation of the proteolytic pathway in skeletal muscle and potentially identifies a pathway to modify protein mobilization. Ghaffari et al. (2019) evaluated skeletal muscle degradation and synthesis throughout the transition period in normal and overconditioned cows via gene expression of the ubiquitin-protease system and mammalian target for rapamycin (**mTOR**) pathway. Cows with BCS above 3.75 at dry off showed an increase in mRNA abundance of key components of both the mTOR and ubiquitin system, compared with thinner cows during the same time, suggesting greater protein turnover. Overconditioned dairy cows catabolize increased amounts of protein, as evidence of upregulation of key proteins of the ubiquitin-proteasome system, and prevent excessive skeletal muscle loss through increased anabolism, as evidence of increases in key components of the mTOR pathway. Thus, these data show catabolism and anabolism rates may vary in transition cows depending on body composition.

Specific AA have different fates within the periparturient cow. The AA alanine and glutamine have a preferential usage for pathways such as gluconeogenesis (Drackley et al., 2001). Moreover, compared with milk, skeletal muscle is lower in branched-chain AA (**BCAA**), which make up almost half of the EAA in milk. Therefore, skeletal muscle may be mobilized in excess to meet specific AA requirements for milk protein synthesis in early lactation. Kuhla et al. (2011) suggests that many of the EAA are reduced in plasma in early lactation compared with prepartum due to the relative increased uptake of some EAA by the mammary gland. Therefore, compared with prepartum, postpartum cows have an increase in NEAA concentrations in plasma. In a protein-deficient diet in early lactation, the mammary gland reduces the catabolism of certain EAA, instead using them for milk protein synthesis (Larsen et al., 2014). This stresses the importance of providing cows the appropriate balance of AA in early lactation to maximize milk protein synthesis and prevent excessive muscle mobilization.

Prepartum Use of AA

During the prepartum period, the dry cow is making necessary adjustments in AA utilization for the subsequent lactation and the developing neonate. These include the AA requirements of the developing fetus and for the proliferation of the mammary gland for its upcoming lactation demands.

From 190 d of gestation until parturition, the uterus increases in size, which coincides with an increase in the growth rate and CP requirements of the uterus. The CP requirement of the fetus and gravid uterus increases exponentially over the last trimester (Bell et al., 1995). To compare, at 190 d of gestation, CP requirements are 62 and 36 g/d for the uterus and fetus, respectively, whereas at full term, the CP requirements are comparable for the uterus and fetus, 117 and 110 g/d, respectively. Likewise, during the last weeks of gestation, the mammary gland undergoes development of the mammary secretory cells and colostrogenesis to prepare for the upcoming lactation. Based on work done by Capuco et al. (1997) evaluating mammary development during the dry period, Bell et al. (2000) estimated 120 g/d MP is required during the last 3 wk of gestation for increased epithelial cell turnover in the mammary gland. This would be in addition to MP requirements for milk protein synthesis in colostrum, which are dependent on quantity and quality of colostrum produced.

Postpartum Use of AA

In the transition to lactation, there are heightened demands for AA for processes to support the high milk yield of the dairy cow. During this homeorhetic transition, several tissues have an increased AA demand to reach their heightened physiological state. Using data from 5 production studies in early lactation, Tamminga et al. (1997) reported mobilization and retention of body reserves with assumptions for gut fill factor and protein:water ratio. Based on the calculations they used, protein mobilization was greatest for the first week of lactation, and cows transitioned from net mobilizers of protein to net retainers of protein by wk 5 of lactation. Even with a relatively large number of cows per week (range n = 76 to 219), the SD reported indicates there was significant variation between cows, with some cows even retaining muscle in the first few weeks of lactation. Gibb et al. (1992) performed a

serial slaughter study from 0 to 29 wk postpartum with empty BW reducing by 1.1 kg/d over the first 8 wk of lactation. Only 6.6% of the CP pool was mobilized during this 8-wk period, representing 0.123 kg/d CP depletion from the carcass and net accretion in the liver and digestive tract. These values for CP mobilization are lower than others have reported (Botts et al., 1979; Komaragiri and Erdman, 1997) and thought to be because of higher CP concentration in diets reducing protein mobilization or differences in milk production between studies.

