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Postpartum longissimus dorsi muscle loss, but not back fat, is associated with resumption of postpartum ovarian activity in dairy cattle

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ABSTRACT

The objectives of this observational cohort study were to assess the effect of body condition score change, back fat depth change, and muscle diameter change on the time to commencement of luteal activity and first estrus in commercial pedigree Holstein cows. A total of 140 of 200 commercial pedigree Holstein cows were enrolled in one dairy herd in Somerset, UK, over 52 wk in 2021 to 2022. The herd used 4 automatic milking machines with in-line progesterone measurement capability to determine commencement of luteal activity and time to first estrus. Cows were followed until at least 60 d postpartum, and milk progesterone was measured daily starting from 10 DIM. Body condition scoring and ultrasound measurements of back fat depth and longissimus dorsi muscle diameter were performed on cows twice, within 7 d of both calving and 60 DIM. Other explanatory variables assessed included parity, 60-d and 305-d milk yield, and subclinical ketosis (β-hydroxybutryate ≥1.2 mmol/L). Occurrence of clinical disease <60 DIM was forced into all models as a binary variable. Data were analyzed using multivariable Cox proportionate survival analyses. Muscle loss was associated with commencement of luteal activity and time to first estrus. A reduction in muscle diameter by 1.5 to 5 mm was associated with the shortest time to the start of luteal activity and first estrus. A reduction in muscle diameter >8 mm was associated with the longest times to luteal activity and first estrus. In addition to being affected by muscle loss, commencement of luteal activity was delayed by subclinical ketosis, clinical disease, and failure to gain body condition to 60 DIM. Cows that had a BCS loss of 0.25 or more between calving and 60 DIM were at least 52 ± 22% less likely to have commenced luteal activity compared with those that gained BCS. Interestingly, cows that had no change in body condition score commenced luteal activity later than those that gained body condition score. Muscle loss was associated with time to first estrus irrespective of clinical disease status. Cows that lost >8 mm of muscle diameter showed estrus behavior later than cows that lost 1.5 to 5 mm. In conclusion, our findings indicate that extensive muscle loss postpartum was associated with a delayed start to luteal activity and first estrus, irrespective of body condition change, clinical disease, and subclinical ketosis. Marginal muscle loss and a gain in body condition, however, were associated with an earlier start to luteal activity and first estrus.
Key words: fertility, dairy cattle, muscle, fat

INTRODUCTION

Body condition scoring has become a key tool in the management of dairy herds due to its association with negative energy balance (**NEB**) and metabolic disease in early lactation (Schröder and Staufenbiel, 2006). Changes in BCS are widely used as a practical tool in dairy farm management due to their ease of use, association with production diseases, and potential detrimental effect on fertility (Pires et al., 2013). Loss of BCS during the periparturient period has been found to negatively affect fertility and to increase nonesterified fatty acid (**NEFA**) and BHB concentrations in serum (Santos et al., 2009; Barletta et al., 2017). Cows that experienced a BCS loss postpartum had a 40% decline in first service conception rate when compared with cows that did not suffer as severe a BCS loss (Butler and Smith, 1989). Conversely, a gain in BCS during the transition period had positive effects on fertility, including an earlier return to normal cyclicity, reduced health problems, and lower circulating serum concentrations of NEFA and BHB (Barletta et al., 2017).

We know less, however, about the role of muscle tissue in NEB and its association with measures of fertility (Mann et al., 2016). Muscle tissue breakdown appears to start around calving before the mobilization of adipose tissue (van der Drift et al., 2012), but the physiological benefits and metabolic process are unclear. Leaner cows that lost BCS in the postpartum

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period had increased plasma indicators of protein mobilization due to the relative lack of adipose tissue (Pires et al., 2013). Any loss of muscle mass would inevitably affect the BCS of the individual and so would be important to quantify along with adipose tissue during the periparturient period. Findings by Pires et al. (2013) have recently been supported by Hatfield et al. (2022) in which those animals with a lower BCS precalving lost more muscle tissue and also had an increased time to first service.

The utility of monitoring dairy cattle BCS to optimize reproductive success has been well described (Barletta et al., 2017), but the inclusion of methods to monitor back fat and muscle changes has not. Ultrasonic measurement of longissimus dorsi diameter and back fat depth has been described as an accurate method to evaluate body tissue mobilization during the periparturient period (Megahed et al., 2019). The goal of our study, therefore, was to characterize the effects of BCS change, muscle diameter change, and back fat depth change on time to commencement of luteal activity and first estrus. These fertility outcomes were determined by a rise or fall of milk progesterone concentrations, respectively, and indicate the resumption of ovarian activity postpartum. The ability to resume normal estrus cycles early postpartum is necessary for farms aiming to achieve a calving interval of one year (Butler, 2003), which is considered economically optimal for many farms (Inchaisri et al., 2010).

