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Association between hyperketolactia and production in early-lactating dairy cows

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ABSTRACT

Study aims were to investigate associations of hyperketolactia (HYKL) status of Holstein dairy cows between 6 and 60 d in milk (DIM), defined by milk acetone (mACE) and β-hydroxybutyrate (mBHB) content, with daily milk yield and composition. Milk samples (~5.0 million) were collected over a 5-yr period (2014–2019) within the milk recording system in Poland. Concentrations of mACE and mBHB determined by Fourier-transform infrared spectroscopy were used to categorize samples into 4 ketolactia groups. Based on threshold values of ≥0.15 mmol/L mACE and ≥0.10 mmol/L mBHB, ketolactia groups were normoketolactia (NKL; mACE <0.15 mmol/L and mBHB <0.10 mmol/L), BHB hyperketolactia ($HYKL_{BHB}$; mACE <0.15 mmol/L and mBHB ≥0.10 mmol/L), ACE hyperketolactia ($HYKL_{ACE}$; mACE ≥0.15 mmol/L and mBHB <0.10 mmol/L), and ACE and BHB hyperketolactia ($HYKL_{ACEBHB}$; mACE ≥0.15 mmol/L and mBHB ≥0.10 mmol/L). To investigate ketolactia association with production outcomes, a linear model was developed, including ketolactia group, DIM, parity, their interactions, year-season as fixed effects, and random effects of herd and cow. Among all milk samples, 31.2% were classified as HYKL, and of these, 52.6%, 39.6%, and 7.8% were $HYKL_{ACEBHB}$, $HYKL_{BHB}$, and $HYKL_{ACE}$, respectively. Ketolactia groups differed for all traits studied in all parities and DIM. Among HYKL groups, lowest milk yield was found in $HYKL_{ACEBHB}$ cows, except for 6 to 30 DIM in first- and second-lactation cows. Milk yield of $HYKL_{BHB}$ cows was higher than that of NKL cows until 20 to 30 DIM, and then it was lower than NKL cows. Milk yield of $HYKL_{ACE}$ cows was mostly lower than NKL cows. Energy-corrected milk (ECM) yield of $HYKL_{ACEBHB}$ cows was higher than that of NKL cows until 30 to 35 DIM for second lactation and third lactation or greater, and in the whole study period for first lactation. The yield of ECM for $HYKL_{BHB}$ cows was mostly higher than that of NKL cows, whereas $HYKL_{ACE}$ cows had higher ECM than NKL cows until 15 to 25 DIM and then was lower for the $HYKL_{ACE}$ group. Milk composition differed among HYKL groups. Highest milk fat (MF) and lowest milk lactose (ML) contents were observed in $HYKL_{ACEBHB}$ cows. Cows in $HYKL_{ACEBHB}$ and $HYKL_{BHB}$ groups had higher MF and lower milk protein (MP; except in 6–8 DIM in first lactation) and ML content than NKL cows. Milk fat content was higher in $HYKL_{ACE}$ than NKL cows in first lactation and during the first 30 to 40 DIM in older cows. Lactose content was lower in $HYKL_{ACE}$ than in NKL cows within 30 to 40 DIM; afterward it was higher in NKL cows. Lower MP content was found in $HYKL_{ACE}$ than in NKL cows, except during 6 to 9 DIM for cows in first lactation and third lactation or greater. In conclusion, HYKL is associated with altered milk production in all parities, but a range of these negative relations depends on ketone status addressing both ACE and BHB contents. Further research is needed to ascertain underpinning biochemical defects of HYKL from elevated ACE, alone or in combination with BHB, during early lactation.

Key words: milk acetone, milk β-hydroxybutyrate, hyperketolactia, production outcomes

INTRODUCTION

Altered production outcomes associated with hyperketonemia, defined as an increased concentration of blood ketone bodies, are well described, with reduced

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milk yield, poor reproduction, higher risk of infectious, metabolic diseases, and displaced abomasum, as well as an increased rate of culling being the most often documented (Duffield et al., 2009; Ospina et al., 2010; McArt et al., 2012; Raboisson et al., 2014; Abdelli et al., 2017; Rodriguez et al., 2022). Adverse hyperketonemia outcomes have always been associated with elevated blood BHB concentration, as this analyte is considered the gold standard for hyperketonemia diagnosis (Duffield 2000; Ospina et al., 2010). This perspective does not consider the presence of other circulating ketone bodies, such as acetone (**ACE**) and acetoacetic acid, which are important molecules in ketogenesis (Bergman, 1971). Omission of ACE in diagnostic models is a consequence of difficulties in quantitative blood assessment (Tyopponen and Kauppinen, 1980; Fritzsche et al., 2001).

In contrast to blood, ACE is stable in milk and can be assessed by Fourier-transform infrared (**FTIR**) spectroscopy (Hansen, 1999; Heuer et al., 2001; de Roos et al., 2007). This analytical technique has been developed for use in milk recording systems to measure milk components, including milk BHB (**mBHB**) and ACE (**mACE**) concentrations (de Roos et al., 2007; van der Drift et al., 2012). Although FTIR has some well-known limitations (Caldeira et al., 2020), determination of mACE and mBHB via FTIR makes milk analysis a potential monitoring tool for hyperketolactia (**HYKL**), defined as elevated content of ketone bodies in milk (Emery et al., 1968; Pralle and White, 2020). Diagnostic HYKL models are based on phenotypic correlations between milk and blood concentrations of ACE ($r = 0.96$) or BHB ($r = 0.66$; Enjalbert et al., 2001) as well as between mACE or mBHB determined by either milk standard chemical analyses or FTIR (de Roos et al., 2007).

Because FTIR allows for mACE determination, it may also allow for identification of cows with elevated mACE content exclusively (acetone-alone type hyperketolactia; $\mathbf{HYKL_{ACE}}$) or in combination with elevated mBHB ($\mathbf{HYKL_{ACEBHB}}$), which may represent different metabolic consequences compared with elevated mBHB alone ($\mathbf{HYKL_{BHB}}$). Previously we reported approximately 8% of HYKL milk samples from early-lactating Polish Holstein-Friesian cows defined solely by elevated mACE (≥0.15 mmol/L) without elevated mBHB (<0.10 mmol/L; Kowalski et al., 2021). Milk ketone thresholds were based on previous reports (de Roos et al., 2007). Acetone-based HYKL was especially high (about 15%) among samples originating from primiparous cows, collected within 6 to 21 DIM (Kowalski et al., 2021).

Unlike hyperketonemia, studies showing cow production and reproductive performance outcomes associated with HYKL cows are infrequent. Impaired reproductive performance was associated with elevated mACE or mBHB concentrations (Albaaj et al., 2019). Excessive mBHB has been linked with fresh cow diseases such as dystocia, retained placenta, and mastitis (Hejel et al., 2018). Santschi et al. (2016) reported that cows with elevated mBHB (≥0.20 mmol/L; mACE was not considered) between 5 and 35 DIM had lower milk yield, protein concentration and yield, higher milk fat concentration and yield, and higher SCC compared with cows without elevated mBHB. Moreover, milk yield difference between $HYKL_{BHB}$ negative and positive cows increased with progressing weeks of lactation. This contrasts with cows developing hyperketonemia in the first week postpartum having lower milk yield than cows that developed hyperketonemia after the first week of lactation (McArt et al., 2012). In other studies milk yield and protein concentration declined, and fat concentration and fat-to-protein ratio increased with increasing mBHB or mACE concentrations (Chandler et al., 2018; Klein et al., 2020). Others found that cows above a defined mBHB cut point produced 2.1 kg/d more average daily milk yield during the first 15 wk of lactation compared with cows below the cut point (Bach et al., 2019). In these studies, HYKL was characterized by mBHB content (Santschi et al., 2016; Hejel et al., 2018; Bach et al., 2019), and none considered cows having elevated mACE alone.

Objectives of this population study were to investigate the associations of different HYKL states, defined by mACE and mBHB concentrations, with milk yield and composition of Polish Holstein-Friesian cows during 6 to 60 DIM. We hypothesized that (1) HYKL occurring in early lactation is associated with impaired cow milk performance; (2) HYKL states originating from elevated mACE and mBHB, mBHB alone, or mACE alone are differently associated with performance outcomes; and (3) these effects depend on DIM and parity.

MATERIALS AND METHODS

Because no human or animal subjects were used, this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Data Sets

The initial study data set was made available by the Polish Federation of Cattle Breeders and Dairy Farmers, which provides monthly milk recording of cows in Poland. Milk samples from only Holstein-Friesian cows were considered for this study. The initial data set con-

sisted of milk samples collected over a 5-yr period (Jan. 4, 2014, to Mar. 31, 2019), from approximately 23,443 dairy herds (1–1,402 cows/herd, average 40.1 cows/herd). According to milk recording system procedures, d 6 was the first day after calving that milk samples were collected. Milk samples originating from the first 2 test-days postpartum were included; thus all study milk samples ranged between 6 and 60 DIM. Milk samples collected within 6 to 30 and 31 to 60 DIM were classified as obtained on first (**1TD**) and second (**2TD**) test-days, respectively.

Given that compiled data cover a 5-yr period and, in the system of monitoring of ketosis in Poland, 2 test-days in the first 2 months of lactation are considered, an individual cow could have between 1 and 12 milk samples in the data set. In total, milk samples were collected from 1,602,831 cows representing 3,102,201 cow lactations.

