



Identification and characterization of dairy cows with different backfat thickness antepartum in relation to postpartum loss of backfat thickness: A cluster analytic approach

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ABSTRACT

The objectives of this study were (1) to characterize the interindividual variation in the relationship between antepartum (ap) backfat thickness (BFT) and subsequent BFT loss during early lactation in a large dairy herd using cluster analysis; (2) to compare the serum concentrations of metabolites (nonesterified fatty acids, β -hydroxybutyrate), metabolic hormones (leptin and adiponectin), and an inflammatory marker (haptoglobin) among the respective clusters; and (3) to compare lactation performance and uterine health status in the different clusters. An additional objective was (4) to investigate differences in these serum variables and in milk yield of overconditioned (OC) cows that differed in the extent of BFT loss. Using data from a large study of 1,709 multiparous Holstein cows, we first selected those animals from which serum samples and BFT results (mm) were available at d 25 (± 10) ap and d 31 (± 3 d) postpartum (pp). The remaining 713 cows (parity of 2 to 7) were then subjected to cluster analysis: different approaches based on the BFT of the cows were performed. *K*-means (unsupervised machine learning algorithm) clustering based on BFT-ap alone identified 5 clusters: lean (5–8 mm BFT, $n = 50$), normal (9–12 mm, $n = 206$), slightly fat (SF; 13–16 mm, $n = 203$), just fat (JF; 16–22 mm, $n = 193$), and very fat (VF; 23–43 mm, $n = 61$). Clustering by difference between BFT-ap and BFT-pp (Δ BFT) also revealed 5

clusters: extreme loss (17–23 mm Δ BFT, $n = 16$), moderate loss (9–15 mm, $n = 119$), little loss (4–8 mm, $n = 326$), no loss (0–3 mm, $n = 203$), and gain (–8 to –1 mm, $n = 51$). Based on the blood variables measured, our results confirm that cows with greater BFT losses had higher lipid mobilization and ketogenesis than cows with less BFT loss. The serum variables of cows that gained BFT did not differ from normal cows. Milk yield was affected by the BFT-ap cluster, but not by the Δ BFT cluster. Cows categorized as VF had lesser milk yield than other clusters. We further compared the OC cows that had little or no BFT loss (i.e., 2% of VF, 12% of JF, and 31% of SF, OC-no loss, $n = 85$) with the OC cows that lost BFT (OC-loss, $n = 135$). Both NEFA and BHB pp concentrations and milk yield were greater in OC-loss cows compared with the OC-no loss cows. The serum concentration of leptin ap was greater in OC-loss than in the OC-no loss cows. Overall, OC cows lost more BFT than normal or lean cows. However, those OC cows with a smaller loss of BFT produced less milk than OC cows with greater losses.

Key words: dairy cows, cluster analysis, transition period, body condition

INTRODUCTION

Dairy cows face physiological challenges during the transition from late pregnancy to lactation (Drackley, 1999). In early lactation, dairy cows are typically in a state of negative energy balance (NEB) because feed intake is insufficient to meet the increased nutrient requirements for milk synthesis (Drackley, 1999). Although metabolizing body fat for milk production is very important, it is known that cows with a high body condition score (BCS) around calving are at greater risk of developing metabolic disorders, mainly ketosis,

Received October 15, 2021.

Accepted March 2, 2022.

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and impaired fertility (Bernabucci et al., 2005; Roche et al., 2009; Rathbun et al., 2017). In addition to the BCS around calving, the magnitude of BCS loss during the transition from pregnancy to lactation may be even more important for metabolic health than BCS alone (Rathbun et al., 2017).

In addition to BCS, body fat reserves can also be assessed by ultrasound measurements of backfat thickness (**BFT**; Schröder and Staufienbiel, 2006). In early-lactating dairy cows, the different fat depots differ in their mobilization velocity, with visceral adipose tissues having the highest mobilization compared with subcutaneous adipose tissue (von Soosten et al., 2011; Mann, 2022). The correlation between BCS and visceral adipose tissues are only moderate, but a relatively strong correlation exists between BCS and total body fat (Mann, 2022). The accumulation of body fat reserves and the extent to which these reserves are mobilized varies among individuals. The reasons for this variation are largely unknown.

Body condition around calving is considered as the main determinant of BCS loss; that is, the greater BCS is at calving, the more body reserves are mobilized postpartum (**pp**), increasing the risk of metabolic disorders (Roche et al., 2007a). A loss of 1 unit of BCS (a 5-point scale) from late gestation (d 28 antepartum, **ap**) to early lactation (d 18 pp) may increase the risk of hyperketonemia (Rathbun et al., 2017). Underconditioned cows (BCS <2.8) may not be able to mobilize enough energy for maximum lactation (Otto et al., 1991) and may have greater post-calving muscle mass mobilization compared with normally conditioned cows (Pires et al., 2013). Excessive loss of body energy reserves has also been linked to impaired immune function, which in turn increases the risk of infectious diseases such as endometritis (Esposito et al., 2014). Therefore, it is generally recommended to avoid overconditioning before calving and to protect against excessive losses during the transition period.

With this background, we used a cluster analytic approach to identify clusters of cows based on either antepartum BFT (**BFT-ap**) or the BFT change during the transition period (BFT loss, **ΔBFT**), with thresholds that were not arbitrary but derived solely from the data set used. We used data and samples from a previously described feeding trial (Süss et al., 2019). The objectives of the present study were (1) to characterize the inter-individual variation in the relationship between BFT-ap (d 25 ± 10 ap, means ± SD) and subsequent BFT loss pp (d 31 ± 3 pp, means ± SD) in a large dairy herd; (2) to estimate into which **ΔBFT** cluster cows from the different BFT-ap clusters would develop; and (3) to compare milk yield, uterine health (mainly prevalence of endometritis and hyperketonemia), and metabolic

and inflammatory status [serum variables: nonesterified fatty acids (**NEFA**), BHB, leptin, adiponectin, and the acute-phase protein haptoglobin, **Hp**] among the different clusters. The descriptive comparisons of cows from a large dairy herd with divergent BFT-ap status extend the knowledge about biological variations in ap body condition and their aftereffects on the mobilization of body reserves. Information about variations within the same herd kept under the same management conditions on a commercial dairy farm is relevant for researchers and dairy farmers for finding strategies of either eliminating less efficient animals or aiming at “personalized” feeding and management systems.