The discussion to follow will show the various areas that have heightened AA requirements during the postpartum period. Over the transition period, the digestive tract increases in size and mass to meet the transition from the low energy dry cow diet to the high intake of conventional farm lactation diets (Johnson et al., 1990). In a study by Reynolds et al. (2004), the investigators slaughtered multiparous cows at various time points over the periparturient period to measure changes in gastrointestinal tract weight. They observed the overall weight of the cow stomach does not significantly change from 21.32 kg at 21 d before parturition to 22.21 kg at 22 DIM. However, relative to other compartments of the stomach, the reticulorumen gained a significant amount of tissue, from 12.25 to 14.20 kg between 21 d prepartum and 22 DIM. They observed a trend for increased liver mass through the transition period; the liver weighed 9.00 kg at 21 d before calving and 9.59 kg at 22 DIM.

Meanwhile, the intestines undergo homeorhetic tissue growth with the shifting nutrient demands of the animal. Overall, the length of the small intestine remained largely unchanged during the transition period, yet there is a significant gain in tissue mass of the small intestine (8.87 vs. 9.54 kg) and a trend for increased mass of the large intestine (4.87 vs. 5.94 kg) from 21 d prepartum to 22 DIM (Reynolds et al., 2004). Similarly, Andrew et al. (1994) performed a serial slaughter study to investigate changes in chemical composition across stages of lactation. They reported that although the total body protein remains largely unchanged from approximately 7 d prepartum to early lactation (mean = 63 DIM), there is a change in the distribution of the protein. Compared with prepartum cows, early lactation cows have an increase in protein in the gastrointestinal tract and less protein in the carcass. Using the values for changes in CP in the gastrointestinal tract from prepartum to early lactation (Andrew et al., 1994) and conversion values from dietary CP to body protein from the NRC (2001), we calculated the approximate AA requirements for the growth of the gastrointestinal tract to be 50.6 g/d from 7 d prepartum to approximately 60 DIM.

Postcalving cows are in a negative MP balance, because their protein consumption levels are outpaced by milk protein output. Based on calculations of individual cows, the negative MP balance lasted more than 21 DIM (Bell et al., 2000). One of the reasons for this may be high-producing dairy cows in early lactation will not consume adequate DMI to produce enough glucose precursors. One metabolic adaptation the dairy cow may use to make up for this gap is to convert more AA into glucose. Due to being in a negative MP balance and not producing sufficient propionate to support large amounts of milk production, muscle becomes an important source for supporting AA requirements in early lactation. To adapt to the increasing demands of glucose after calving, the dairy cow has an increased ability to convert AA to glucose postpartum compared with the prepartum state (Overton et al., 1999). All AA except for leucine and lysine, which are strictly ketogenic, can be used for glucose synthesis at varying rates. Alanine and glutamine are the AA that have been found to be used in the greatest quantity for gluconeogenesis (Drackley et al., 2001). Although AA can be glucogenic, a vast majority of the glucose derived during early lactation is from gluconeogenesis from lactate-derived carbons (Larsen and Kristensen, 2013). Therefore, there is likely a relatively minimal amount of AA used for glucose synthesis in early lactation; instead, AA are used preferentially for protein anabolism.