The initial data set consisted of chemical composition of 5,041,140 milk samples, including concentrations of fat, crude protein, lactose, mACE, and mBHB. Data on mBHB and mACE concentrations were collected within the ketosis monitoring system, which was introduced in Poland in 2013, as a part of milk recording (Kowalski et al., 2015). All milk samples were collected on test-days by trained sampling technicians. Details on sampling procedures and sample handling are presented in Kowalski et al. (2021). The FTIR instruments (MilkoScan FT 6000; Foss Analytical A/S, Hillerød, Denmark) were standardized monthly following International Committee on Animal Recordings (ICAR) guidelines for routine milk components. Instruments were calibrated for mACE and mBHB determination according to de Roos et al. (2007).

Samples with abnormal values of any milk parameter, except mACE or mBHB, were censored from the data set. We considered abnormal values as errors in milk analysis. Censored values included 1 or more of the following from any milk sample: no data on milk composition; protein content less than 2% or more than 7%; fat content less than 1% or more than 12%; and lactose content less than 3% or more than 6%. To create the basic data set, consisting of 5,017,004 milk samples, we excluded 24,136 samples from the initial data set.

Within the data set, each milk sample had data on DIM of the test-day, daily milk yield (**MY**, kg/d), milk fat content (**MF**, %), milk fat yield (**MFY**, kg/d), milk crude protein content (**MP**, %), milk protein yield (**MPY**, kg/d), ECM yield (kg/d), milk lactose content (**ML**, %), and milk lactose yield (**MLY**, kg/d), together with lactation number. Test-day number was assigned based on DIM as described. Yield of ECM was calculated as ECM = 0.327 × MY + 12.95 × MFY + 7.65 × 0.93 × MPY (Tyrrell and Reid, 1965).

Ketolactia Groups and Subpopulations

As in our previous study (Kowalski et al., 2021), published mACE (≥0.15 mmol/L) and mBHB (≥0.10 mmol/L) threshold concentrations considered to define HYKL status (de Roos et al., 2007) were used to categorize milk samples of the basic data set into ketolactia groups: normal with nonelevated milk ketone bodies (**NKL**; mACE <0.15 mmol/L and mBHB <0.10 mmol/L) and hyperketolactic (HYKL; mACE ≥0.15 mmol/L or mBHB ≥0.10 mmol/L). Within the HYKL group, samples were categorized into 3 subpopulations based on elevated mACE alone ($HYKL_{ACE}$; mACE ≥0.15 mmol/L and mBHB <0.10 mmol/L), mBHB alone ($HYKL_{BHB}$; mACE <0.15 mmol/L and mBHB ≥0.10 mmol/L), or both ($HYKL_{ACEBHB}$; mACE ≥0.15 mmol/L and mBHB ≥0.10 mmol/L). In the statistical analysis, the NKL group was considered as the fourth subpopulation.

Statistical Analyses

Descriptive statistics for milk components, including mACE and mBHB concentrations, were calculated using the R environment (R Core Team, 2020). The R package ggplot2 (Wickham, 2016) was used to create all figures.

Univariate analyses were performed to determine the association of ketolactia subpopulation, parity, DIM, and their interactions with MY and components, using the following linear model:

$$y_{ijklmo} = YS_i + K_j + P_k + DIM_l + K_j \times P_k + P_k \times DIM_l + K_j \times DIM_l + K_j \times P_k \times DIM_l + H_m + C_o + \varepsilon, \quad [1]$$

where y_{ijklmo} = dependent variable of interest (i.e., MY, milk component contents or yields); YS_i = fixed effect of year-season of calving (i = 1–20; 5 years × 4 seasons, i.e., winter = January, February, March; spring = April, May, June; summer = July, August, September; autumn = October, November, December); K_j = fixed effect of ketolactia subpopulation (j = NKL, $HYKL_{ACEBHB}$, $HYKL_{BHB}$, or $HYKL_{ACE}$); P_k = fixed effect of parity (k = 1, 2, or ≥3); DIM_l = fixed effect of DIM (l = 6–60); H_m = random herd effect (m = 1–23,443) proportional to $N\left(0,\mathrm{I}\sigma^2_H\right)$, where σ^2_H is the variance of herd effects; C_o = random cow effect (o = 1–1,602,831) proportional to $N\left(0,\mathrm{I}\sigma^2_c\right)$, where σ^2_c is the variance of cow effects; and ε = residual error $\sim N\left(0,\mathrm{I}\sigma^2_e\right)$, where σ^2_e is the residual variance. The PREDICT statement of Echidna software (Gilmour,

2021) was used to generate least squares means (**LSM**) for effects of parity, DIM, and ketolactia subpopulation × parity × DIM.

Because year-season of calving (*YS*) subclasses were large and included cows that fell into each ketolactia subpopulation, *YS* was treated as a fixed effect to account for environmental effects. The herd was treated as random because some herds are small and may not have cows from every ketolactia subpopulation. To check whether milk yield and composition parameters are different for analysis of 4 subpopulations (NKL, $HYKL_{ACEBHB}$, $HYKL_{BHB}$, $HYKL_{ACE}$), a *t*-test with Bonferroni adjustment was used for LSM multiple comparisons. In this adjustment, the accepted statistical significance ($P \leq 0.05$) was revised by the number of comparisons N ($P < 0.05/N$). Comparison number accounted for 4 ketolactia subpopulations, 3 parities, and 55 DIM, for a total of 660 comparisons. Data are presented as LSM ± standard error of the mean, except where noted.

Given the large data set, each DIM was considered a class variable, allowing for characterization of change in parameters studied over time. Specific day-to-day mean comparisons were not of interest, except where noted, but changes in their values over the study time period (6–60 DIM) were of primary interest.

In place of ketolactia subpopulation, we also regressed MY and milk component contents or yields on the concentrations of mACE and mBHB with the following model:

$$y_{iklmno} = YS_i + P_k + DIM_l + P_k \times DIM_l + \sum_{j=1}^{n} b_{aj} \times mACE^n \text{ (or } mBHB) + b_{apd} \times mACE \text{ (or } mBHB) \times P_k \times DIM_l + H_m + C_o + \varepsilon, \quad [2]$$

where y_{iklmno}, YS_i, P_k, DIM_l, H_m, C_o, and ε are as described above; b_{aj} = regression coefficients on mACE (or mBHB); b_{apd} = regression coefficient on mACE (or mBHB) nested in $P_k \times DIM_l$. Because of the large number of observations, regression coefficients were significant on high-order polynomials (n tested up to the fifth order) of mACE and mBHB. However, ε was not reduced, and inferences related to the level of mACE and mBHB did not change for n >1, so we elected to set n = 1 for simplicity and ease of interpretation. For the same reason, we limited interactions to the first order of mACE and mBHB. The PREDICT statement of Echidna software (Gilmour, 2021) was used to generate LSM for effects of parity, DIM, mACE (mACE = 0, 0.01, 0.02, . . . , 0.40), or mBHB (mBHB = 0, 0.01, 0.02, . . . , 0.40) × parity × DIM.

RESULTS

Milk Sample Demographics

The study was conducted with approximately 5 million milk samples (Table 1). Approximately 33.7% (n = 1,690,731), 26.0% (n = 1,305,677), and 40.3% (n = 2,020,596) of milk samples were from primiparous cows, second-lactation cows, and cows in third lactation or greater (**≥3**), respectively (Table 1). Mean (±SD) number of milk samples per cow was 3.1 ± 1.9 (median 3.0; range 1–12). A total of 54 cows had 12 samples. Overall, raw population mean mACE and mBHB concentrations were 0.10 ± 0.16 mmol/L (median 0.06; range 0.00–4.89) and 0.08 ± 0.09 mmol/L (median 0.06; range 0.00–3.94), respectively.

A mACE concentration ≥0.15 mmol/L was found in 18.8% of all milk samples (Figure 1). Frequency of milk samples with mACE ≥0.15 mmol/L was similar in samples originating from first- (20.0%) and ≥3-lactation cows (20.9%), and it was lowest for second-lactation cows (14.2%). A mBHB concentration ≥0.10 mmol/L was found in 28.7% of all milk samples. Frequency of milk samples with mBHB ≥0.10 mmol/L was similar in samples originating from first- (25.4%) and second-lactation cows (25.5%), and it was greater for ≥3-lactation cows (33.6%).

Among all milk samples, 68.8 and 31.2% were classified as NKL and HYKL, respectively (Table 2). Proportion of HYKL milk samples was highest for ≥3-lactation cows (35.7%), whereas no difference occurred between first- (28.8%) and second-lactation cows (27.3%). Among all HYKL milk samples, we found 52.6%, 39.6%, and 7.8% of $HYKL_{ACEBHB}$, $HYKL_{BHB}$, and $HYKL_{ACE}$, respectively. Of the 1,565,293 total milk samples classified as HYKL, 122,717 were classified as $HYKL_{ACE}$. The greatest percent of $HYKL_{ACE}$ milk samples was found among primiparous cows (11.7% of HYKL).

More HYKL milk samples were found in 1TD (38.8%; Table 2) than in 2TD (24.5%). Among HYKL milk samples collected on 1TD, $HYKL_{ACEBHB}$ dominated (60.7%), whereas among those collected on 2TD, $HYKL_{BHB}$-classified samples were most frequent (53.2%). Percentage of $HYKL_{ACE}$ samples was greater among 1TD than 2TD milk samples (9.4 vs. 5.6%, respectively).