MATERIALS AND METHODS

This study was approved by the Slovakian Regional Veterinary Food Administration, and by the institutional ethics committee of the University of Veterinary Medicine, Vienna, Austria (ETK-09/02/2016). The animal experiment was conducted from March 2016 to November 2017 on a commercial dairy farm in Slovakia, where about 2,400 Holstein-Friesian cows are kept.

Animals, Diets, Records, and Samplings

The current study extends the study by Süss et al. (2019) in which the effect of dietary supplementation with rumen-protected methionine (**MET**) on reproductive performance was tested in 1,709 multiparous Holstein cows. The field trial was conducted from March 2016 to November 2017 on a dairy farm in Slovakia that housed approximately 2,400 Holstein-Friesian cows. The cows were milked twice daily with a rotary milking parlor. The average energy-corrected milk yield was 9,260 kg (based on a milk fat content of 4% and a protein content of 3.4%). Because the cows were housed on a different farm during their first lactation, only multiparous cows were included in this study. Each barn consisted of groups of approximately 250 cows and was equipped with concrete floors and cubicles. All animals received the same basal diet as a TMR during pregnancy and lactation as reported previously (Süss et al., 2019). Cows were offered the TMR twice daily and adjusted to achieve refusal rates of 5 to 10%. After calving and leaving the fresh group (between d 8 and 40 pp), the cows were assigned to either a basal diet (control) or the basal diet plus approximately 25 to 27 g/d of rumen-protected MET (Mepron, Evonik Operations GmbH), which was added to the mineral and vitamin premix and fed in the TMR until the end of the observation period (70 d pp). Mepron is coated with ethyl cellulose and contains 85% DL-methionine, resulting in a rumen bypass of 80% (Overton et al., 1996)

and intestinal digestibility coefficient of 90% (Schwab, 1995). As a result, each cow in the MET group received approximately 18 or 19 g of absorbable methionine per day.

On d 25 ± 10 ap and d 31 ± 3 pp (means \pm SD), BFT was assessed by ultrasound (Easi-Scan, IMV Imaging; 7.5-MHz linear probe with wrist display) as previously described (Schröder and Staufenbiel, 2006). Cows were evaluated for metritis at d 5 pp by assessment of vaginal discharge and body temperature, and for endometritis at d 31 ± 3 pp by vaginal examination and uterine cytology (Süss et al., 2019). Reproduction management procedures were defined in dairy farm-specific standard operating procedures. Voluntary waiting period was set at 50 DIM. Estrus detection was performed using an automated monitoring device and visual animal observation by farm workers twice daily. Animals that were not in estrus and not bred were assigned to an Ovsynch protocol started at 70 DIM. In addition, a Resynch protocol was started 1 wk before pregnancy testing and continued for nonpregnant cows. Selected fertility parameters of multiparous dairy cows were 74 ± 13 d to first AI, 101 ± 40 d to conception, a calving interval of 377 ± 36 d, 55% first AI submission rate (percentage of cows receiving at least 1 AI in the first 3 wk after the voluntary waiting period), 45% first AI conception risk (number of first AI resulting in pregnancies relative to total number of first AI \times 100), and 25% pregnancy rate. Endometritis was diagnosed using a modified vaginal discharge score (Williams et al., 2005) to classify vaginal mucus as (E0) clear mucus; (E1) $\leq 50\%$ non-white or white mucopurulent material; and (E2) $\geq 50\%$ off-white or white mucopurulent material. Furthermore, uterine cytology samples were collected using the Cytobrush method, as described by Kasimanickam et al. (2004). These samples were then prepared by rolling the brush onto a clean glass slide. On the farm, the slides were immediately fixed and stained (LT-SYS, Labor und Technik, Berlin, Germany) and evaluated under the microscope ($\times 400$ magnification) by counting a total of 300 cells to determine the percentage of PMN (Melcher et al., 2014). The cut-off point for diagnosing subclinical endometritis was 5% PMN (Madoz et al., 2013). Based on the vaginal examination and uterine cytology, cows were classified as healthy (vaginal discharge score = E0, proportion of PMN $< 5\%$) or affected by endometritis: subclinical endometritis (vaginal discharge score = E0, proportion of PMN $\geq 5\%$) or clinical endometritis (vaginal discharge \geq E1).

Blood samples were collected from a coccygeal vessel using evacuated tubes coated with a clot activator for serum collection (Süss et al., 2019), on the same day as the BFT measurements. In the samples, serum BHB

was used to classify cows as normal or hyperketonemia using a BHB threshold of 1.2 mM (Süss et al., 2019). Two metabolic hormones (leptin and adiponectin), an acute-phase protein (Hp), and 2 metabolites (BHB and NEFA) were measured in serum as described subsequently. Cows were milked twice daily in a rotating milking parlor, and milk production was recorded daily throughout the study. Milk production (kg/d) is expressed as a weekly average. Only cows from which all records and samples were available and which were assigned to the control group or MET treatment no later than d 20 pp were included in the present study, resulting in a total of 713 cows.

Clustering of Cows According to BFT and BFT Loss. The *k*-means (unsupervised machine learning algorithm) analysis procedure of the SAS package (PROC FASTCLUS; SAS Institute Inc.) was used with the mean, median, sum, and standard deviation of BFT at 25 ± 10 d ap (BFT-ap) or BFT loss (Δ BFT = BFT at d 25 ap – BFT at d 31 pp) as key features for each cluster procedure. The number of clusters was determined by tests based on the separation between clusters and homogeneity within clusters. To validate the clusters, we estimated the optimal number of clusters for *k*-means clustering using the direct method in R (version 4.0.3; R Core Team, 2019). This method aims to optimize a criterion, the sum of squares within clusters or the average silhouette, using the method of elbows and average silhouette (Rousseeuw, 1987). Statistical power was defined as the probability of the cluster analysis to reject the null hypothesis (no clustering found), which was defined by an average silhouette width above 0.5 (Kaufman and Rousseeuw, 1990).