MATERIALS AND METHODS

Animals

The study was approved by the University of Liverpool Veterinary Research Ethics Committee (Reference Number: VREC1060). This observational cohort study enrolled all animals calving on one pedigree Holstein dairy farm over a period of 12 mo in Somerset, UK. All cows were eligible to be enrolled in the study, thereby minimizing selection bias. A sample size calculation estimated that 155 cows were needed at 80% power to detect a delay of 3 DIM to time to luteal activity in cows that lost excessive BCS (≥1 BCS) from calving to 60 DIM relative to cows that did not lose as much BCS. A standard deviation of 10 DIM and 11% of the herd were assumed to have excessive BCS loss. The herd consisted of 204 dairy cows milked an average of 2.7 times per day by one of 4 automatic milking systems. All cows were housed in a single freestall barn and produced high quality milk for a supermarket processor. Lactating cows were fed a TMR consisting of grass silage (5.8 kg DM/d), maize silage (4.4 kg DM/d), concentrate blend (4 kg DM/d), canola meal (3.5 kg DM/d), rolled wheat (3.5 kg DM/d) and minerals (0.3 kg/d). This provided a diet of 10.2 MJ/kg ME and 17.7% CP. Shortfall in energy requirements based on yield was made up by concentrate fed in the automatic milking machines. All cows <60 DIM were fed 7 kg DM of concentrates per day during milking. From 60 DIM, cows were fed concentrates during milking on a linear scale according to milk yield with cows producing 44 L of milk per day receiving the maximum of 8 kg of concentrates per day. Close-up dry cows (<2 wk from calving) were housed in loose straw yards in small groups of up to 4 cows in each bay (dimensions 4.6 × 14 m) and far-off dry cows were loose housed. Cows were dried off approximately 55 d before calving and were fed a TMR of 4.7 kg DM/d maize, 3.1 kg DM/day hay, 2.8 kg DM/d oat straw, 2.4 kg DM/d of canola meal, 0.2 kg/d of magnesium chloride and 0.2 kg/d of a dry-cow mineral. This provided a diet of 9.3 MJ/kg ME and 13.3% CP.

Progesterone (P4) and BHB were measured using a commercially available in-line milk analysis system [Herd Navigator (**HN**), DeLaval, Tumba, Sweden]. The system automatically samples an individual cow's milk at intervals based on prior results and stage of lactation as per the biometric model. The pooled milk samples from the individual cow are then mixed before a representative subsample of milk is collected for analysis in the unit directly on the farm. The P4 concentration is measured using a dry-stick enzyme immunoassay technique (Pemberton et al., 1998). The raw measures are converted into smoothed P4 concentrations based on a bio-model that reduces random noise attributable to environmental conditions (Friggens and Chagunda, 2005). This system was used to determine the days from calving to commencement of luteal activity (smoothed milk P4 concentrations ≥3 ng/mL for 2 consecutive days), first estrus event (time to first estrus defined as previous day P4 >5 ng/mL followed by a next day P4 <5 ng/mL) and maximal P4 concentration in the first luteal phase. Start of luteal activity and first estrus were mutually exclusive events based on the algorithm. Estrus detection using this system has a sensitivity of 93% and specificity of 94% (Friggens et al., 2008). Samples for milk BHB were taken once daily following parturition until 60 DIM and the raw values smoothed. Progesterone measurements were taken from 10 DIM on a set sampling frequency to assess cyclicity. Sampling continued on d 5, 9, 14, and 30 after insemination and continued for 25 d to detect a sustained elevation of P4 (not <5 ng/mL) consistent with a continued pregnancy. Pregnancy was confirmed by the author (JR) using transrectal ultrasonography

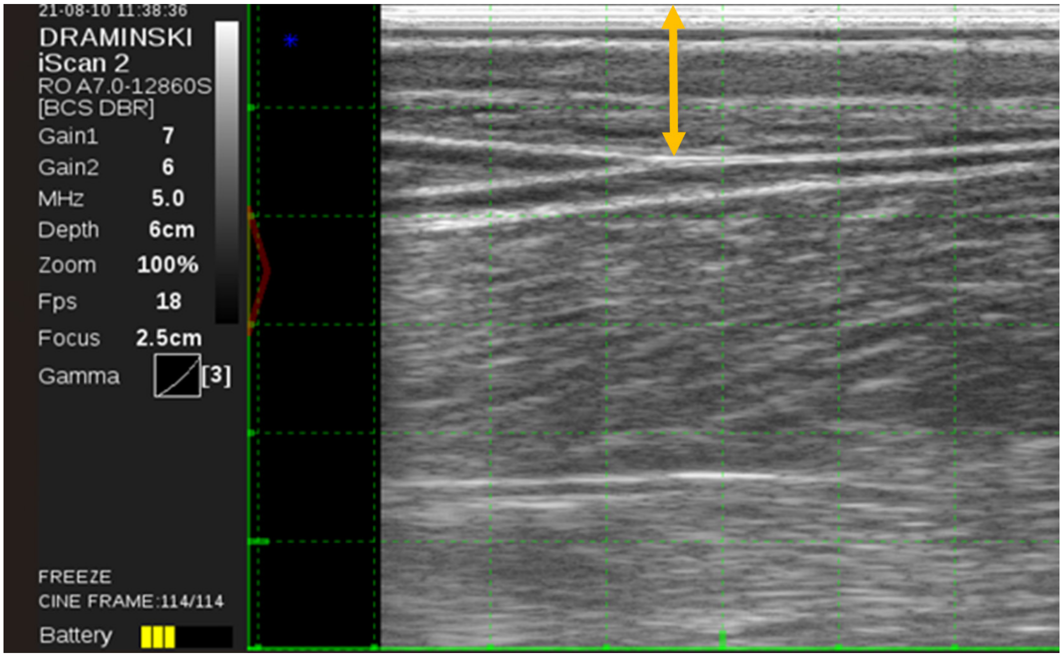

Figure 1. Ultrasound image showing location (arrow) of back fat depth measurement taken between biceps femoris and gluteus medius.

from 30 d postinsemination. Milk P4 measurement was automatically performed at d 100 and 200 after insemination using in-line testing.