Considering all milk samples in the data set, fixed effects of HYKL subpopulation, parity, DIM, and their

interactions influenced milk yield and composition parameters (all $P < 0.001$).

Milk Yield

Mean MY (LSM ± SE) within 6 to 60 DIM for cows in lactation 1, 2, and ≥3 were 24.0 ± 0.13, 29.6 ± 0.17, and 30.5 ± 0.13 kg/d, respectively. Mean MY (kg/d) of $HYKL_{BHB}$ (28.7 ± 0.11), $HYKL_{ACE}$ (27.6 ± 0.29), and $HYKL_{ACEBHB}$ cows (26.9 ± 0.11) were lower compared with NKL cows (28.9 ± 0.06). In all parity categories, MY of $HYKL_{BHB}$ cows was higher than NKL cows until 20 to 30 DIM, and then it was lower (Figure 2). In contrast, MY of $HYKL_{ACEBHB}$ cows in first and second lactations, but not lactation ≥3, was higher than NKL cows until 10 to 14 DIM, and then it was lower, being 90% to 93% of NKL cows' MY at approximately 30 to 60 DIM. In the second month of lactation, MY of $HYKL_{ACEBHB}$ cows was lowest among HYKL groups. Across parities, MY of $HYKL_{ACE}$ cows was always lower than NKL cows, except for 6 to 9 DIM for second-lactation cows. The difference between MY of NKL and $HYKL_{ACE}$ cows was greatest in cows in lactation ≥3, especially between 18 and 60 DIM, where MY was 92% to 96% that of NKL cows.

Mean ECM yield (kg/d) within 6 to 60 DIM of cows in lactation 1, 2, and ≥3 was 26.0 ± 0.14, 32.0 ± 0.18, and 33.2 ± 0.14 kg/d, respectively. Mean ECM yield of $HYKL_{BHB}$ and $HYKL_{ACEBHB}$ of first-lactation cows were always higher than NKL cows (Figure 3). For $HYKL_{ACEBHB}$ ECM yield was higher than NKL for second- and ≥3-lactation cows within 6 to 33 DIM, and then it was lower. Yield of ECM of $HYKL_{BHB}$ of second- and ≥3-lactation cows was always higher than NKLcows, except for some days within 48 to 60 DIM for second-lactation cows. Yield of ECM of $HYKL_{BHB}$ was always higher than other HYKL groups, except for 6 to 28 DIM in all parity groups. For $HYKL_{ACE}$ cows ECM yield was higher than NKL cows until 15 to 30 DIM, and then it was lower, especially in lactation 2 and ≥3. For these parity groups during the 30 to 60 DIM period, ECM yield was 90% to 94% that of NKL cows.

Milk Composition

Mean MF concentration was always higher in milk samples originating from HYKL compared with NKL cows, except for $HYKL_{ACE}$ cows in second and ≥3 lactation in the second month of lactation (Figure 4). In all parity categories, and in all 6 to 60 DIM, the highest MF content was always found among samples of $HYKL_{ACEBHB}$ cows (mean MF 4.73 ± 0.013%), and it was 118% to 126% that of MF for NKL cows (3.86 ± 0.006%). Cows of the $HYKL_{BHB}$ group (4.27 ± 0.013%) had higher MF concentration (110%) compared with NKL cows. Across parity groups, $HYKL_{ACE}$ cow MF content (4.06 ± 0.034%) decreased compared with NKL cows with increasing DIM from 18 or 25 to 60 DIM.

Table 1. Raw characteristics of Polish Holstein-Friesian cows and milk samples collected in the 5-yr period from Jan. 4, 2014, to Mar. 31, 2019[1]

Item	Number	Mean	Median	Minimum	Maximum	SD	CV
Number of milk samples							
All	5,017,004						
From first-lactation cows	1,690,731						
From second-lactation cows	1,305,677						
From ≥third-lactation cows	2,020,596						
From cows within 6–30 DIM	2,365,536						
From cows within 31–60 DIM	2,651,468						
Cows characteristics on test-days							
Number of cows	1,602,831						
Number of cow lactations	3,102,201						
Parity		2.5	2.0	1.0	12.0	1.7	65.6
DIM		31.9	32.0	6.0	60.0	15.8	49.6
Milk (kg/d)		31.7	30.8	0.2	99.8	9.3	29.3
ECM[2] (kg/d)		34.0	33.1	0.2	141.6	10.0	29.4
Number of milk samples per cow		3.1	3.0	1.0	12.0	1.9	59.2
Milk composition							
Protein (%)		3.12	3.08	2.00	7.00	0.36	11.6
Fat (%)		4.10	3.98	1.00	12.00	0.95	23.3
Lactose (%)		4.81	4.83	3.00	5.92	0.22	4.5
Fat/protein		1.32	1.28	0.22	5.19	0.30	23.0
mACE (mmol/L)		0.10	0.06	0.00	4.89	0.16	166.0
mBHB (mmol/L)		0.08	0.06	0.00	3.94	0.09	114.8

[1]mACE = milk acetone; mBHB = milk BHB.

[2]ECM calculated as 0.327 × MY + 12.95 × MFY + 7.65 × 0.93 × MPY, where MY = milk yield, MFY = milk fat yield, and MPY = milk protein yield.

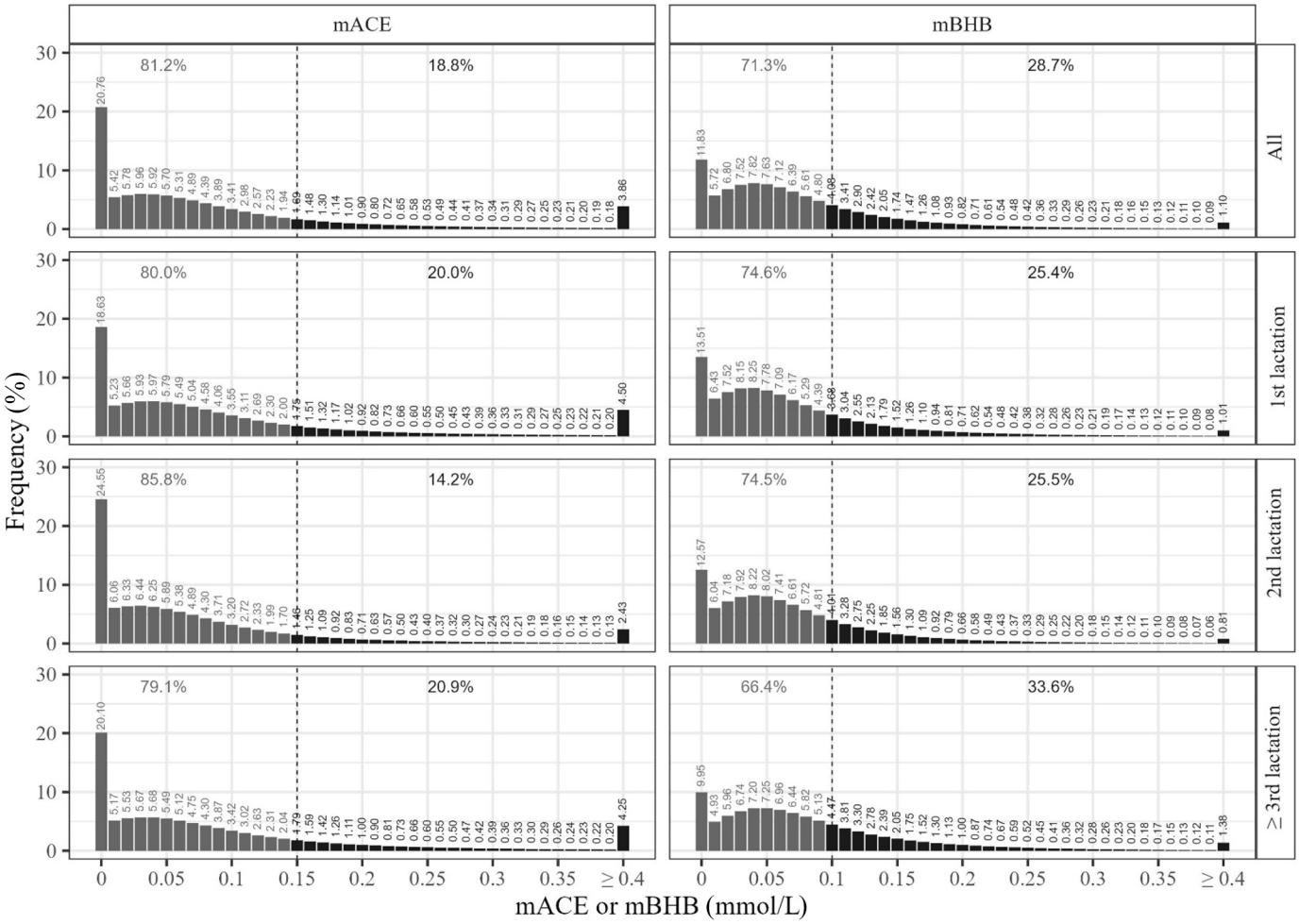

Figure 1. Frequency distribution of milk acetone (mACE) and milk BHB (mBHB) concentrations in all milk samples and in milk samples classified by parity of cows. Percentage of milk samples with mACE and mBHB concentrations above or below 0.15 mmol/L and 0.10 mmol/L, respectively, are indicated.