Laboratory Analysis of All 713 Cows (ap and pp Samples). The concentrations of BHB and NEFA were measured spectrophotometrically (HORIBA ABX SAS) at the Research Institute for Farm Animal Biology in Dummerstorf, Germany, using the following kits: BHB (no. RB1008, Randox Laboratories Limited) and NEFA (no. 434-91795, Wako Chemicals GmbH). Adiponectin, leptin, and Hp were measured using ELISA methods developed in-house (Sauerwein et al., 2004; Hiss et al., 2009; Mielenz et al., 2013). For the adiponectin ELISA, the intra- and interassay coefficients of variation (CV) were 9.8 and 13.4%, respectively. The intra- and interassay CV were 9.2 and 13.4% for leptin and 8.9 and 11.6% for Hp, respectively.

Statistical Analyses

A repeated-measures model was fitted to the data (both blood and milk) using a linear mixed-effects model with SPSS software (SPSS Statistics 24.0, IBM Corp.), using treatment (BFT-ap clusters, Δ BFT clus-

ters, or BFT loss), time (day, week), time \times treatment interaction, MET supplementation (control or MET), uterine health status (healthy, subclinical, or clinical endometritis), and parity as fixed effects, and cow as a random effect. In the preliminary data analysis, 2-way interactions (treatment \times uterine health status; treatment \times MET supplementation; time \times MET supplementation; time \times uterine health status) were included in the model as fixed effects, but the results of this preliminary statistical analysis showed no significant effect of the 2-way interactions on the tested variables in this study. Therefore, these 2-way interactions were disregarded in the final statistical analysis of the data. All data were tested for normal distribution using the UNIVARIATE procedure of SAS (Shapiro-Wilk test), and variables that were not normally distributed were \log_{10} -transformed to meet the assumptions of normality and homoscedasticity of the residuals. Data are presented as means \pm standard error of the mean, and Tukey-Kramer adjustment was applied to account for multiple comparisons. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Characteristics of the Clusters Obtained for BFT-ap

Five clusters were determined based on BFT-ap: lean (**LEN**, BFT: 5–8 mm; BCS: 2.7 ± 0.3 , mean \pm SD), normal (**NOR**, BFT: 9–12 mm; BCS: 3.1 ± 0.3 , mean \pm SD), slightly fat (**SF**, BFT: 13–16 mm; BCS: 3.4 ± 0.3 , mean \pm SD), just fat (**JF**, BFT: 16–22 mm; BCS: 3.7 ± 0.3 , mean \pm SD), and very fat (**VF**, BFT: 23–43 mm; BCS: 4.0 ± 0.3 , mean \pm SD). Figure 1A shows a histogram indicating the frequency (number of animals) in the obtained clusters. The Gaussian distribution showed anomalous skewness and kurtosis coefficients, and the Shapiro-Wilk test for the obtained clusters was significant, indicating that the data were not normally distributed (Figure 1A). In our data set, relatively fewer cows were grouped in the LEN ($n = 50$, 7%) and VF ($n = 61$, 8.6%) clusters, with the majority appearing in the NOR ($n = 206$, 28.9%), SF ($n = 203$, 28.5%), and JF ($n = 193$, 27.1%) clusters, indicating a relatively high portion of cows in the clusters with mild to moderately overconditioned cows (BFT ≥ 13 mm and < 23 mm). For the different BFT-ap clusters, milk yield and serum parameters are presented in Figures 1B and 1C, respectively. Milk yield was affected by the BFT-ap cluster, time, parity, and uterine health ($P < 0.01$). Cows categorized as VF had lesser milk yield than other clusters. Milk yield increased with time ($P < 0.01$) and reached a plateau around 7 to 8 wk of lactation. The serum concentration of BHB was affected

by BFT-ap cluster ($P < 0.01$), time ($P < 0.01$), cluster \times time interaction (trend, $P = 0.06$), and MET ($P = 0.02$). The concentration of NEFA was affected by BFT-ap cluster ($P < 0.01$), time ($P < 0.01$), cluster \times time interaction (trend, $P = 0.08$), and parity ($P < 0.01$). The concentrations of NEFA and BHB after calving were greater in VF and JF than in the other clusters. Leptin concentrations were affected by BFT-ap cluster and time ($P < 0.01$; Figure 1C). Compared with the other treatment groups, the VF group had the highest concentration of ap leptin, whereas the LEN group had the lowest. The adiponectin concentrations in serum were affected only by time and decreased after calving. The concentrations of Hp in serum were not affected by the main effects or by the cluster \times time interaction. As expected, circulating concentrations of NEFA and BHB increased, whereas leptin and adiponectin decreased from ap to pp.

In addition, no differences were found among clusters when other factors, including MET treatment, health status, and parity, were considered (Supplemental Table S1; <https://doi.org/10.6084/m9.figshare.19294499.v1>). The incidence of endometritis was assessed at d 31 pp, but we did not observe any differences among the 5 different BFT-ap clusters in uterine health, calving or conception intervals (Supplemental Table S2; <https://doi.org/10.6084/m9.figshare.19294499.v1>).

Characteristics of the Clusters Obtained for Δ BFT

Clustering by difference between BFT-ap and BFT-pp (Δ BFT) also revealed 5 clusters (Figure 2): extreme loss (**EL**, $n = 16$; Δ BFT: 17–23 mm; Δ BCS: 1.2 ± 0.4 , mean \pm SD), moderate loss (**ML**, $n = 119$; Δ BFT: 9–15 mm; Δ BCS: 1.1 ± 0.4 , mean \pm SD), small loss (**SL**, $n = 326$; Δ BFT: 4–8 mm; Δ BCS: 0.8 ± 0.4 , mean \pm SD), no loss (**NL**, $n = 203$; Δ BFT: 0–3 mm; Δ BCS: 0.4 ± 0.3 , mean \pm SD), and gain (**GN**, $n = 51$; Δ BFT: -8 to -1 mm; Δ BCS: 0.2 ± 0.4 , mean \pm SD). Evaluation of the Gaussian distribution showed anomalous skewness and kurtosis coefficients, and the P -value of the Shapiro-Wilk test for the clusters obtained for Δ BFT was less than 0.01, indicating that the data were not normally distributed (Figure 2A). A smaller number of cows were observed in the EL (2.2%) and GN (7.2%) clusters, whereas the majority of cows were found in the SL (45.7%), ML (16.7%), and NL (28.2%) clusters.