Data Collection

Weekly farm visits were carried out by the author (JR) to collect all measurements except those obtained by the HN system. All cows in the study were individually assessed for BCS within 7 d of their calving due date and within 7 d of reaching 60 DIM according to the technique described by Edmonson et al. (1989), using a 5-point scale at 0.25 increments. Cows that calved more than 7 d before their expected calving date were scored within 7 d after calving. At the same time as the BCS assessments, each animal also had ultrasound measurements of back fat depth and muscle diameter. Back fat was measured at the muscle juncture between the biceps femoris and the gluteus medius (Figure 1) using the method described by Schröder and Staufenbiel (2006). Longissimus dorsi muscle diameter was measured at the level of the transverse process of the fourth lumbar vertebra (Figure 2) according to the technique described by van der Drift et al. (2012).

Ultrasound measurements were performed on the right side of the animal using a scanner (Draminski iScan2, Poland) with a linear transducer (7.0 MHz L60, Draminski). Before measurement the hair was brushed clean but not clipped, and sprayed with a mixture of 70:30 ethanol:water before a liberal amount of ultrasound gel was applied (Aquasonic 100 Ultrasound Transmission Gel). The following settings were preprogrammed into the scanner: 5 MHz frequency, 6 cm depth, 18 frames/second, and 2.5 cm focus. Three images per site were saved for subsequent measurement and analysis.

Longissimus muscle diameter was measured at the transverse process of the fourth lumbar vertebra with the linear transducer placed perpendicular to the vertebral column. The largest diameter of the muscle between the fascial planes at this point was measured. Subcutaneous back fat depth was measured at the pelvic region using the tuber ischii, tuber sacrale, and greater trochanter of the femur as landmarks. The linear transducer was placed parallel to the vertebral column one hands breadth before the tuber ischii, above the greater trochanter in the region of the thurl. A triangular shaped structure representing the meeting of the fascia of the gluteus medius and biceps femoris was used as the landmark for measurement.

Cows were classified into 3 groups based on BCS at the point of calving: thin (<2.75), moderate (2.75–3.5) and fat (≥3.75) as in (Garmo et al., 2009a) and (Pires et al., 2013). Four categories of BCS loss that occurred between calving and 60 DIM were used as previously described by others (Chebel et al., 2018; Gobikrushanth et al., 2019): excess loss as category 3 (≥0.75 loss), moderate loss as category 2 (≥0.25 ≤ 0.5 loss), no change as category 1, or gain as category 0 (≥0.25 gain). Changes in back fat depth and muscle diameter were categorized

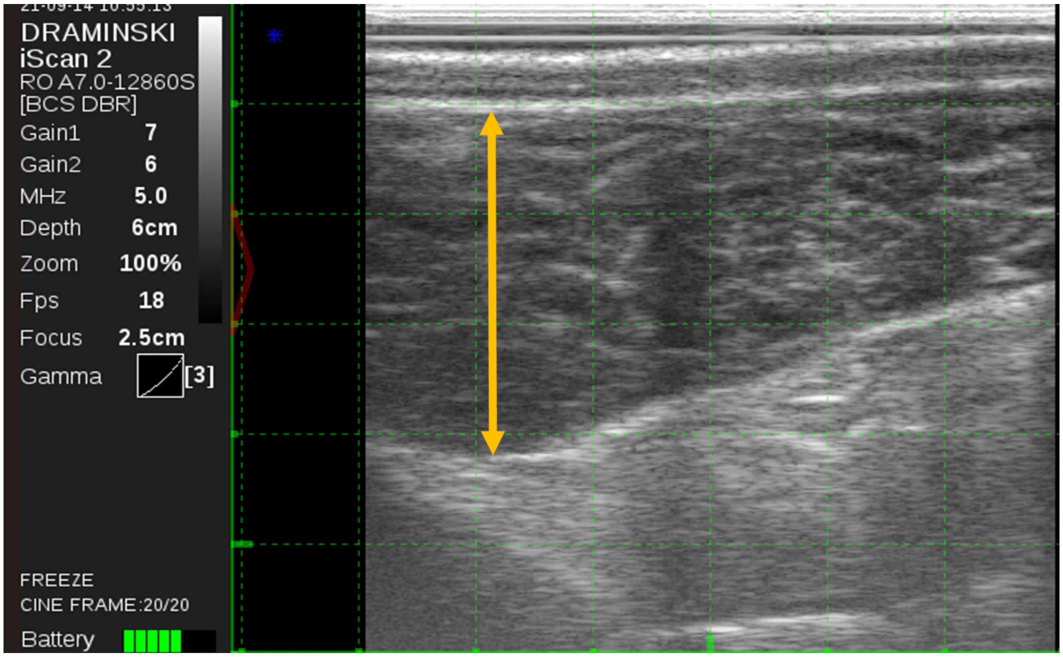

Figure 2. Ultrasound image showing location of longissimus dorsi muscle diameter measurement (arrow).

into quartiles. Back fat depth change categories were as follows: category 0 (>0 mm gain), category 1 (≥0 to <2 mm loss), category 2 (≥2 to <4 mm loss), and category 3 (≥4 mm loss). Muscle diameter change categories were as follows: category 0 (<1.5 mm loss), category 1 (≥1.5 to 5 mm loss), cateogry 2 (≥5 to <8 mm loss), and category 3 (≥8 mm loss). These classifications were done to enable comparison of the differences between groups relative to baseline on time to first estrus and commencement of luteal activity.

Back fat depth and muscle diameter were measured using imaging analysis and measurement software (ImageJ, LOCI, University of Wisconsin), taking a geometric mean average of the 3 images obtained from each site (back fat and muscle) to minimize measurement error. The same assessor performed all on-farm measurements to avoid confounding associated with assessor. The assessor recorded BCS on the day of the evaluation, but to reduce the potential for recall bias inherent in our study design, the assessor did not view the records again until the end of the study. The same assessor also evaluated all ultrasound images at the end of the study.