Mean MFY was always higher in HYKL cows than in NKL cows, except for $HYKL_{ACE}$ cows in the second month of lactation (Figure 5). Highest MFY was always found among $HYKL_{ACEBHB}$ cows (1.27 ± 0.006 kg/d), and in relation to MFY for NKL cows (1.10 ± 0.003 kg/d), it decreased from 126% to 128% at the start of lactation to 110% to 112% at 60 DIM. Yield of MF in $HYKL_{BHB}$ cows (1.23 ± 0.006 kg/d) was 110% to 118% of the MFY of NKL cows. Across parity groups, MFY of $HYKL_{ACE}$ cows (1.10 ± 0.015 kg/d) decreased from 110% at the start of lactation to 86% to 88% on 50 to 60 DIM compared with NKL cows.

Mean MP content was always lower in milk samples of HYKL cows than in NKL (3.10 ± 0.002%), except for milk samples originating from $HYKL_{ACE}$ and $HYKL_{ACEBHB}$ cows in first lactation and for $HYKL_{ACE}$ cows in ≥3 lactation, at 6 to 8 DIM (Figure 6). Among first-lactation cows, lowest MP content was always found in $HYKL_{BHB}$ and $HYKL_{ACEBHB}$ samples. Although statistically significant, differences between HYKL subpopulations among cows in second and ≥3 lactation, within DIM studied, were not considered biologically meaningful.

Mean MPY was always lower in HYKL than NKL samples (0.89 ± 0.002 kg/d), except for $HYKL_{BHB}$ and $HYKL_{ACEBHB}$ of first-and second-lactation cows within 6 to 18 DIM (Figure 7), and for $HYKL_{ACE}$ of first-lactation cows at 6 DIM. Lowest MPY was found among $HYKL_{ACEBHB}$ cows (0.81 ± 0.003 kg/d), except for $HYKL_{ACE}$ up to 20 to 30 DIM for cows in all parities. In relation to MPY of NKL cows, MPY of $HYKL_{ACEBHB}$ cows decreased from about 100% to 102% at the start of lactation to 88% at 60 DIM, except for first-lactation cows in 6 to 21 DIM. The MP yield in $HYKL_{BHB}$ cows (0.86 ± 0.004 kg/d) decreased from the start of lactation until 60 DIM, when it was about 94% of MPY of

Table 2. Effect of parity and test-day on number and proportion of hyperketolactic (HYKL) milk samples collected in the 5-yr period from Jan. 4, 2014, to Mar. 31, 2019, from Polish Holstein-Friesian cows within 6–60 DIM

		HYKL[2]			
Item	NKL[1]	$HYKL_{ACEBHB}$[3]	$HYKL_{BHB}$[4]	$HYKL_{ACE}$[5]	All
Parity 1					
N milk samples	1,203,828	280,899	149,083	56,921	1,690,731
% of all	71.2	16.6	8.8	3.4	100.00
% of HYKL	—	57.7	30.6	11.7	—
Parity 2					
N milk samples	949,546	163,143	170,006	22,982	1,305,677
% of all	72.7	12.5	13.0	1.8	100.00
% of HYKL	—	45.8	47.7	6.5	—
Parity ≥3					
N milk samples	1,298,337	379,357	300,088	42,814	2,020,596
% of all	64.3	18.8	14.9	2.1	100.00
% of HYKL	—	52.5	41.6	5.9	—
All					
N milk samples	3,451,711	823,399	619,177	122,717	5,017,004
% of all	68.8	16.4	12.3	2. 5	100.00
% of HYKL	—	52.6	39.6	7.8	—
Test-d 1					
N milk samples	1,448,648	556,274	274,396	86,218	2,365,536
% of all	61.2	23.5	11.6	3.6	100.00
% of HYKL	—	60.7	29.9	9.4	—
Test-d 2					
N milk samples	2,003,063	267,125	344,781	36,499	2,651,468
% of all	75.6	10.1	13.0	1.4	100.00
% of HYKL	—	41.2	53.2	5.6	—

[1]NKL = normoketolactia; milk acetone (mACE) <0.15 mmol/L and milk BHB (mBHB) <0.10 mmol/L.
[2]HYKL; mACE ≥0.15 mmol/L or mBHB ≥0.10 mmol/L.
[3]$HYKL_{ACEBHB}$ = hyperketolactia with elevated ACE and BHB; mACE ≥0.15 mmol/L and mBHB ≥0.10 mmol/L.
[4]$HYKL_{BHB}$ = hyperketolactia with elevated BHB only; mACE <0.15 mmol/L and mBHB ≥0.10 mmol/L.
[5]$HYKL_{ACE}$ = hyperketolactia with elevated acetone only; mACE ≥0.15 mmol/L and mBHB <0.10 mmol/L.

NKL cows. Across parity groups, MPY of $HYKL_{ACE}$ (0.84 ± 0.009 kg/d) cows decreased from 100% at the start of lactation to 90% to 94% of MPY of NKL cows at 30 to 60 DIM.

Mean ML content was always lower in milk samples originating form HYKL compared with NKL cows, except for $HYKL_{ACE}$ in the second month of lactation (Figure 8). Across parity groups and DIM, lowest ML content was always found in milk samples of $HYKL_{ACEBHB}$ cows (4.73 ± 0.003%), at ∼98% of NKL cows (4.82 ± 0.001%). Lactose content of $HYKL_{BHB}$ milk samples was always lower than in NKL. Lactose content of $HYKL_{ACE}$ cows (4.81 ± 0.007%) was lower than NKL cows only within 6 to 35 DIM.

Mean MLY of $HYKL_{BHB}$ cows (1.37 ± 0.006 kg/d) exceeded MLY of NKL cows in the first month of lactation (Figure 9). Yield of ML in $HYKL_{ACE}$ (1.33 ± 0.014 kg/d) and especially $HYKL_{ACEBHB}$ (1.27 ± 0.006 kg/d) cows was always lower than in NKL (1.40 ± 0.003 kg/d). Regardless parity and DIM, MLY of $HYKL_{ACE}$ cows was about 94% to 96% of NKL cows MLY.

Mean MY losses caused by each 0.01 mmol/L increase in mACE were 0.039, 0.074, and 0.083 kg/d (0.15%, 0.23%, and 0.26%), respectively, for cows in lactations 1, 2, and ≥3 (Figure 10A and 10C). Moreover, in all parity categories, MY losses due to increase in mACE increased with progressing DIM. However, mean MY losses caused by each 0.01 mmol/L increase in mBHB were 0.056, 0.118, and 0.125 kg/d (0.22%, 0.37%, and 0.38%), respectively, for cows in lactations 1, 2, and ≥3 (Figure 10B and 10D). Similarly, as in mACE, in all parity categories, MY losses caused by increase in mBHB increased with progressing DIM. However, for first- and second-lactation cows, within 6 to 10 DIM, increasing mBHB by 0.01 mmol/L resulted in MY increases of 0.010 kg/d (0.05%) and 0.006 kg/d (0.02%), respectively.

DISCUSSION

Findings of this study suggest that altered milk production performance and milk component content in early-lactating Holstein-Friesian cows are associated