Figure 2 shows milk yield (Figure 2B) and serum variables (Figure 2C) associated with the Δ BFT clusters. Milk yield was affected by time, parity, and uterine health ($P < 0.01$), but not by the Δ BFT cluster, MET, or the cluster \times time interaction. The BHB concentration in serum was affected by Δ BFT cluster ($P < 0.01$), time ($P < 0.01$), cluster \times time interaction ($P = 0.02$),

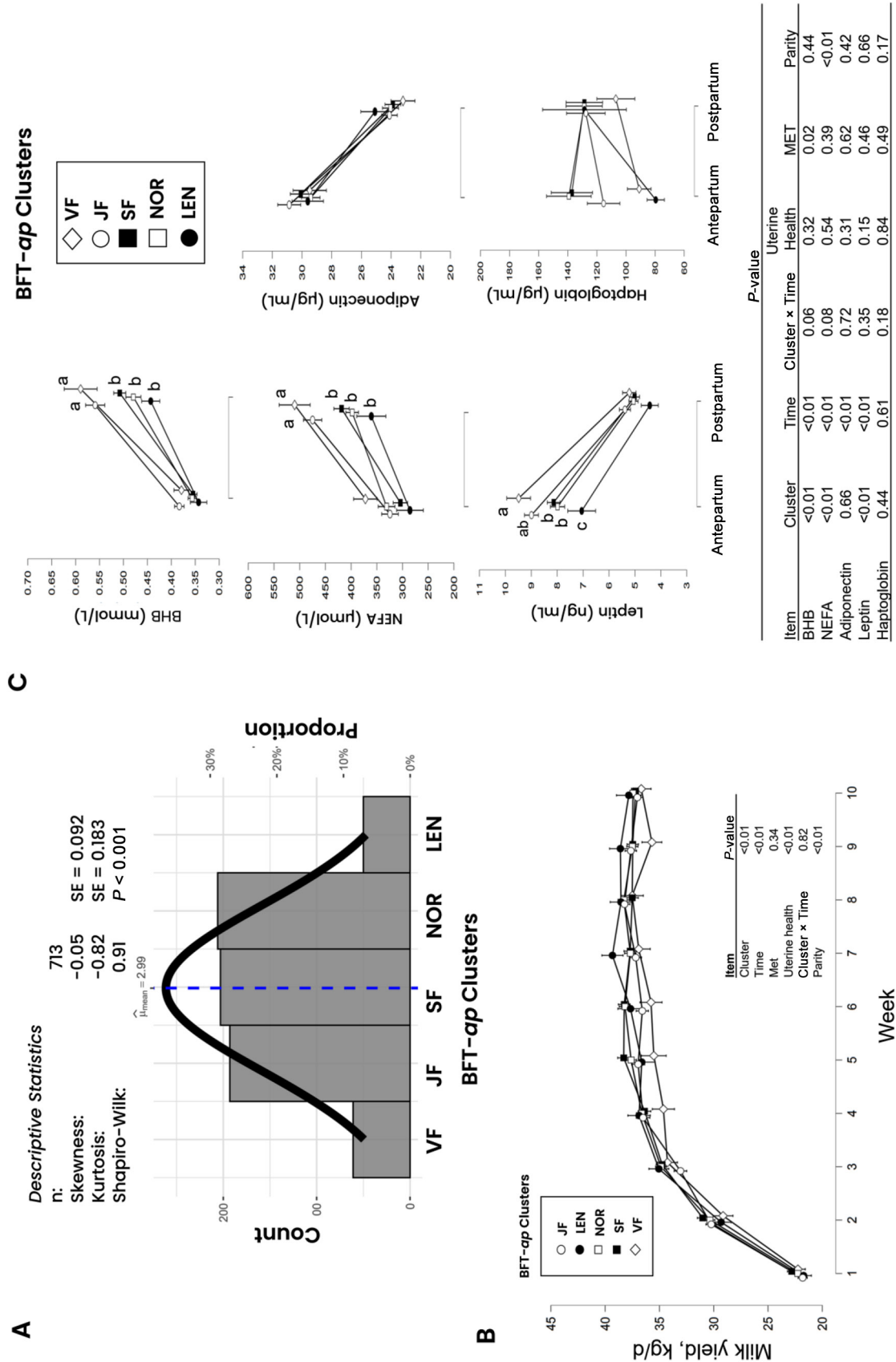


Figure 1. Characteristics of the clusters based on backfat thickness antepartum (BFT-ap): LEN = lean, BFT 5–8 mm; NOR = normal, BFT 9–12 mm; SF = slightly fat, BFT 13–16 mm; JF = just fat, 16–22 mm; VF = very fat, 23–43 mm. (A) Histogram of the distribution; (B) milk yield (means \pm SEM); (C) serum concentration (means \pm SEM) of various metabolites [nonesterified fatty acids (NEFA) and BHB], metabolic hormones (leptin and adiponectin), and acute-phase protein (haptoglobin) in samples collected antepartum (30 d before calving) and postpartum (30 d after calving). Uterine health = cows were classified as healthy or affected by endometritis based on vaginal examination and uterine cytology. Vaginal discharge score: clear mucus proportion of PMN $<5\%$ or affected by endometritis = subclinical endometritis (vaginal discharge score $\leq 50\%$ off-white or white, proportion of PMN $\geq 50\%$) or clinical endometritis ($\geq 50\%$ off-white or white mucopurulent material). MET = methionine treatment.