Progesterone Sampling

Commencement of luteal activity was defined as whole milk P4 concentrations ≥3 ng/mL for 2 consecutive days but not earlier than 10 DIM (Garmo et al., 2009a), which was when HN commences in-line milk P4 sampling. Estrus (Heat Alarm in HN) was defined within HN as a previous day high P4 (>5 ng/mL) followed by a next day P4 <5 ng/mL, which triggered an estrus alarm in HN. The first estrus alarm was recorded as the first estrus postpartum for each cow. The estrus alarm within HN signals the onset of estrus, not ovulation, and informs the HN bio-model P4 sampling regimen by acting as an anchor point for the next expected estrus event.

The start of P4 sampling was set to 10 DIM specifically for the purpose of this study, rather than the customary 20 DIM. Sampling for cows more than 90 DIM reverted to the normal HN sampling protocol. Luteal activity was detected before an estrus alarm being triggered in HN (Figure 3), indicating that cows were commencing luteal activity before estrus as would be expected.

Variables of Interest

Milk BHB was measured daily for the first 60 DIM of each cow's lactation. A ketosis alarm was recorded in HN in the first 60 DIM when milk BHB levels ≥0.08 mmol/L, which are equivalent to blood levels over 1.2 mmol/L, a commonly accepted threshold for subclinical ketosis. This is a lower milk BHB threshold than the 0.15 to 0.19 mmol/L thresholds that have been proposed by others (Koeck et al., 2014; Santschi et al., 2016). Cows were recorded as having ketosis if they experienced at least one ketosis alarm in the first 60 DIM

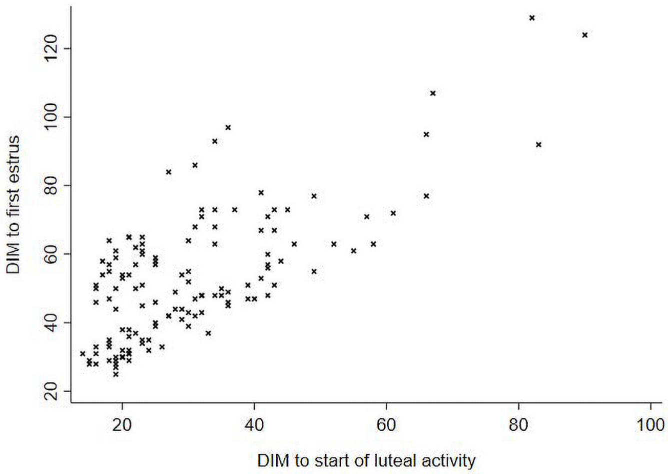

Figure 3. Scatterplot of DIM to first estrus against DIM to commencement of luteal activity.

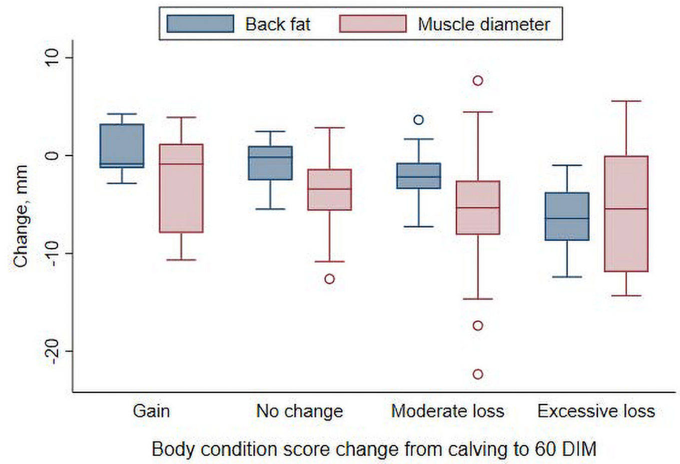

Figure 4. Boxplot showing the relationship between change in back fat depth and longissimus dorsi muscle diameter and the category of BCS change, defined as gain of $\geq$0.25, no change, moderate loss of $\geq$0.25 but $\leq$0.5, or excess loss of $\geq$0.75 BCS. From inside to outside, the horizontal lines represent the median, upper (75th percentile) and lower (25th percentile) quartiles, and maximum and minimum values within 1.5 times the interquartile range of the nearer quartile, respectively. Circles represent outliers: observations occurring over 1.5 times the interquartile range away from the nearest quartile.

and recorded as a variable of interest. At this point HN calculates a ketosis risk percentage for each cow to aid the farmer in decision making for treatment.

Diseases encountered in the study were recorded and scored according to the farm's data recording methods. Cows were treated as per the treatment protocols in the herd health plan. Other data relating to the cow collected included parity and 305-d lactation yield (predicted or realized where complete).

Statistical Analysis

All data were stored securely on the University of Liverpool's password-protected network, in its raw format as a Microsoft Excel spreadsheet. Statistical analyses were performed using Minitab v.19 (Minitab LLC, State College, PA) and Stata v.15 (College Station, TX). Data entry errors were evaluated by histogram, scatterplot, or box plots for each variable before any statistical analyses.

Univariable Cox proportionate hazard analyses were performed on each of the explanatory variables to assess their effect on time to commencement of luteal activity and time to first estrus. Variables that had a P-value less than or equal to 0.2 were included in the full multivariable model. Back fat depth and muscle diameter changes from calving to 60 DIM were categorized into quartiles based on the amount of change, as described above. Time to event data were centered on 10 d consistent with the start of the at-risk period when P4 measurements began. Backward stepwise elimination in a Cox proportional hazard model was then performed until all remaining variables had P-value less than 0.05. Occurrence of clinical disease within 60 DIM was forced into the model as a binary variable as a known risk factor for infertility. Days between measurements was also forced into models to account for temporal differences as measurements were taken within 7 d of calving and within 7 d of 60 DIM. Likelihood ratio tests were performed to determine overall statistical significance of categorical variables. Interactions were not evaluated as we did not have sufficient a priori reasoning to do so and did not want to increase the risk of making a type II error. Collinearity between BCS change and back fat depth (Figure 4) was avoided as back fat depth did not have sufficient univariable association with either fertility outcomes to be included in the full model. Models were validated by graphical assessment of the raw data for outliers that may be attributable to data entry error and graphical evaluation of the Cox-Snell residuals.