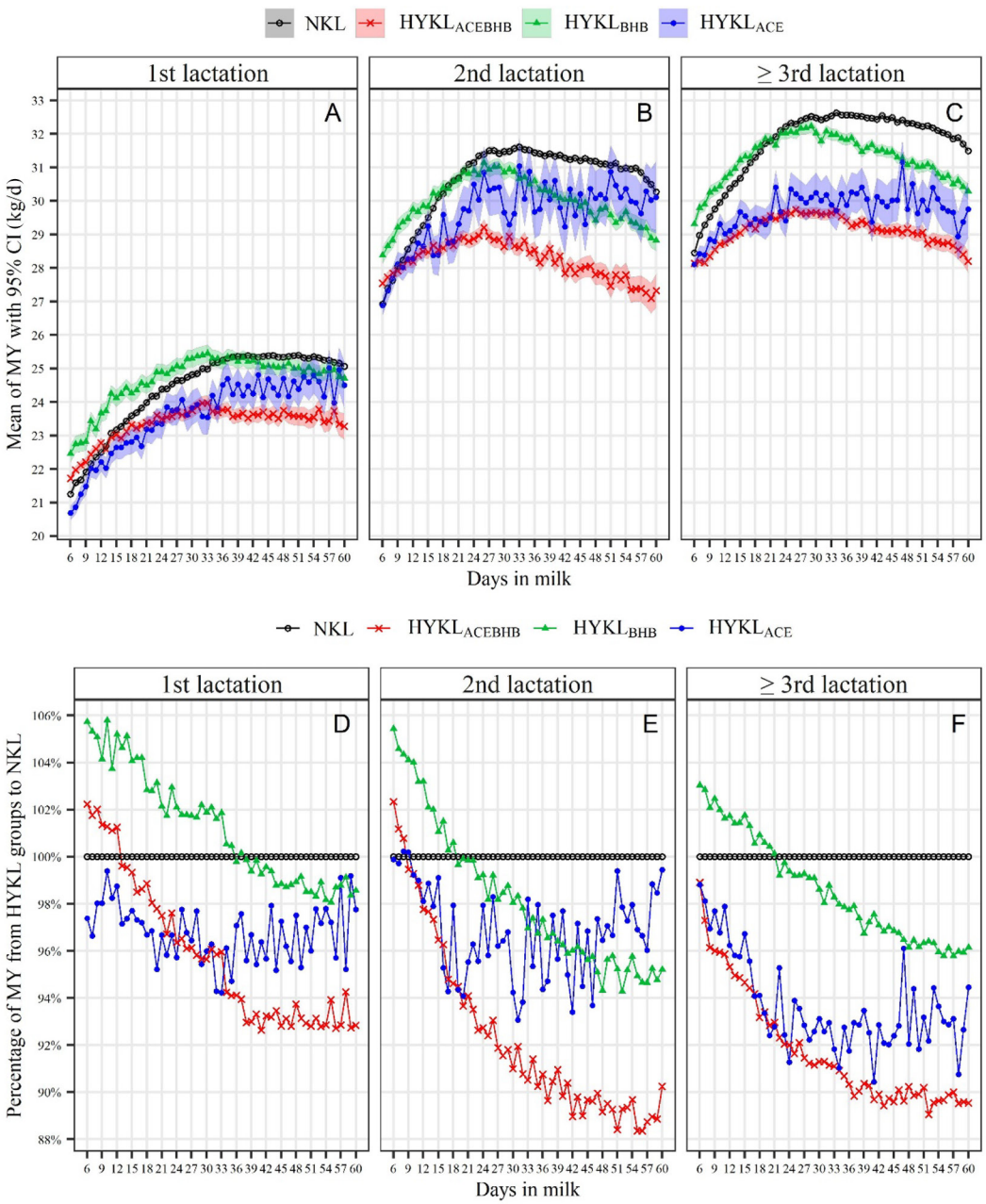

Figure 2. Least squares means, daily milk yield (MY, kg/d; panels A, B, C) or as percentage of MY of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

with increased milk concentrations of ACE, BHB, or their combination, relative to milk samples without ketone body elevations. A general assumption is that hyperketolactic cows produce less milk than cows without elevated milk ketones. However, study findings suggest that daily MY and milk content relationships between HYKL and NKL cows depend on the subpopulation of HYKL cows. To our knowledge, this is the first study presenting cow production outcomes classified into HYKL$_{ACEBHB}$, HYKL$_{BHB}$, and HYKL$_{ACE}$ subpopulations. Of interest is the differentially associated response relative to the defined HYKL subgroups, suggesting some underlying metabolic differences in ketogenesis.

Hyperketolactia Statistics

The proportion of milk samples originating from hyperketolactic cows in the present experiment (31.2% of all samples) is consistent with other reports for the prevalence of either hyperketonemia (22.9–47.2%; Vanholder et al., 2015; Mann et al., 2016) or HYKL (29.3% in Hejel et al., 2018; 39% in Berge and Vertenten, 2014; 22.6% in Santschi et al., 2016; 21% in Tatone et al., 2017), but higher than in other studies (e.g., 11% in van der Drift et al., 2012). In studies by Hejel et al. (2018) and Berge and Vertenten (2014), HYKL was

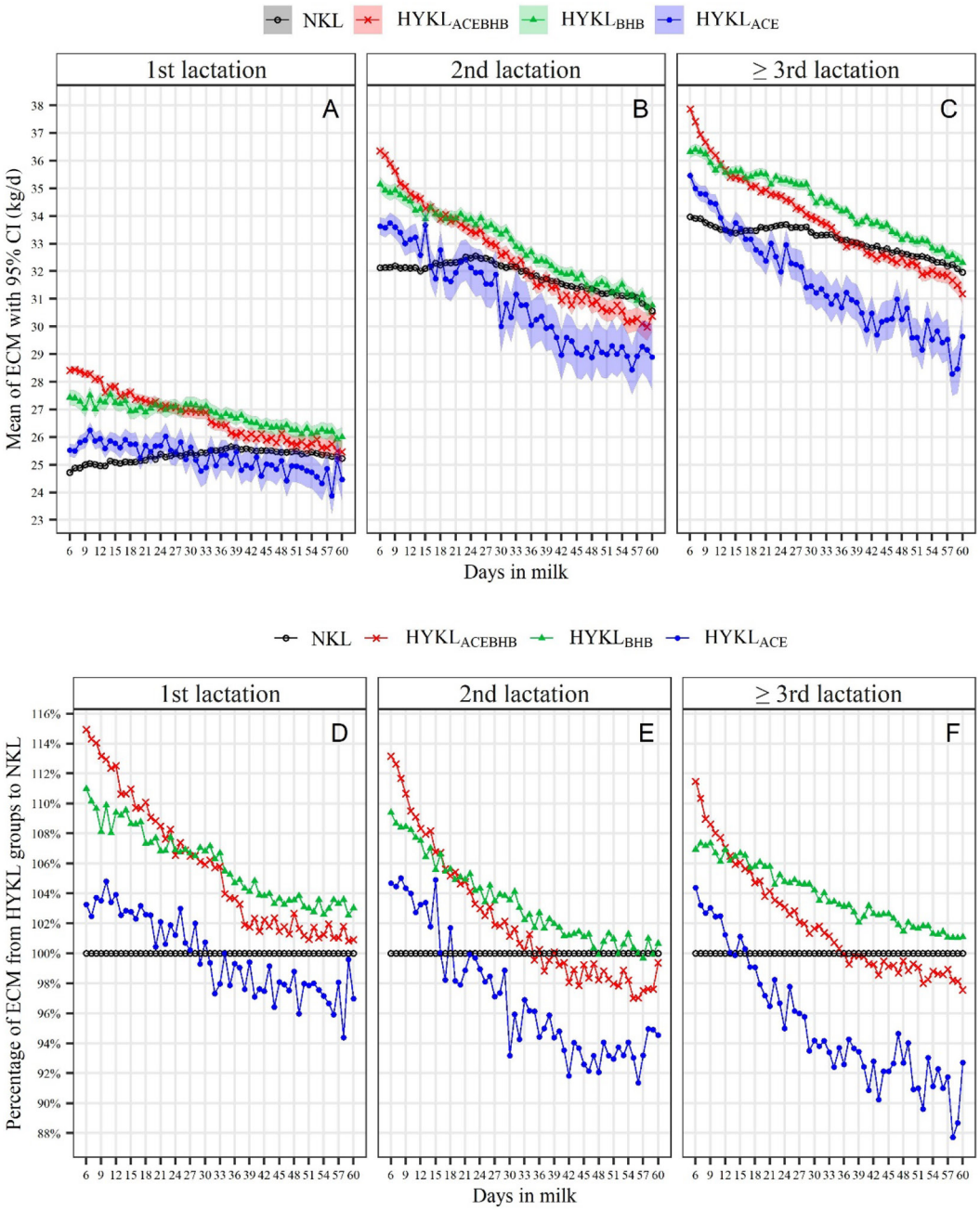

Figure 3. Least squares means, daily ECM yield (kg/d; panels A, B, C) or as percentage of ECM of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

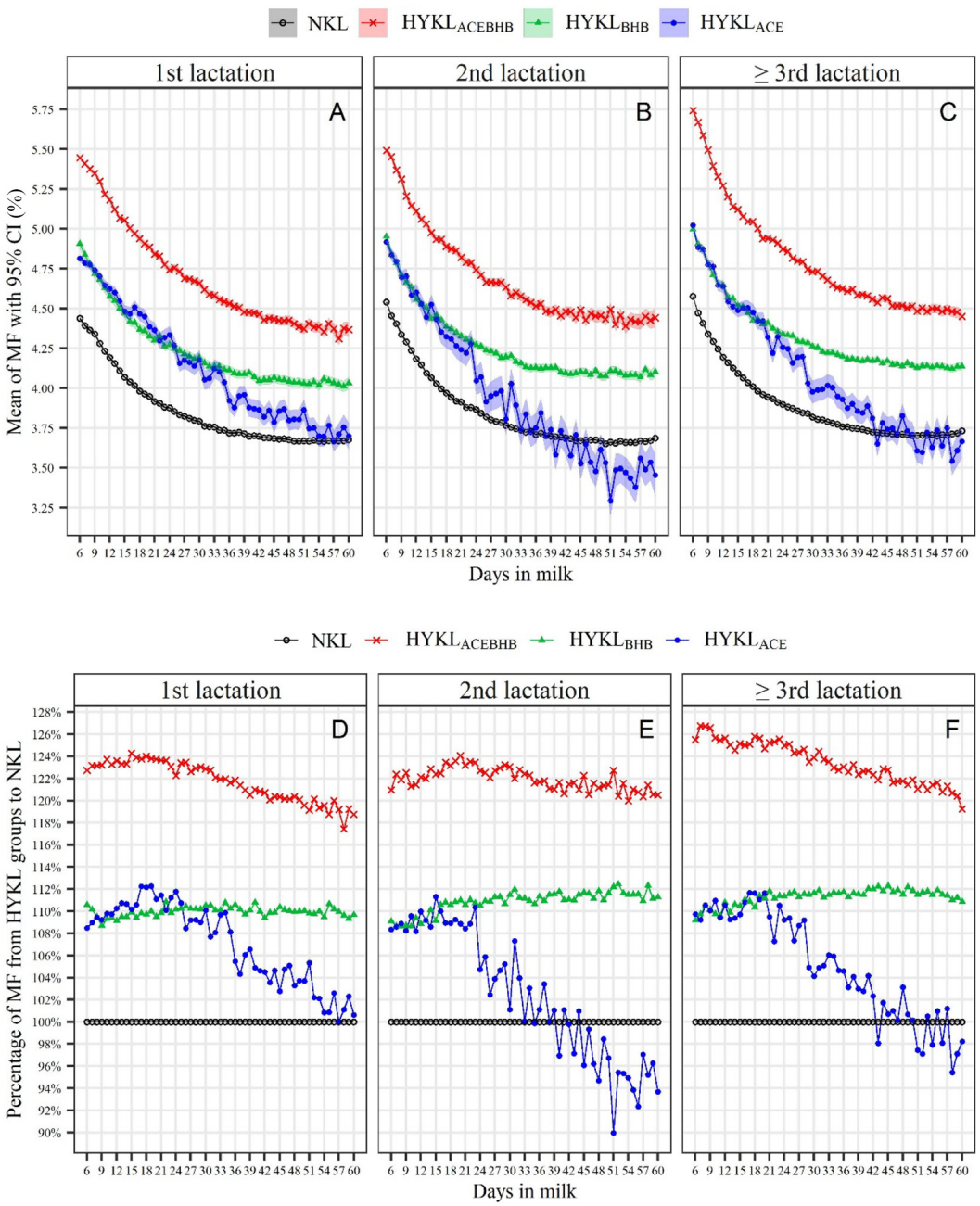

Figure 4. Least squares means, milk fat content (MF, %; panels A, B, C) or as percentage of MF of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

detected by measuring mBHB concentration by test strips, whereas Santschi et al. (2016) and Tatone et al. (2017) classified cows as HYKL based on FTIR milk analysis. None of these studies considered mACE in the evaluation of HYKL prevalence.