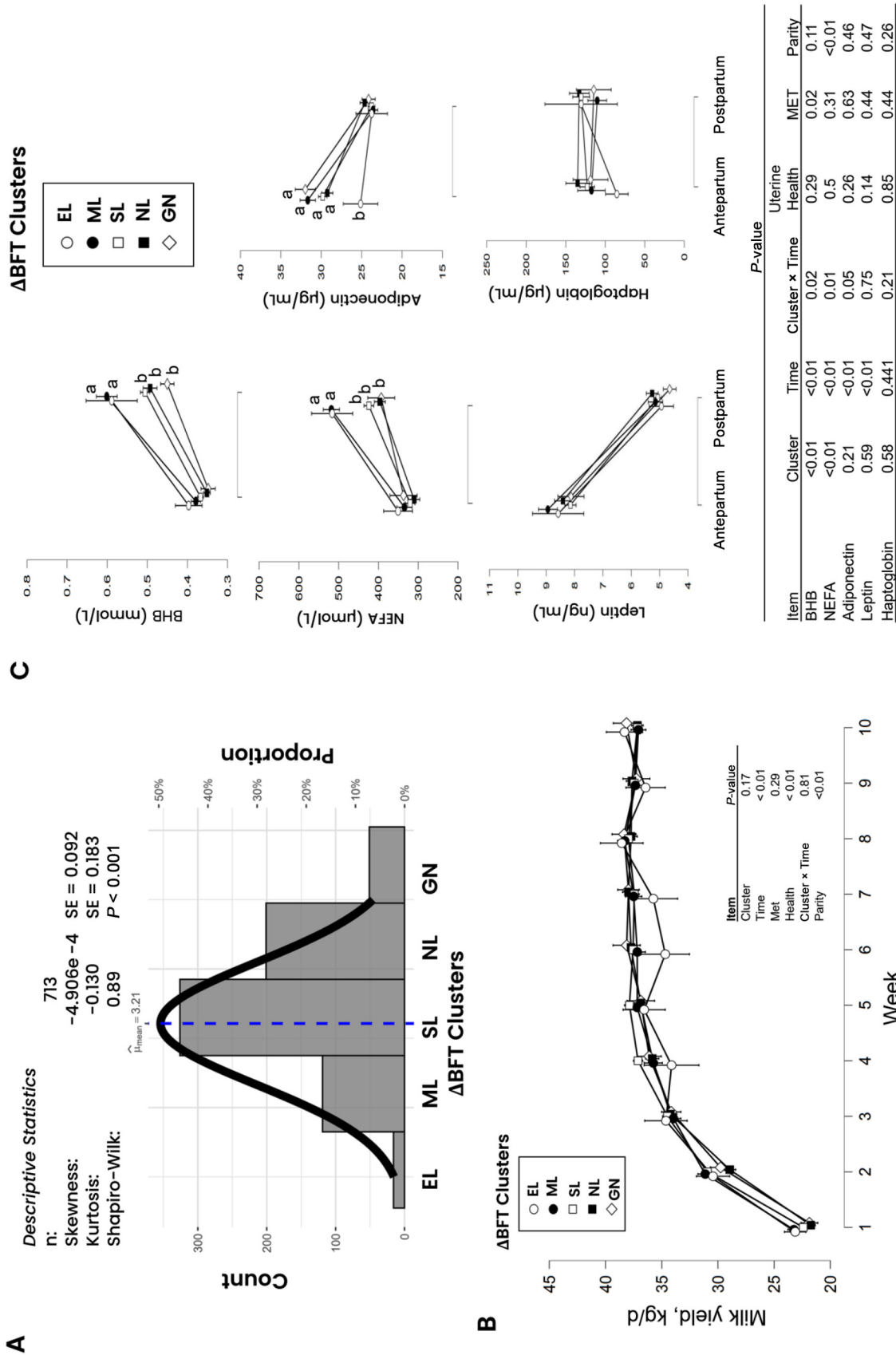


Figure 2. Characteristics of the clusters based on backfat thickness loss from ante-partum to post-partum (Δ BFT): EL = extreme loss (Δ BFT 17–23 mm); ML = moderate loss (9–15 mm); SL = small loss (4–8 mm); NL = no loss (–8 to –1 mm); GN = gain (–8 to –1 mm). (A) Histogram of the distribution; (B) milk yield (means \pm SEM); (C) serum concentration (means \pm SEM) of various metabolites [nonesterified fatty acids (NEFA) and BHB], metabolic hormones (leptin and adiponectin), and acute-phase protein (haptoglobin) in samples collected ante-partum (30 d before calving) and post-partum (30 d after calving). Uterine health = cows were classified as healthy or affected by endometritis based on vaginal examination and uterine cytology. Vaginal discharge score: clear mucus proportion of PMN <5% or affected by endometritis = subclinical endometritis (vaginal discharge score \leq 50% off-white or white, proportion of PMN \geq 5%) or clinical endometritis (\geq 50% off-white or white mucopurulent material). MET = methionine treatment.



Figure 3. Sankey plot for representing the apportionment of animals in 5 antepartum backfat thickness (BFT-ap) clusters (lean = BFT 5–8 mm; normal = BFT 9–12 mm; slightly fat = BFT 13–16 mm; just fat = 6–22 mm; very fat = 23–43 mm) to the 5 backfat thickness loss (Δ BFT) clusters (extreme loss = Δ BFT 17–23 mm; moderate loss = Δ BFT 9–15 mm; small loss = Δ BFT 4–8 mm; no loss = Δ BFT 0–3 mm; gain = Δ BFT –8 to –1 mm).

and MET ($P = 0.02$), but not by parity and uterine health. The NEFA concentrations were influenced by Δ BFT cluster ($P < 0.01$), time ($P < 0.01$), cluster \times time interaction ($P = 0.01$), and parity ($P < 0.01$), but not by MET and uterine health. The NEFA and BHB concentrations pp were greater in EL and ML than in the other clusters. Serum leptin concentrations were affected by time ($P < 0.01$), but not by Δ BFT cluster, cluster \times time interaction, parity, MET, and uterine health. Serum adiponectin concentrations were affected only by the Δ BFT cluster and cluster \times time interaction, and were lower in EL cows compared with other clusters ap. Serum Hp concentrations were not affected by the main effects or by the cluster \times time interaction.