The reduction in circulating insulin concentrations and IGF-1, as well as insulin resistance in insulin-sensitive tissue, promotes the mobilization of AA in early lactation (Bell et al., 2000; De Koster and Opsomer, 2013). The increased demand and lack of dietary AA requires protein mobilization, which leads to AA mobilization from muscle and the subsequent increase in concentration in plasma during the transition from late gestation to early lactation. Several EAA are in lower concentration in plasma the day immediately following parturition, including all BCAA and gluconeogenic AA, indicating their uptake from the blood to meet the requirements for lactation (Kuhla et al., 2011; Zhou et al., 2016). Based on the AA composition of milk reported by Waghorn and Baldwin (1984), EAA represent almost half of the total AA in milk protein. The BCAA comprise over 44% of the EAA in milk; however, they are lower in muscle protein, representing 35% of the total EAA in muscle (Doepel et al., 2004; Table 1). Many EAA are taken up by the mammary gland in excess of milk output (Larsen et al., 2014). Generally, BCAA and arginine are extensively catabolized in the mammary gland, although Leu and Ile also play a role in mTOR regulation for protein synthesis in the mammary gland (Appuhamy et al., 2012). Other AA that are important regulators of gene transcription and translation, which include glutamine, glutamate, and remaining NEAA, are synthesized in the mammary gland (Rezaei et al., 2016). Although muscle is an important AA source to support milk protein production in early lactation, the differences in AA profile between milk protein and skeletal muscle protein may lead to greater than expected mobilization to support milk protein when dietary protein intake is insufficient.

In 2 studies conducted by Larsen et al. (2014, 2015), casein or a mixture of AA similar to the profile of casein were abomasally infused into cows from 1 to 29 d after parturition. They reported an over 7 kg/d increase in milk

Table 1. Amino acid composition of EAA in milk and skeletal muscle of dairy cattle

AA (g/100 g of AA)	Empty body AA[1]	Milk protein AA[2]	Ratio of empty body AA/milk protein AA
Arg	7.3	3.40	2.15
His	2.7	2.74	0.99
Ile	3.1	5.79	0.54
Leu	7.4	9.18	0.81
Lys	7.0	7.64	0.92
Met	2.2	2.71	0.81
Phe	3.9	4.75	0.82
Thr	4.3	3.79	1.13
Trp	0.8	1.51	0.53
Val	4.4	5.89	0.75

[1]Reported by Doepel et al. (2004; adapted from Williams, 1978; Rohr and Lebzien, 1991; and Ainslie et al., 1993).
[2]Waghorn and Baldwin (1984).

production per day when AA were infused in both studies. The authors initially speculated that increased AA supply would prevent the negative protein balance usually observed right after parturition (Larsen et al., 2014). However, when more AA were supplied through AA infusions postpartum, there was no change in MP balance due to an increase in milk protein synthesis. Contrary to initial speculation, increased supply of AA postpartum may lead to additional mobilization of protein, especially in the first week following parturition due to increased utilization of AA by the mammary gland (Larsen et al., 2015). Results from these 2 early lactation studies make us consider whether feeding diets higher in MP, rather than infusions of AA with a profile similar to milk protein, would increase milk protein synthesis without considerably changing MP balance.

As estimated by Bell et al. (2000), as much as 1 kg of muscle may be mobilized to support milk production in the first 7 to 10 DIM. The AA liberated from muscle can be used for the synthesis of milk protein as well as precursors for gluconeogenesis required for copious milk production. When calculating the MP balance of cows in early lactation, researchers estimated that the nadir of MP balance occurs around 7 DIM and was −600 g/d (Bell et al., 2000). Moreover, these estimates of muscle mobilization to meet AA requirements in early lactation are more than 2 decades old. In the past 20 yr, annual milk production per cow in the United States has increased by 2,705 kg (5,963 lb, 1998 compared with 2018 milk per cow data) or an almost 35% increase in milk production per cow from 1998 (USDA, 2019). Therefore, we must formulate early-lactation diets to minimize negative MP balance and take advantage of these gains in milk protein synthesis and production achieved over time.