Consistent with other studies addressing hyperketonemia (Duffield et al., 1997; Vanholder et al., 2015; Rathbun et al., 2017) and HYKL (Santschi et al., 2016; Hejel et al., 2018), older cows (lactation ≥3) were always at greater risk. It is worth noting that the

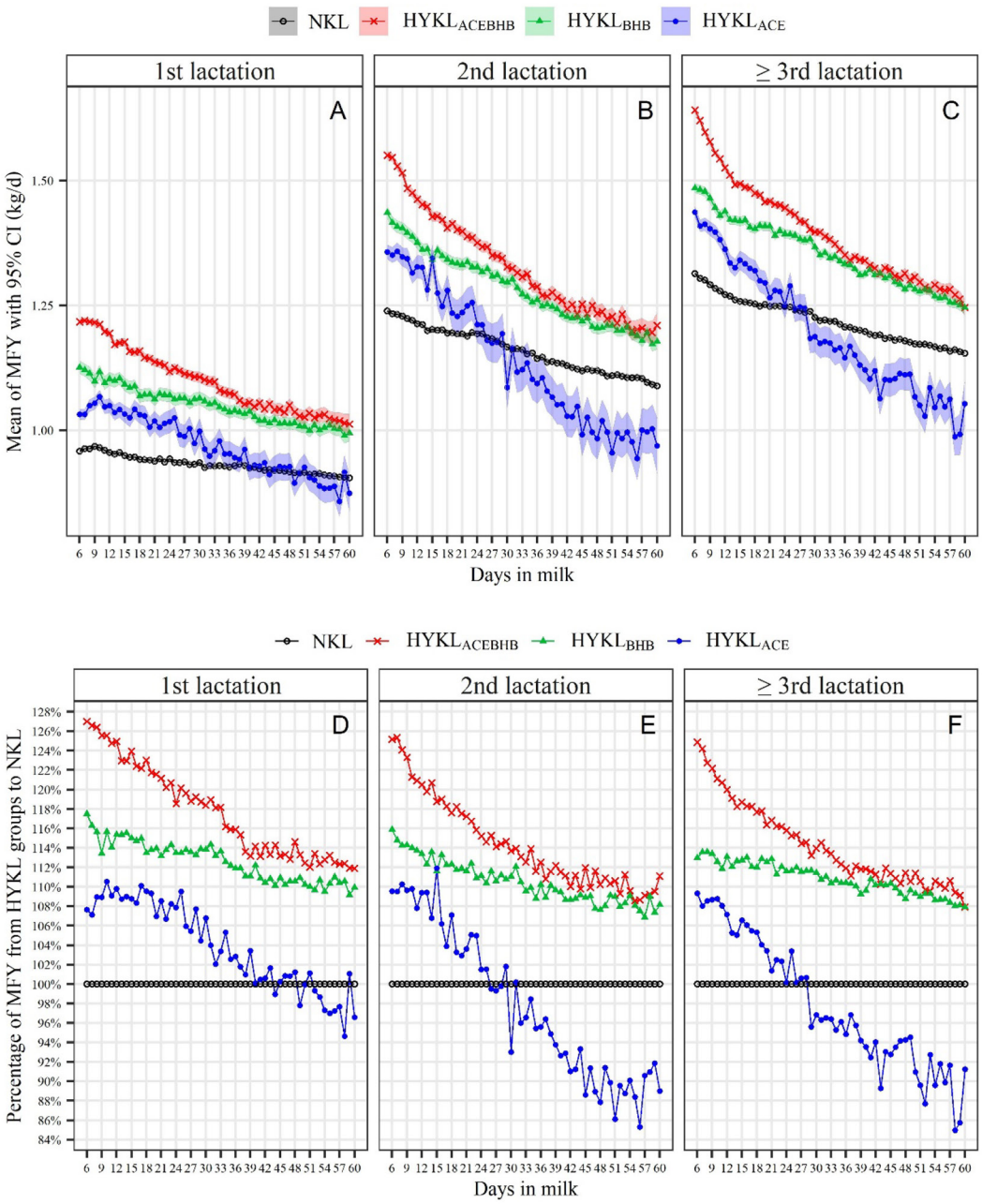

Figure 5. Least squares means, daily milk fat yield (MFY, kg/d; panels A, B, C) or as percentage of MFY of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

lowest proportion of HYKL cows was found for second-lactation cows. The reason for such a parity effect is not fully understood, but a lack of linear effect of increasing parity on increasing prevalence of HYKL might have resulted from the observed higher prevalence of HYKL among primiparous cows, not because of a special effect of the second lactation itself.

As in our previous study (Kowalski et al., 2021), milk samples having both elevated mACE and mBHB concentrations (subpopulation HYKL$_{ACEBHB}$) repre-

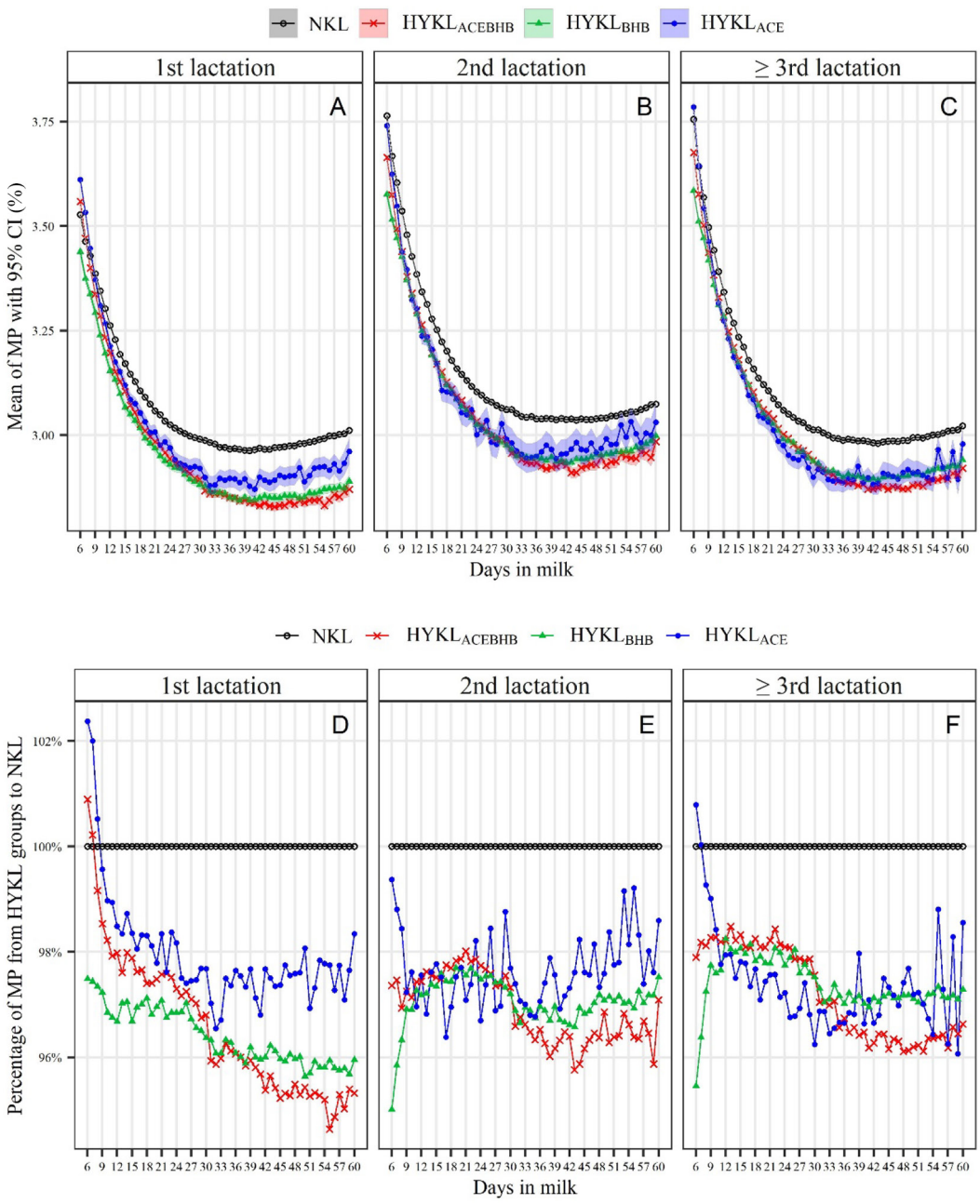

Figure 6. Least squares means, milk protein content (MP, %; panels A, B, C) or as percentage of MP of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

sented the majority of HYKL samples, especially in milk samples taken on 1TD. For milk samples taken on 2TD, increased mBHB concentration only (subpopulation HYKL$_{BHB}$) was most common, reflecting decreasing mACE content with increasing DIM, as was shown in our previous study (Kowalski et al., 2021). Higher HYKL$_{BHB}$ prevalence in the second month of lactation (2TD) may also be related to rumen contribution related to increased DM intake in this period compared with the first month of lactation (1TD). At least a part of mBHB may result from rumen butyrate production (Borrebaek et al., 1990). Santschi et al. (2016) also