One-sided, 2-sample t-tests were performed to determine if back fat loss and muscle loss were greater in cows with subclinical ketosis <60 DIM. We expected ketotic cows to lose more back fat and muscle mass than nonketotic cows in association with the NEB. We also expected that back fat and muscle loss would increase with increasing BCS loss; consequently, a one-way ANOVA test followed by Tukey's test of pairwise differences were used to assess the difference between categories of BCS change and the amount of back fat and muscle loss. Paired t-tests were used to determine differences in back fat and muscle measurements between calving and 60 DIM. Paired Pearson correlation analyses were performed on back fat depth and muscle diameter at calving.

The relationship between 60-d and 305-d milk yield and body tissue mobilization was investigated as evidence suggests that the magnitude of body weight loss is positively associated with milk yield in early lactation (Zachut and Moallem, 2017). Linear regression analyses were performed with muscle diameter change, back fat thickness change, and BCS change included as explanatory variables. Changes in back fat thickness change and BCS were included in separate analyses due to collinearity (Figure 4), and the best fitting model was determined from Akaike's and Schwarz's Bayesian information criteria. Parity and occurrence of clinical disease and ketosis between calving and 60 DIM were forced into all models as fixed effects. Likelihood ratio tests were performed to determine overall statistical significance of categorical variables.

RESULTS

Data were collected from 133 of the 140 cows enrolled. Three cows were culled (broken leg, lameness, mastitis) and 4 were missed at their 60 DIM evaluation due to an animal handling error. Their data were removed from all analyses. The mean (±SD) parity was 2.1 ± 1.2 with 58 first lactation heifers, 31 cows in their second lactation, and 44 cows in their third or greater lactation. The oldest cow was in her sixth lactation. The mean 305-d milk yield (77 realized and 63 predicted) was 9,754 ± 2,092 L (mean ± SD; range 6,001 to 16,652 L). The mean BCS at calving was 3.0 ± 0.4 (range 2.25 to 4.0) with a mean loss of 0.4 ± 0.4 BCS units (range 1.25 loss to 0.25 gain) to 60 DIM. Calving-to-conception was 91.5 d (median) and 102 ± 41 d (mean ± SD). The median number of serves per pregnancy was 3.

On average, cows experienced a reduction in back fat depth (2.2 ± 0.3 mm) and muscle diameter (5.0 ± 0.4 mm) to 60 DIM. Mean back fat depth reduced from 14.1 ± 0.3 mm at calving to 11.9 ± 0.1 mm at 60 DIM ($P < 0.001$). Mean muscle diameter reduced from 42.2 ± 0.3 mm at calving to 37.2 ± 0.4 mm at 60 DIM. There was no pairwise correlation between back fat and muscle diameter at calving (r = 0.05; $P = 0.59$) but weak correlation at 60 DIM (r = 0.34; $P < 0.001$). Muscle diameter change and back fat depth change to 60 DIM were not correlated (r = 0.12, $P = 0.16$). Days from calving to commencement of luteal activity and time to first estrus was 31 ± 15 d (range 14 to 90 DIM) and 53 ± 19 d (range 25 to 129 DIM), respectively.

On average, BCS measurements were taken 67 ± 9 d (mean ± SD) apart with the second measurement being taken at 65 ± 7 DIM. The average coefficients of variation for back fat depth and muscle diameter measurements taken were 0.03 and 0.02 respectively, indicating a small amount of variation between the repeat ultrasound images taken.

Body Condition, Muscle, and Back Fat Loss Were Associated with Ketosis

Sixty-six cows (49%) had at least one milk BHB ≥0.08 mmol/L, and 48 cows (36%) had a clinical disease event in the first 60 DIM. In descending order of frequency, these were metritis (n = 25), mastitis (n = 13), digital dermatitis (n = 5), retained fetal membranes (n = 2), displaced abomasum (n = 2), and one dystocia that required veterinary assistance. Ketotic cows had greater back fat loss and muscle loss than nonketotic cows. A ketotic cow lost on average 3.2 ± 0.36 mm of back fat thickness, whereas a nonketotic animal lost 1.4 ± 0.31 mm ($P < 0.001$). A ketotic cow lost on average 5.8 ± 0.68 mm of muscle tissue, whereas a nonketotic animal lost 4.1 ± 0.49 mm of muscle tissue ($P = 0.024$). Cows that lost greater than 0.75 of a BCS (Category 3) had 5.0 ($P = 0.09$), 4.6 ($P = 0.02$), and 4.6 ($P = 0.01$) times greater odds of developing subclinical ketosis compared with cows that gained ≥0.25 BCS, no change in BCS, and moderate loss of BCS (≥0.25 but ≤0.5 loss), respectively.

Cows that had the greatest loss in BCS also had the greatest loss in back fat depth ($P < 0.001$; Figure 4). There was no association between BCS loss and muscle diameter change ($P = 0.19$). Cows with ≥0.25 gain, no BCS change, or a moderate loss of BCS (≥0.25 ≤ 0.5 loss) had 6.7 ± 1.0 mm ($P < 0.001$), 5.4 ± 0.7 mm ($P < 0.001$), and 4.2 ± 0.6 mm ($P < 0.001$) gains in back fat depth to 60 DIM relative to cows that cow lost ≥0.75 BCS units, respectively. There was no relationship between BCS group at calving (thin, moderate, fat) and time to first estrus ($P = 0.63$) or commencement of luteal activity ($P = 0.93$).