Ways to Measure Protein Mobilization

Deuterium Oxide. The gold standard for determining empty body composition is to directly measure protein after slaughter of several animals over the periparturient period. However, this method requires significant animal slaughter and is labor intensive; therefore, we will discuss several alternatives to measuring protein content that could be applied to measure protein mobilization over the transition period. Injections of deuterium oxide have been used to predict body composition in animals. Deuterium oxide (D_2O) infusion into the jugular vein followed by serial blood sampling results in D_2O dilution space and can be used to predict body composition and energy consumption (Odwongo et al., 1984; Andrew et al., 1995; Brown, 2013). Andrew et al. (1995) compared mature Holstein cows at different stages of lactation and found D_2O dilution could estimate body protein from empty BW with less than 5 kg of error. Odowongo et al. (1984) developed a series of equations to predict total BW, empty BW, body protein, and body fat using D_2O space based on results from actual measurements at slaughter. These equations were developed across growing animals as well as animals at different stages of lactation. Two studies have previously looked at using this method for measuring changes in empty body protein, energy, and fat in cows supplemented with protein or fat (Komaragiri and Erdman, 1997, Komaragiri et al., 1998). In both studies cows underwent D_2O analysis at −2, 5, and 12 wk relative to parturition. The authors found that the greatest changes in body protein and fat occurred between 2 wk prepartum and 5 wk postpartum. The same cows continued to lose body fat weight from 5 to 12 wk in lactation, but there was no additional loss of protein at 12 wk. This indicates that protein mobilization occurs for a shorter period of time after parturition than fat mobilization, but given the limitation of sampling time points, the exact point protein mobilization ends could not be confirmed from these studies.

The equations derived from D_2O space in previous studies have been used to predict body composition; however, it is uncertain how sensitive the values would be due to changes in gut fill, body condition, and whether changes over short periods of time could be detected. Additionally, the cost and amount of D_2O required to predict body composition may be prohibitive for use on transition dairy cows. Therefore, due to these variable measurements, additional costs, and the large amount of labor required to perform these experiments, it is impractical to perform this method on a large number of cows.

Metabolic Indicators. From a metabolic point of view, changes in creatinine concentration can be used to determine proteolysis around parturition. Creatinine is a waste product produced by muscle from the nonenzymatic breakdown of creatine and phosphocreatine at a relatively constant rate and can be used as an indicator of muscle mass (Wyss and Kaddurah-Daouk, 2000). Increases in creatinine concentrations indicate an increase in muscle

mass or muscle accretion, whereas decreases in creatinine concentrations indicate a reduction in muscle mass or muscle mobilization. Concentrations of creatinine can be determined from urine, plasma, or serum samples. In urine, creatinine levels appear to have significant diurnal variation, indicating that timing of sampling significantly effects results, and spot sampling may not be appropriate to determine creatinine concentrations (Shingfield and Offer, 1998). Cows across BCS showed reduced plasma creatinine concentrations from 4 wk prepartum to 7 wk postpartum, showing reduced muscle mass from before to after parturition (Pires et al., 2013). In addition, cows at lower BCS (2.33) had lower creatinine concentrations before calving than cows with medium or high BCS (3.13 and 4.17, respectively), indicating that cows with low body condition also had less muscle mass.

During actin and myosin degradation, the by-product 3-methylhistidine (**3-MH**) is produced and serves as a known indicator of muscle mobilization (Chibisa et al., 2008). Concentrations of 3-MH are excreted at a rate relative to muscle breakdown; at times when muscle catabolism is elevated, increases in 3-MH are observed. When analyzing for 3-MH, it is important to be able to separate out 1-methylhistidine from 3-MH (Houweling et al., 2012). The former may be difficult to differentiate from 3-MH because of their similar chemical properties but is not thought to be specifically related to protein mobilization. Being unable to separate out methylhistidine products would result in elevated and inaccurate values for 3-MH and would not accurately represent protein degradation.