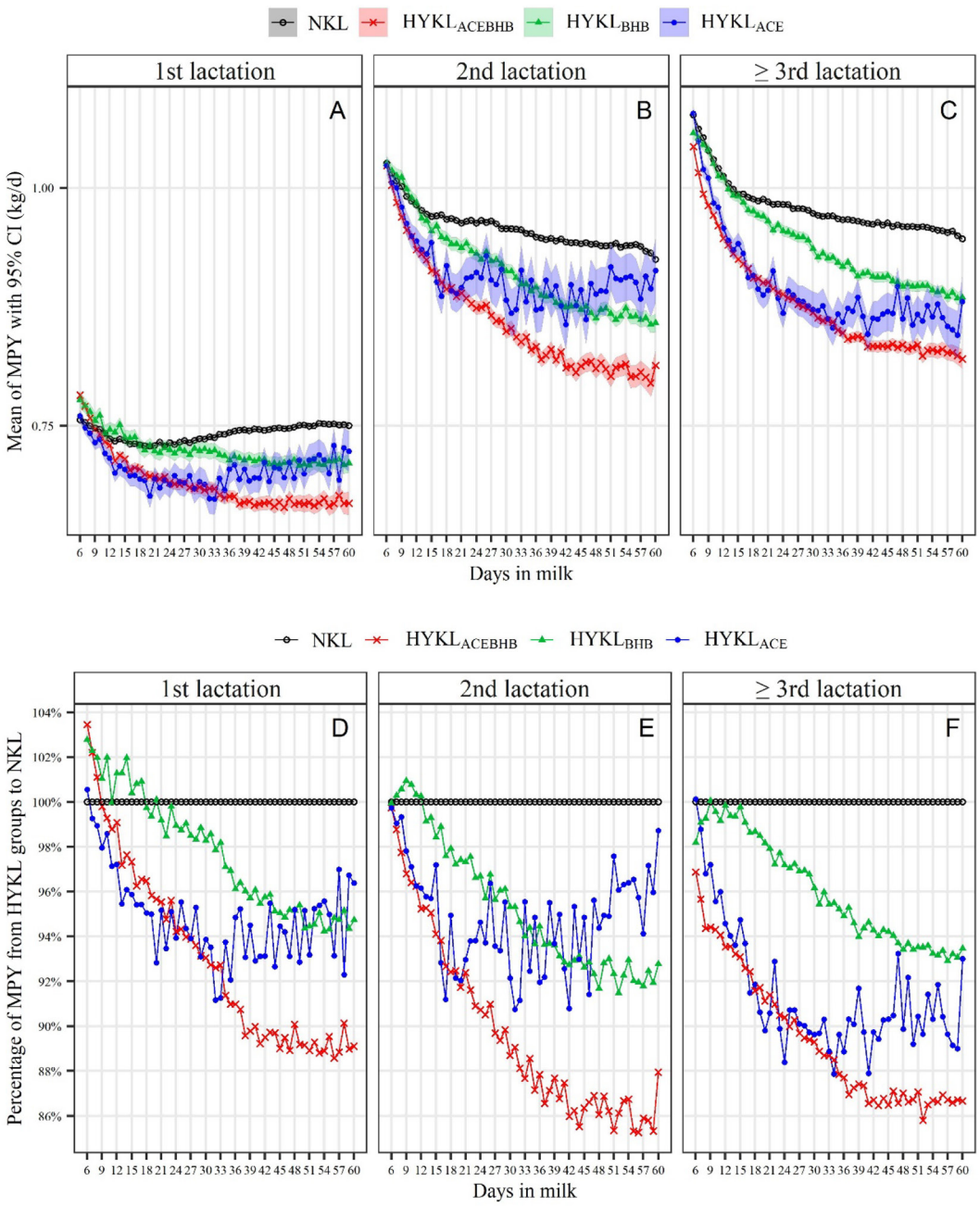

Figure 7. Least squares means, daily milk protein yield (MPY, kg/d; panels A, B, C) or as percentage of MPY of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

showed a higher prevalence of elevated mBHB later in the early lactation period (i.e., second and third week of lactation) compared with the first week postpartum. However, this effect was observed only in older cows, whereas among primiparous cows a high prevalence of elevated mBHB was found in the very first days of lactation.

Proportion of milk samples (approximately 8%) with elevated mACE without elevated mBHB (subpopulation HYKL$_{ACE}$) did not change by increasing the num-

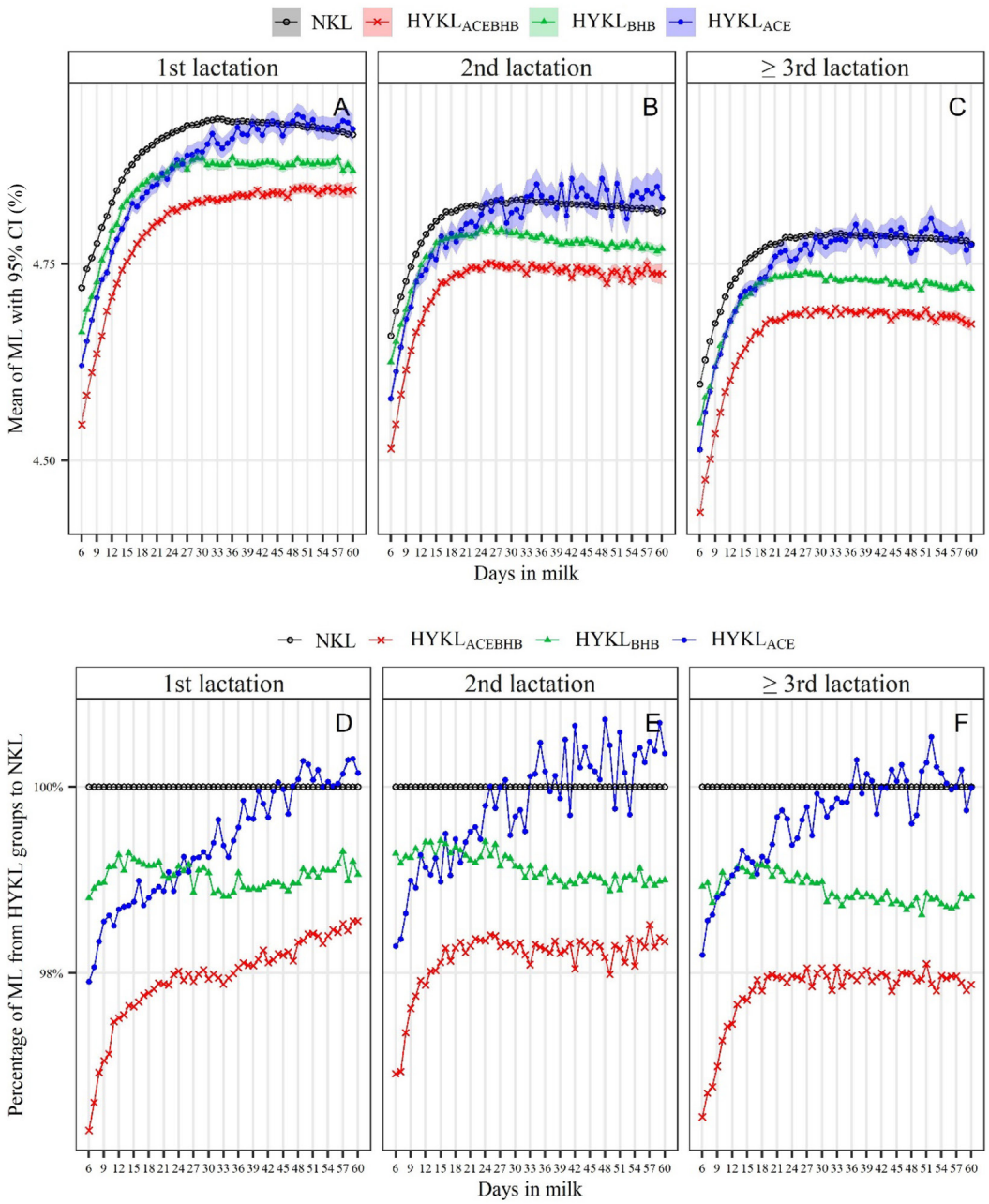

Figure 8. Least squares means, milk lactose content (ML, %; panels A, B, C) or as percentage of ML of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

ber of milk samples from 3.9 million in our previous study (Kowalski et al., 2021) to 5 million in the current study. Samples with elevated mACE only were more common among milk samples originating from primiparous cows, as well as among samples collected on 1TD. The relatively small proportion of cows of this population at 2TD should be acknowledged. This type of HYKL was of interest because milk analysis for ACE is the only way to detect cows having elevated ACE but not BHB. Due to the instability of ACE in the blood (Työppönen and Kauppinen, 1980; Fritzsche et al., 2001), such cows cannot be detected by blood analy-