Apportionment of Cows in the BFT-ap Clusters to Δ BFT Clusters

To illustrate the presumed dependence of BFT loss on BFT-ap, we used a Sankey plot diagram (Figure 3) to show the proportions of dairy cows from the different BFT-ap clusters that were classified into the different Δ BFT clusters. We observed that 98% of VF cows, 88% of JF cows, and 69% of SF cows were assigned to the Δ BFT clusters with greater losses, compared with NOR or LEN cows. We further used the Sankey plot results to compare the overconditioned (OC) cows that had little or no BFT loss (i.e., 2% of VF, 12% of JF,

and 31% of SF; $n = 85$) with the OC cows that lost BFT ($n = 135$).

The resulting groups, designated as OC with little or no BFT loss (OC-no loss) and OC with severe to normal loss (OC-loss), were then compared for milk yield (Figure 4 A) and serum concentrations of the assessed variables (Figure 4B). Milk yield was affected by the OC- Δ BFT groups (OC with different BFT losses), time, parity, and uterine health ($P < 0.01$), but not by MET supplementation, or group \times time interaction. Milk yield was greater in OC-loss cows than in OC-no loss cows ($P < 0.01$). Serum BHB concentration was affected by OC- Δ BFT group ($P < 0.01$), time ($P < 0.01$), group \times time interaction (trend, $P = 0.09$), and MET (trend, $P = 0.07$), but not by parity and uterine health. The NEFA concentration was affected by OC- Δ BFT group ($P < 0.01$), time ($P < 0.01$), group \times time interaction (trend, $P = 0.10$), and parity (trend, $P = 0.07$), but not by MET and uterine health. The pp concentrations of NEFA and BHB were greater in the OC-loss than in the OC-no loss cows. Serum adiponectin was affected only by time ($P < 0.01$), and leptin was affected by OC- Δ BFT group, time, and uterine health ($P < 0.01$), but not by the group \times time interaction, parity, or MET. The ap concentration of leptin was greater in the OC-loss cows than in the OC-no loss cows, whereas the concentrations of Hp in serum were not affected by the main effects.

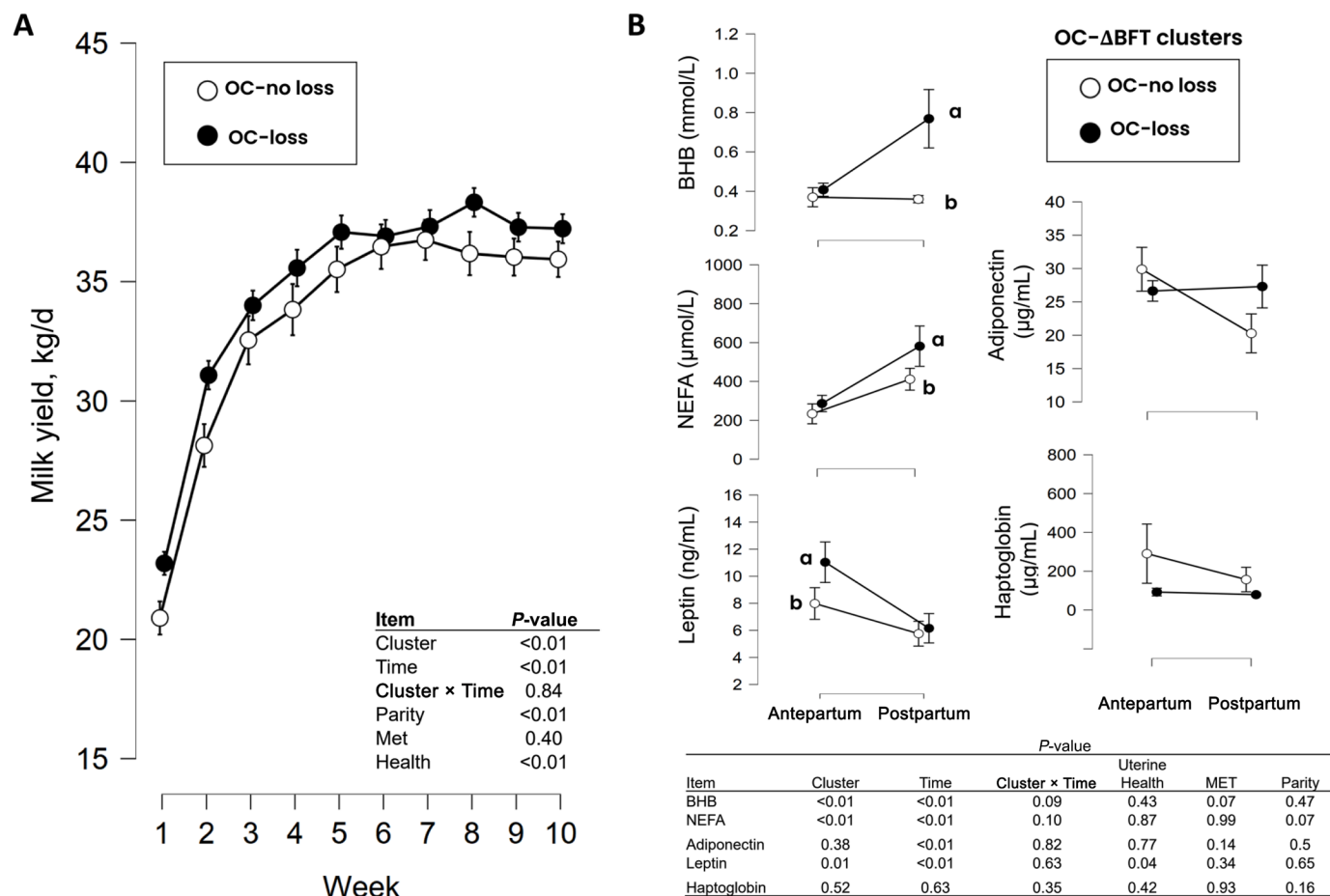


Figure 4. (A) Milk yield (means \pm SEM); (B) serum concentration (means \pm SEM) of various metabolites [nonesterified fatty acids (NEFA) and BHB], metabolic hormones (leptin and adiponectin), and acute-phase protein (haptoglobin) in samples collected antepartum (30 d before calving) and postpartum (30 d after calving) in overconditioned cows with different backfat thickness loss (Δ BFT). OC-no loss = overconditioned cows with little or no BFT loss; OC-loss = overconditioned cows with severe to normal loss. Uterine health = cows were classified as healthy or affected by endometritis based on vaginal examination and uterine cytology. Vaginal discharge score: clear mucus proportion of PMN $<5\%$ or affected by endometritis = subclinical endometritis (vaginal discharge score $\leq 50\%$ off-white or white, proportion of PMN $\geq 5\%$) or clinical endometritis ($\geq 50\%$ off-white or white mucopurulent material). MET = methionine treatment.