To correct for differences in muscle mass between cows and between stage of gestation or lactation, using the ratio of 3-MH to creatinine allows the comparison of protein mobilization per unit of muscle mass. Muscle mobilization measured as 3-MH and the ratio of 3-MH:creatinine were both elevated in the first 2 wk before calving compared with 4 wk before calving and 7 wk after parturition (Pires et al., 2013). It is important to note that creatinine concentrations are weakly correlated with muscle depth at any given time point (Megahed et al., 2019). A better way to approximate the amount of protein mobilization is through assessing 3-MH concentrations over time to track the changes in empty body protein. This method requires access to laboratory analysis, but in today's commercial herds, we must use a technique that can rapidly assess protein mobilization in several animals.

Quantifying Muscle Mobilization—Ultrasound

As mentioned before, during the transition period, the body's protein requirement is larger than its potential protein intake. Quantifying the amount of lean muscle cows mobilize during this time can help determine the optimal level of mobilization for production and health purposes. As a proxy for s.c. fat, BCS are used to assess changes in energy balance (Wildman et al., 1982) as they have been shown to be correlated with backfat thickness and whole-body fat content (e.g., Bullock et al., 1991; Domecq et al., 1995). However, while this scoring system can be easily implemented at the herd level, it cannot directly quantify the amount of protein mobilized and is potentially subjective due to differences in the individual scoring cattle (Edmonson et al., 1989). Alternatively, ultrasonography can be used to determine both backfat thickness and muscle depth with 2 common areas measuring fat thickness above the longissimus dorsi muscle (Figure 1) and the gluteus medius (Schroder and Staufenbiel, 2006). A display of an ultrasound image from above the longissimus dorsi muscle is shown in Figure 2. In other production animals, ultrasonography is used to assess carcass traits before slaughter or for breeding stock. For example, in Angus steers, ultrasound images were taken within 5 d before slaughter and then compared with carcass measurements. Correlation coefficients between ultrasound measurements taken between the 12th and 13th rib above the longissimus dorsi muscle were 0.89 for carcass fat and 0.86 for LM area (Greiner et al., 2003). However, the results do not necessarily quantify protein mobilization, but an approximation of protein mobilization can be inferred by comparing sequence scans over time to track the change in muscle depth during the periparturient period.

Regression equations from ultrasound measurements of dairy cattle across DIM showed that across different breeds of cattle, muscle is lost over the length of the transition period and the smallest LM area was found after the conclusion of the peripartum period between 30 and 60 DIM (Sloniewski et al., 2004). van der Drift et al. (2012) used ultrasound measurements to record changes in muscle depth from late gestation to early lactation. They observed a reduction in muscle depth starting in the last weeks of gestation through the first weeks of lactation, and the smallest LM thickness was observed between 4 to

Figure 1. Image of the location and orientation of a 17-cm linear array 3.5-MHz transducer used to measure the longissimus dorsi depth and backfat thickness above the 12th intercostal space.

8 wk of lactation. Cows mobilized on average 0.74 cm of muscle depth or 18% of their muscle depth from 4 wk before calving to 8 wk after calving with significant variation between cows, depending on muscle depth in late gestation. Interestingly, cows with more muscle depth mobilized a greater amount of muscle, whereas cows with more backfat at the start of the trial mobilized more backfat from 4 wk before calving to 8 wk postpartum (van der Drift et al., 2012). Ultrasound images of the longissimus dorsi muscle are noninvasive and can be performed quickly on dairy cattle at multiple time points over the transition period. However, they do not allow quantification of body protein mobilization and instead should be viewed as a tool to compare changes in muscle depth over time and between treatments.

Future Research Needed

There is no ideal method for quantifying protein mobilization; however, utilizing some of the methods discussed will increase our knowledge of the timing and extent of protein mobilization through the periparturient period. Ideally, we would like to know the extent of mobilization from protein and the terminal use of those AA so that we can provide the optimal AA to transition dairy cows. Potential first steps are to provide nutritional modifications during the periparturient period to determine their effects on milk production and extent of muscle degradation. Incorporating methods that measure protein degradation, changes in muscle mass, or changes in muscle depth into transition-cow studies will allow us to understand in greater depth how the dairy cow responds to dietary treatments or environmental conditions. Work in this area has the potential to improve milk production and animal health by providing additional information about of the use and requirements for AA by the periparturient cow. Future research focused on determining whether there is a genetic component to the extent and timing of protein mobilization may also be warranted.