Longissimus Dorsi Diameter and BCS at Calving Were Associated with Muscle Loss to 60 DIM

The BCS group at calving (thin, moderate, fat) was associated with the amount of muscle diameter present at calving ($P < 0.001$). Fat and moderate BCS cattle had 2.0 ± 2.0 mm ($P = 0.32$) and 3.3 ± 0.8 mm ($P < 0.001$) greater muscle diameter than thin cattle at calving, respectively. Even when accounting for BCS group at calving, muscle diameter at calving was associated with muscle loss to 60 DIM. On average, every 1 mm of muscle diameter present at calving was associated with a loss in muscle diameter of 0.7 ± 0.1 mm by 60 DIM ($P < 0.001$) irrespective of BCS group

Table 1. Cox proportional hazard model on time to commencement of luteal activity with longissimus dorsi muscle diameter reduction, clinical disease, subclinical ketosis, and body condition score change as predictors

Item	Hazard ratio	SE	95% CI	P-value
Muscle diameter reduction,[1] mm				0.01
<1.5	0.59	0.15	0.36, 0.97	0.04
≥5 to <8	0.88	0.23	0.52, 1.48	0.63
≥8	0.47	0.12	0.28, 0.78	0.004
Clinical disease <60 DIM	0.68	0.14	0.47, 1.04	0.07
Subclinical ketosis <60 DIM	0.63	0.12	0.43, 0.93	0.02
BCS change[2]				0.02
No change	0.36	0.15	0.15, 0.84	0.02
≥0.25 to ≤0.5 loss	0.28	0.11	0.12, 0.63	0.002
≥0.75 loss	0.48	0.22	0.19, 1.19	0.12

[1]Pairwise comparisons made to ≥1.5- to 5-mm reduction in muscle diameter.

[2]Pairwise comparisons made to a BCS gain ≥0.25.

($P < 0.001$). Thin cows lost 2.3 ± 0.9 mm ($P = 0.02$) and 8.3 ± 2.2 mm ($P < 0.001$) more of muscle diameter when compared with moderate and fat cows, respectively. Moderate cows lost 6.0 ± 2.0 mm more muscle diameter when compared with fat cows ($P = 0.004$). Back fat depth at calving was not associated with muscle loss to 60 DIM ($P = 0.78$). These findings indicate that thin cows catabolize more muscle tissue compared with cows with greater BCS.

Commencement of Luteal Activity Was Associated with Muscle Loss and BCS Loss

Muscle diameter change ($P = 0.01$), subclinical ketosis ($P = 0.01$), BCS category change ($P = 0.13$), milk yield ($P = 0.16$), and clinical disease <60 DIM ($P = 0.07$) were taken forward to the full model, but back fat depth change ($P = 0.26$) and parity ($P = 0.36$) were not. Muscle loss ($P = 0.01$), BCS category change ($P = 0.02$), and BHB ≥1.2 mmol/L ($P = 0.02$) and clinical disease <60 DIM ($P = 0.08$) delayed commencement of luteal activity. Cows in muscle loss category 1 (1.5–5 mm of muscle loss) were most likely to commence luteal activity so this group was used as the reference category (Table 1). Cows that lost ≥8 mm of muscle were the least likely to commence luteal activity. Cows that had clinical disease or subclinical ketosis were 30 ± 14% ($P = 0.08$) and 37 ± 12% ($P = 0.02$), respectively, less likely to have commenced luteal activity compared with those that did not. Cows that had a BCS loss of 0.25 or more were at least 52 ± 22% less likely to have commenced luteal activity compared with those that gained BCS (Table 1).

Time to First Estrus Was Associated with Muscle Loss But Not BCS Change or Back Fat Loss

Muscle diameter change ($P = 0.13$), parity ($P = 0.12$), and milk yield ($P = 0.17$) were taken forward to the full model, but BCS change ($P = 0.27$), back fat depth change ($P = 0.23$) and BHB being ≥1.2 mmol/L ($P = 0.65$) were not taken forward, as their univariable statistical association with time to first estrus was >0.2. Clinical disease <60 DIM was forced into the full model DIM ($P = 0.46$) as periparturient disease is known to influence fertility (Dalanezi et al., 2020; Cruz et al., 2021; Ranasinghe et al., 2011).

The final model consisted of muscle loss ($P = 0.007$) and clinical disease ($P = 0.16$). Cows that lost >8 mm of muscle were the least likely to commence estrus, and cows that lost ≥1.5 to 5 mm were most likely. Cows that lost >8 mm, ≥5 to <8 mm, and <1.5 mm were 55 ± 12% ($P = 0.002$), 1 ± 25% ($P = 0.96$), and 28 ± 18% ($P = 0.19$) less likely to show estrus behavior than cows that lost ≥1.5 to 5 mm irrespective of disease status. Clinical disease tended to reduce the likelihood of first estrus occurring by 24 ± 15%.

Severity of Tissue Mobilization Was Positively Associated with Milk Yield

Loss of BCS, back fat depth and muscle diameter were associated with greater milk yield to 60 DIM. A loss of 1 mm of back fat depth from calving to 60 DIM was associated with 43 ± 19 L increase in milk yield to 60 DIM ($P = 0.02$) irrespective of muscle loss ($P = 0.07$). The Akaike information criterion (**AIC**), however, was marginally better for the model containing BCS loss (AIC = 1,524) than back fat depth change (AIC = 1,528) so the results for this model are presented in Table 2.