Supplemental Figure S1 (<https://doi.org/10.6084/m9.figshare.19294499.v1>) shows the frequency of cows supplemented with or without rumen-protected MET in different clusters. A description of the distribution of endometritis and hyperketonemia, as described by Süß et al. (2019), after our clustering based on BFT-ap and Δ BFT is shown in Supplemental Tables S1 and S3 (<https://doi.org/10.6084/m9.figshare.19294499.v1>), respectively. The frequency of cows with different parities in the different clusters is shown in Supplemental Figure S2 (<https://doi.org/10.6084/m9.figshare.19294499.v1>). The calving to conception intervals calculated for the BFT-ap clusters for the lactation cycles preceding and following the current study are presented in Supplemental Table S2 (<https://doi.org/10.6084/m9.figshare.19294499.v1>). In summary, the portion of cows receiv-

ing MET supplementation ranged from 37.5 to 58.8% in all clusters (mean \pm SD: $49.1 \pm 7.9\%$), and we found no apparent shift in the proportions of animals with diagnoses among clusters.

DISCUSSION

The extent of body fat mobilization during this transition varies between cows (Weber et al., 2013) and is usually associated with the ap body condition of the animal (Gobikrushanth et al., 2019). This study was conducted to characterize individual variation in the relationship between BFT-ap and subsequent loss of BFT-pp (d 31 ± 3 pp, means \pm SD) in large dairy herds and to estimate which Δ BFT clusters cows from the different BFT-ap clusters would develop. In addi-

tion, we examined milk yield, uterine health (focusing on endometritis and hyperketonemia), and metabolic and inflammatory status among the different clusters.

In the current study, we observed no differences between the Δ BFT clusters for milk yield, but a significant difference was detectable in milk yield of the clusters obtained for BFT-ap. In our study, VF cows produced less milk compared with the other clusters. This might have been due to lower breeding values for milk yield in these cows. Moreover, less milk yield in the preceding lactation might, in turn, result in a more positive energy balance, thus allowing the animals to accumulate more body fat. Unfortunately, neither breeding values nor milk yields and feed intake of the preceding lactation were available in this study. No conclusive evidence has yet been found on how BCS may influence milk yield in dairy cows. Several studies examining the effect of BCS on milk yield found no significant association, whereas others found an association between BCS and milk production. For example, in an analysis of 2,463 lactation records of pasture-based dairy cows by Roche et al. (2007b), milk yield was found to be nonlinearly related to BCS at calving, and the highest milk yield was found at a calving BCS of 3.50. A positive relationship between BCS loss (between calving and nadir) and milk yield was observed by Roche et al. (2007b). In contrast, Gobikrushanth et al. (2019) reported no association between pp BCS change categories and cumulative milk yield in early lactation up to 25 and 90 DIM, but peak and 305-d milk yield were greater in cows with extreme losses (loss ≥ 0.75 BCS units) compared with cows gaining BCS (≥ 0.25 units). Other studies found no association of BCS at calving or BCS change during early lactation with daily milk yield or daily energy-corrected milk yield in dairy cows [Pires et al., 2013 (up to 7 wk pp); Carvalho et al., 2014 (up to 3 wk pp)].

As shown in the Sankey plot, we observed that most OC cows (98% of VF and 88% of JF cows) were assigned to the Δ BFT clusters with greater losses compared with NOR or LEN cows. Furthermore, in this study, OC cows with little or no BFT loss (OC-no loss) produced less milk than OC cows with severe to normal losses (OC-loss). Likely, OC cows that do not lose their BCS pp (d 31 ± 3 pp) do not have the genetic merit to produce more milk. Smith and McNamara (1990) reported that cows with higher genetic merit for milk production experience greater lipolysis and mobilization of body reserves during early lactation, resulting in greater BCS loss and NEB.

It is well documented that with increased lipolysis and release of NEFA from adipose tissue into the bloodstream, the capacity for fatty acid oxidation is insufficient, and thus re-esterification of fatty acid to triglyc-

erides in the liver increases, which is further augmented by the limited capacity of the liver to export lipids as very-low-density lipoproteins. In consequence, ketogenesis and fatty liver increase (McFadden, 2020; Ghaffari et al., 2021). Increased concentrations of NEFA, BHB, and ceramides (Rico et al., 2015; McFadden and Rico, 2019) are related to greater risk for production diseases. In our study, we observed that BHB and NEFA values increased from parity 2 to 5, but were lesser in parity 6 and 7 compared with parity 5. However, the lesser values in parity 6 and 7 could be due to the relatively small sample size in our analysis. Circulating concentrations of BHB and NEFA have been reported to reach greater levels in multiparous than in primiparous cows (Meikle et al., 2004).

We also observed greater blood concentrations of NEFA and BHB in VF and JF as well as EL and ML cows compared with the other clusters. Moreover, cows with severe to normal loss (OC-loss) had greater NEFA and BHB concentrations than OC-no loss cows, indicating greater NEB and a greater allocation of energy to the mammary gland to produce more milk. Metabolic pathway enrichment analysis in a recent serum metabolomics study by Ghaffari et al. (2019) showed that branched-chain amino acid degradation before calving and mitochondrial β -oxidation of long-chain fatty acids along with fatty acid metabolism after calving were significantly enriched in overconditioned versus normal-conditioned cows. Further studies have found that overconditioning at calving can lead to greater BCS losses after calving, resulting in mitochondrial lipid overload and incomplete fatty acid oxidation in the liver (Ghaffari et al., 2021). Furthermore, using targeted metabolomics, divergent metabolotypes have been identified even within well-characterized phenotypes with high BCS and BFT at calving and severe subsequent BCS and BFT loss (Ghaffari et al., 2020). Thus, individual cows with comparable body fat portions appear to differ in how they manage their fat reserves during the transition period, even when fed and managed in the same manner.