APPLICATIONS

Cows begin to mobilize protein in late gestation due to AA demands for fetal growth, mammary gland development, and colostrum synthesis, as well as decreased AA intake in the days before parturition. After calving, protein continues to be mobilized for milk protein synthesis and to produce energy sources such as glucose and ketone bodies. Although less is known about muscle mobilization than adipose tissue mobilization, ultrasound imaging and measuring proteolysis products (i.e., 3-MH and creatinine) provide an indication of the extent and timing of protein mobilization. There is limited evidence that a relationship exists between the amount of protein present and the extent of protein mobilization. An important consideration for formulating diets for dairy cows is to consider both the accretion of protein as well as the mobilization of body protein. Any protein mobilized during the transition period is likely re-accreted at some point during lactation or the early dry period. Although there are energetic considerations for muscle accretion, there may also be benefits to mobilizing muscle to meet AA and glucose demands in early lactation rather than relying on adipose tissue mobilization as an energy source. More work to understand the mechanisms that regulate tissue mobilization as well as to understand nutritional strategies that will influence muscle mobilization are needed to help drive positive animal health and performance outcomes.

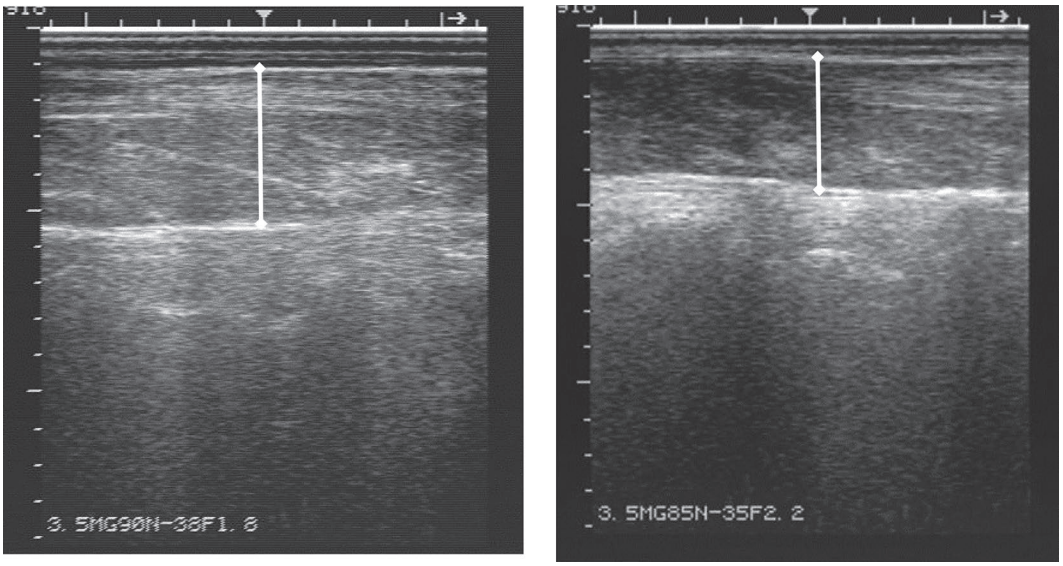

Figure 2. Ultrasound images of the longissimus dorsi of 2 different cows of differing muscle depth. The image on the left represents a cow with 4.33 cm of muscle depth, shown by the white vertical line. The image on the right represents a cow with 3.63 cm of muscle depth, shown by the white vertical line. Ultrasound images were captured using an Aloka SSD 500V Ultrasound (Corometrics Medical Systems, Wallingford, CT).

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