The relationship between body tissue mobilization and milk yield may have continued throughout the lactation. A 1-mm reduction in back fat depth to 60 DIM was associated with a 84 ± 54 L increase in milk yield to 305 DIM ($P = 0.12$) and a 1-mm reduction in muscle diameter to 60 DIM was associated with a 47 ± 31 L increase in milk yield to 305 DIM ($P = 0.14$). The AIC

Table 2. Linear regression analyses showing the relationship between muscle diameter change, BCS change on milk yield to 60 DIM when controlling for clinical disease <60 DIM, subclinical ketosis <60 DIM, and parity

Item	β-Coefficient	SE	95% CI	*P*-value
Muscle diameter change, mm	−17.4	10.5	−38.3, 3.5	0.10
BCS change[1]				0.003
No change	87.4	209.2	−328.3, 503.1	0.67
≥0.25 to ≤0.5 loss	380.5	197.5	−11.9, 772.9	0.06
≥0.75 loss	610.2	228.9	155.3, 1,065.0	0.009
Clinical disease <60 DIM	−155.6	103.7	−361.7, 50.5	0.14
Subclinical ketosis <60 DIM	74.9	109.0	−141.6, 291.4	0.49
Parity[2]				0.002
2	129.6	123.1	−115.0, 374.2	0.295
3	235.7	132.4	−27.5, 498.8	0.08
4	613.3	181.0	253.8, 972.9	0.001
5	849.5	353.2	147.8, 1551.3	0.02

[1]Pairwise comparisons made to a BCS gain ≥0.25.

[2]Pairwise comparisons made to parity 1.

was again marginally better for the model containing BCS loss (AIC = 2,357) than back fat depth change (AIC = 2,359). BCS loss to 60 DIM was positively associated with milk yield to 305 DIM ($P = 0.02$). Cattle with a BCS loss to 60 DIM ≥0.75, moderate BCS loss (≥0.25 ≤ 0.5 loss), and no change in BCS had 305-d milk yields of 860 ± 702 L, 1,347 ± 658 L, and 1,964 ± 740 L greater than cattle that had a BCS gain ≥0.25, respectively.

DISCUSSION

This study found that severe muscle loss in the first 60 DIM was associated with a delay in time taken to first estrus and commencement of luteal activity. This demonstrates the negative association of muscle loss and fertility and supports recent work by Hatfield et al. (2022). Cows that gained in BCS from calving to 60 DIM were more likely to have commenced luteal activity, which is in line with other positive fertility effects associated with a gain in BCS in the postpartum period, including an earlier return to normal cyclicity, reduced health problems, and lower circulating serum concentrations of NEFA and BHB (Barletta et al., 2017). Although BCS is strongly correlated with energy reserves in the dairy cow, directly reflecting adipose stores, and does incorporate muscle tissue, it is not as reliable as ultrasound assessment of the longissimus dorsi muscle diameter and, in turn, information about muscle loss (Schröder and Staufenbiel, 2006; Siachos et al., 2021). Muscle diameter loss was not clearly associated with back fat depth loss in this study, which agrees with other studies (Hatfield et al., 2022). This could be because, in our study, leaner cows were found to mobilize more muscle tissue when compared with cows in better BCS, perhaps due to the relative lack of adipose tissue present. These findings have been supported by Pires et al. (2013) who found increased plasma indicators of protein mobilization in leaner cows that lost BCS in the postpartum period. Another possibility could be attributed to the disproportionate mobilization of adipose tissue postpartum with visceral fat breakdown occurring at a greater rate than subcutaneous fat (Ruda et al., 2019), which may also explain the apparent lack of an association between back fat and the fertility outcomes assessed.

In agreement with prior research, clinical disease negatively affected both time to first estrus and commencement of luteal activity (Ranasinghe et al., 2011; Dalanezi et al., 2020; Cruz et al., 2021). Severe BCS loss is known to lead to health and production disorders, including ketosis and fatty liver, with a decrease of more than one unit of BCS being associated with more days open (López-Gatius et al., 2003; Schröder and Staufenbiel, 2006).

Early lactation is the time of greatest metabolic stress for the dairy cow, due to the competition for energy from increasing milk yields and the appetite depression that accompanies the periparturient period. Cows attempt to bridge the energy deficit by breaking down adipose tissue, which represents the most important body tissue in terms of an energy source for the cow during early lactation (Schröder and Staufenbiel, 2006). Our findings, however, show that leaner cows catabolize muscle tissue more intensely to meet this NEB. This is likely due to the relative lack of adipose tissue and is supported by findings elsewhere (Pires et al., 2013). This muscle diameter change was not reflected in the BCS category change also seen here. This suggests that in leaner cows BCS change in the postpartum period may not be accurately reflecting the metabolic stress and body tissue mobilization, namely muscle, present. Megahed et al. (2019) suggested that ultrasonographic measurement of the longissimus dorsi diameter could be used in conjunction with ultrasonographic measurement of back fat depth to assess energy reserve mobilization in the periparturient period. We believe, however, despite its undoubted usefulness and improved accuracy, this is not currently feasible on most commercial dairy farms because it requires using an ultrasound and specialist training that makes it less practical as a tool than body condition scoring, which can be performed by trained farm staff.