In the current study, serum leptin concentrations decreased after calving in all clusters, as shown in previous studies (Reist et al., 2003; Kokkonen et al., 2005; Schuh et al., 2019), which is associated with the onset of NEB and a decrease in feed intake (Chilliard et al., 2005). The high leptin concentrations in VF cows are consistent with the notion that adiposity is an important determinant of leptinemia (Chilliard et al., 2005). Similarly, in a previous study, ap plasma leptin concentration was highest in the high BCS group and did not differ between the moderate and low BCS groups (Pires et al., 2013). However, no BFT-ap effects were observed on leptin concentrations after calving,

as previously observed (Holtenius et al., 2003; Pires et al., 2013).

In the current study, serum adiponectin concentration decreased after calving. The decrease in blood adiponectin concentration and lower mRNA abundance in tissues after calving may be due to increased lipolysis (Singh et al., 2014). In the current study, serum adiponectin concentration was affected only by the Δ BFT cluster and was lower in EL cows compared with the other clusters. Our results suggest that BFT is more closely related to leptin than adiponectin in dairy cows during the ap period, but this relationship might disappear as parturition approaches because adiponectin and leptin concentrations decrease after calving. Therefore, BFT loss pp associated with NEB may become more important than the degree of ap body fat in determining adiponectin concentration (Giesy et al., 2012; Singh et al., 2014; De Koster et al., 2017). This could explain why the degree of BFT loss was not associated with ap leptin concentration in our experiment. Unfortunately, as a limitation of the current study feed intake could not be recorded, we cannot substantiate the potential relationship of BFT loss with feed intake and energy balance.

Besides being an acute-phase protein, Hp has also been identified as an adipokine in cattle (Saremi et al., 2012). However, given the much lower abundance of Hp mRNA in adipose tissue compared with liver, the contribution of adipose tissue-derived Hp to blood levels is likely well below 1%, even considering the greater tissue mass of adipose tissue (Saremi et al., 2012). Accordingly, we did not detect differences in circulating Hp concentrations in any of the BFT-related comparisons. In terms of animal health, differences might have been expected when comparing cows with different health statuses, especially for uterine infections; however, this was not the case, but the association between Hp concentrations and metritis was also reported to be weak (Hirvonen et al., 1999; Pohl et al., 2015) or even absent (Yasui et al., 2014).

This study was limited by the fact that daily DMI could not be recorded in the commercial herd in which the study was performed, although DMI is likely associated with changes in BCS. As a result, it was not possible to correlate the blood parameters of cows in different BFT clusters with their energy balance. The animals in this study were from a feeding trial in which supplementation with rumen-protected MET was tested. Although the diets contained enough MET, MET was supplemented to see whether more MET could affect reproduction. The MET treatment did not affect performance and health status, as reported previously (Süss et al., 2019). Considering that MET is involved in one-carbon metabolism (Vailati-Riboni

et al., 2020) and regulates lipid metabolism via the AMPK pathway to increase lipid oxidation in NEFA-treated hepatocytes (Shen et al., 2020), MET might affect lipid metabolism in dairy cows. The portion of hyperketotic cows (≥ 1.2 mmol/L BHB) from the basic trial, with a total of 1,709 cows, was not different in the control and the MET-supplemented group (11.7% vs. 12.3%); furthermore, the proportions of cows with BFT < 14 mm were the same in both groups (81.9% and 82.6%, respectively; Süss et al., 2019). In contrast, in the current study, with a subset of these cows, treatment of MET was significant for BHB concentrations in the BFT-ap and Δ BFT comparisons, and we detected a trend for an effect of MET supplementation on blood BHB concentration in the OC-loss versus no loss comparison. The proportions of MET-supplemented and non-supplemented cows were similar in each cluster (Supplemental Figure S1; <https://doi.org/10.6084/m9.figshare.19294499.v1>). However, given that these differences were limited to the ap period, when MET supplementation was not yet started, the significance for MET in the general model, which included both ap and pp values, is not considered as meaningful, and we thus refrain from further interpretation. In general, the lack of differences between the control group and the MET group on any of the variables tested could be related to the relatively late start of supplementation (i.e., only within the first 20 d after calving). Batistel et al. (2017) recommended starting supplementation 1 wk before calving and continuing it for at least 60 d after calving, to observe differences in lactation performance.

CONCLUSIONS

The variables measured in blood confirmed that cows with greater BFT losses had increased lipolysis and ketogenesis. Cows that gained BFT did not differ from normal cows in their serum variables. Differences in milk yield were limited to the BFT-ap clusters, with cows categorized as VF producing less milk than the other clusters. Non-uniform adaptive responses to lactation were particularly observed for the overconditioned cows: OC-no loss cows produced less milk and also had increased lipolysis and ketogenesis compared with OC-loss cows. This study outlines the variability in the intensity of mobilizing body fat reserves in response to metabolic changes related to the onset of lactation in cows of different body fat content before calving. The findings, derived from a relatively large number of cows from the same herd on a commercial dairy enterprise, are of interest for both dairy farmers and researchers; the clustering approaches described herein provide a quick and efficient method to identify extreme groups for specific management and also for outlining further

comparisons, as when planning more expensive and sophisticated analyses such as proteomics or metabolomics on selected samples.

ACKNOWLEDGMENTS

This project received funding from the European Union's Horizon 2020 research and innovation program H2020-MSCA-ITN-2017-EJD: Marie Skłodowska-Curie Innovative Training Networks (European Joint Doctorate) – grant agreement no. 765423 – MANNA (European Joint Doctorate in Molecular Animal Nutrition). The authors have not stated any conflicts of interest.

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