We found that BCS loss and subclinical ketosis deleteriously affected time to commencement of luteal activity. This was not surprising and has been reported by others (Santos et al., 2009; Barletta et al., 2017). Conversely, we also found that a gain in BCS during the transition period was associated with a reduced time to luteal activity and by a considerable magnitude: cows that had no change in BCS were 64 ± 15% less likely to have commenced luteal activity when compared with those that had a BCS gain. Barletta et al. (2017) also reported a beneficial effect of BCS gain on ovarian activity with an earlier return to normal cyclicity, reduced health problems, and lower circulating serum concentrations of NEFAs and BHB in cows that gained BCS relative to those that did not. This implies that not only should we aim to avoid BCS loss and NEB in early lactation, but also promoting the idea that cows should be on a rising plane of nutrition and gaining BCS for optimal fertility akin to flushing in sheep for increased fecundity. This, however, requires further research and is likely to be difficult to achieve in high-yielding dairy cattle. Indeed, our study indicates that the degree of body tissue mobilization is positively associated with milk yield to 60 DIM. Others have reported a similar trade-off between fertility and milk yield with one study reporting greater early lactation milk yields but poor fertility in Holstein cows with high-weight loss postcalving relative to those that lost less weight (Zachut and Moallem, 2017). For this reason, the traditional concept of a 12-mo calving interval as being most cost-effective breeding system (Holmann et al., 1984) is becomingly increasingly scrutinized. Extending the voluntary waiting period in a study of high-yielding Holstein cattle beyond 40 d showed improved estrus detection and first service conception risk, but the overall economic impact of extending the calving interval was not evaluated (Niozas et al., 2019).

Although every effort was made to ensure every cow due to calve was measured within 1 week of her due date and within 1 week of being 60 DIM, it was not always possible for several reasons. For example, several study animals were primiparous heifers that had been served by the bull with no known service date but were moved into the calving shed when they were "bagging up," some animals calved either early or late, or were missed by the automatic sorting gate system at the dairy at 60 DIM. The author attended weekly for data collection to measure every cow due to calve or reach 60 DIM in the next 7 d. Therefore, there could be a difference of 14 d from the intended 60 d between measurements. This is an obvious weakness of the study; however, the median result for days between measurements was 65 d and for days between calving and measurement was 63 d.

In our study, cows that had greater muscle diameter at calving also lost more muscle diameter by 60 DIM. Postpartum muscle catabolism may be a physiological response to the amino acid demands of early lactation that occur simultaneously with NEB and insufficient dietary protein ingestion (Bell et al., 2000). Mobilization of muscle tissue to provide mammary and extramammary AA. It may also be at partly attributable to mobilization of intramuscular fat rather than solely skeletal muscle breakdown (Sadri et al., 2023). Other causes include nutritional protein deficiencies such as those on a low-protein diet, for instance a straw-based dry-cow ration. Dry-cow diet manipulation, namely supplementing protein within the diet, can increase longissimus dorsi muscle diameter in the periparturient period, as well as increasing both calf birth weights and CP milk concentration (Jaurena and Moorby, 2017). Interestingly, in the same study, milk protein yield increased only where fat was also supplemented in the dry-period diet. Animals supplemented with fat in the dry period had increased maximum back fat depth, milk, and protein yields and led to more longissimus dorsi muscle mobilization in the early postpartum period (Jaurena and Moorby, 2017).

Due to our study design, it is unclear if the mobilization of muscle tissue commenced before parturition, as may be the case (van der Drift et al., 2012). Metabolic demand for AA increases both before and after parturition in response to various processes such as mammary development, fetal growth, colostrum, and then milk production (Bell et al., 2000). Other studies have suggested that DMI depression and the initiation of lactation compounds the NEB that ensues, but that NEB is not the primary initiating factor that leads to muscle tissue mobilization as shown by the asynchronous breakdown of adipose and muscle tissue (Hatfield et al., 2022). More work is required to determine the mechanisms and triggers underlying postpartum muscle tissue mobilization, with both animal and environmental factors playing a part.

Throughout this study, a single assessor performed all data collection, as was stated as being preferential in a recent study to limit variation between assessors (Hatfield et al., 2022). Repeatability of BCS was not assessed; however, Edmonson et al. (1989) developed a well-recognized chart for accurately and repeatedly assessing BCS intending to remove subjectivity as much as possible. Ferguson et al. (1994) then went on to attempt to simplify the process by creating a decision tree to assign a BCS; these methods form the basis of the Penn State body condition scoring system (Ferguson et al., 1994). However, less precision was found in the results scored by untrained assessors when compared with the technique employed in this study as described by Edmonson et al. (1989).

Only one farm was studied due to the large amount of individual-animal data that needed to be collected. This limits our ability to make external inferences; however, our findings are largely in agreement with others, and we offer some new insights, such as the beneficial effect of BCS gain on fertility, that warrant further investigation. There is undoubtedly co-linearity among some of the variables in our study that we have attempted to account for in our statistical models. For example, BCS change and muscle loss variables are both measures of a decline in body condition; however, within the BCS change there will be visceral fat, subcutaneous fat as well as muscle diameter change. It is also worth remembering that we only measured the longissimus dorsi diameter and may not reflect muscle catabolism elsewhere. Another limitation of our study is that we did not evaluate genetic merit of the cattle in our study. Establishing the genetic correlations among commencement of luteal activity, time to first estrus, NEB, back fat depth change, or muscle diameter change presents an important next step. Positive changes to time to commencement of luteal activity can occur when both production and fertility are selected for (Garmo et al., 2009b).

CONCLUSIONS

Our findings highlight the importance of minimizing muscle breakdown and BCS loss to improve herd reproductive performance; however, our findings also indicate that body tissue mobilization is generally associated with greater milk yield in Holsteins, particularly in early lactation. Back fat loss was not associated with either of the fertility outcomes evaluated but was associated with 60-d milk yield. We found that ultrasound evaluation has merit for assessing the degree of catabolism of muscle and back fat, but body condition scoring greater practical utility on farms.

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