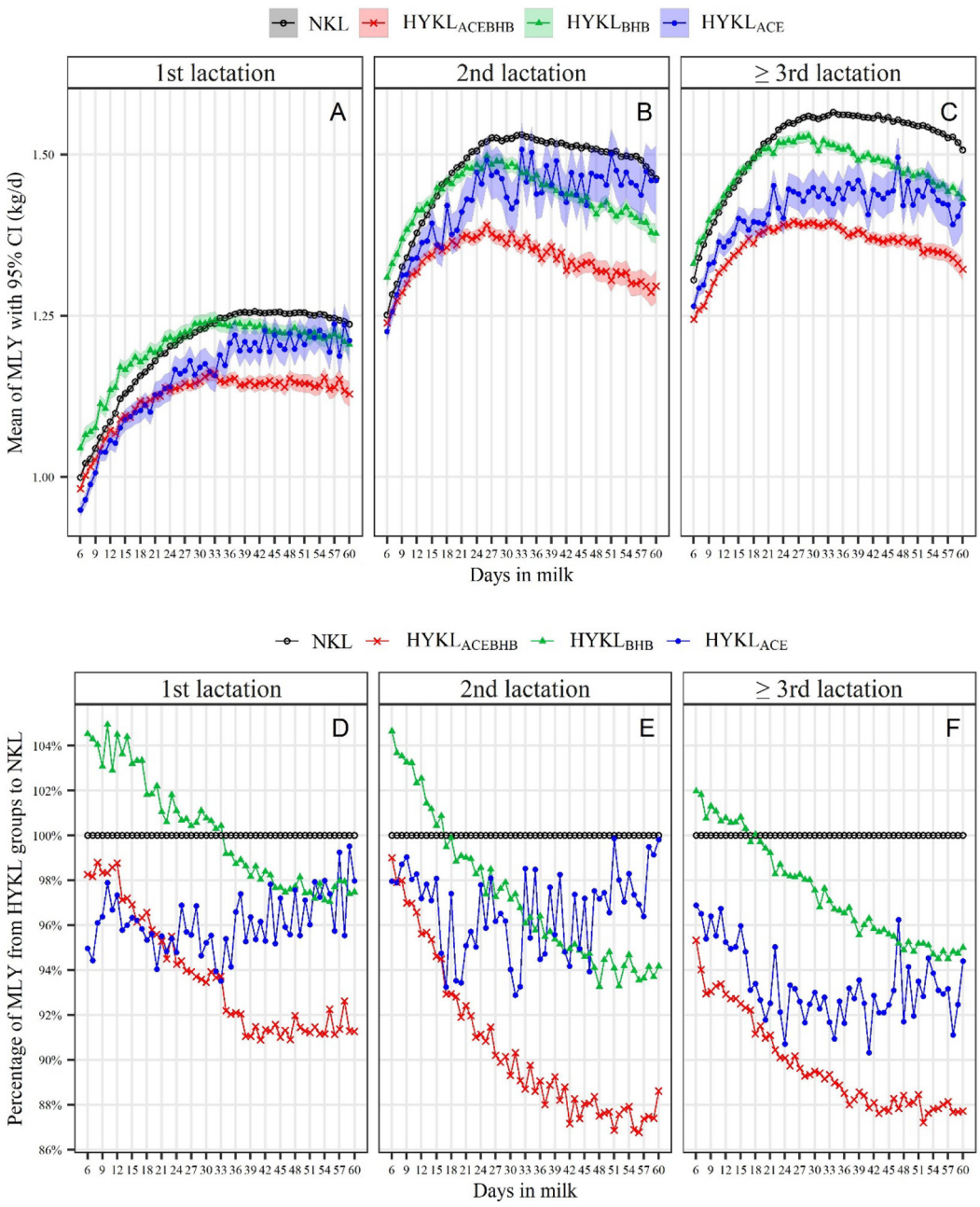

Figure 9. Least squares means, daily milk lactose yield (MLY, kg/d; panels A, B, C) or as percentage of MLY of normoketolactia (NKL) group (panels D, E, F) by ketolactia subpopulation (○ NKL, × HYKL$_{ACEBHB}$, ▲ HYKL$_{BHB}$, and ● HYKL$_{ACE}$), parity (1, 2, and ≥3), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of ketolactia subpopulation ($P < 0.001$), parity ($P < 0.001$), DIM ($P < 0.001$), and all their interactions (all $P < 0.001$). Shaded areas in panels A, B, and C represent 95% CI for each DIM within each subpopulation. Subpopulations based on milk acetone (mACE) and milk BHB (mBHB) concentrations, with hyperketolactia (HYKL) defined as mACE ≥0.15 mmol/L (HYKL$_{ACE}$), mBHB ≥0.10 mmol/L (HYKL$_{BHB}$), or both (HYKL$_{ACEBHB}$).

sis, including those performed using hand-held ketone meters. Similarly, cows having both elevated mACE and mBHB cannot be differentiated from those having only elevated mBHB by current diagnostic approaches. Milk analysis using FTIR technology allows for mACE determination, despite some limitations (Caldeira et al., 2020), making possible the identification of cows in differing metabolic states.

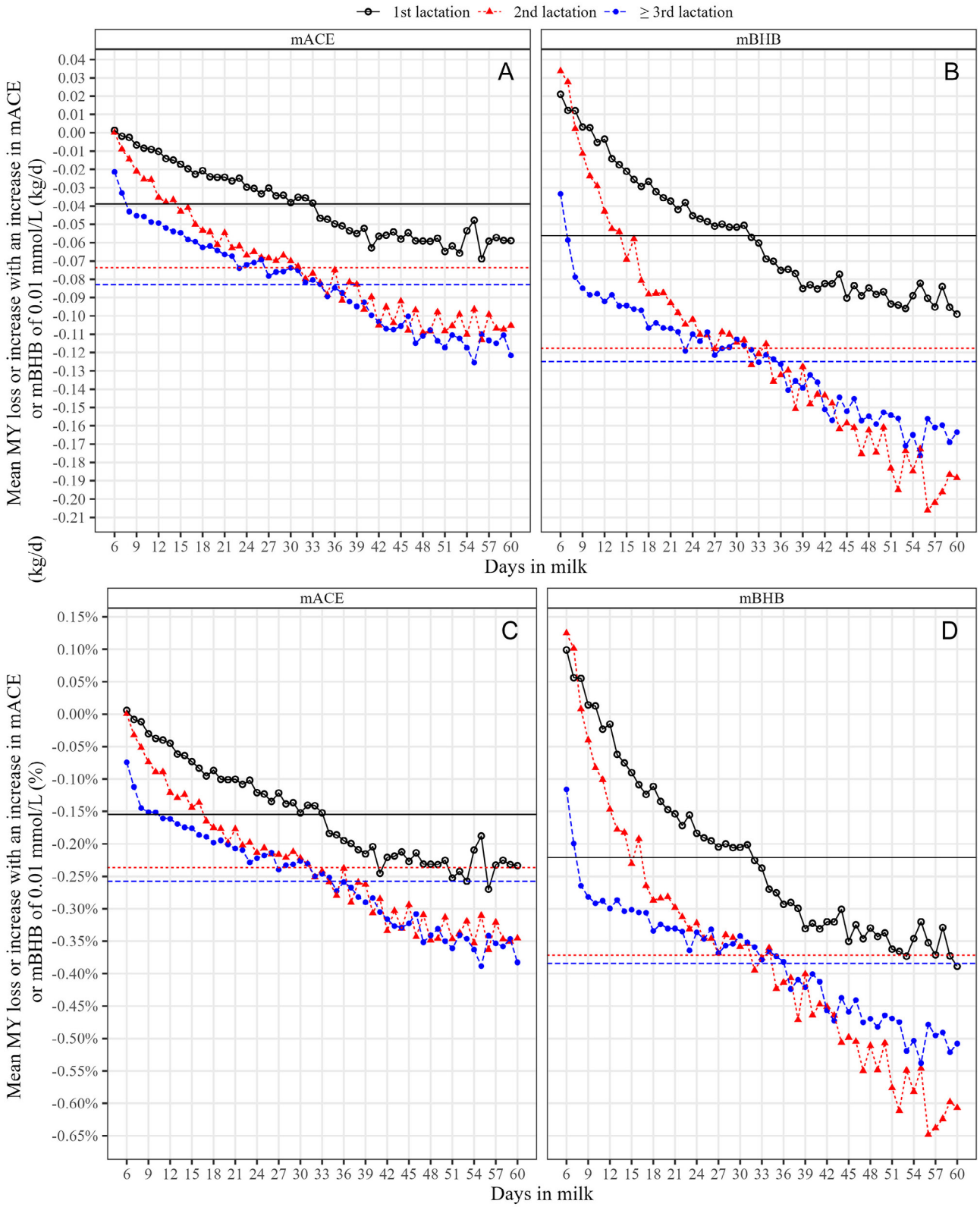

Figure 10. Least squares means, milk yield (MY) loss (−) or increase (+) with each 0.01 mmol/L increase in milk acetone (mACE) or milk BHB (mBHB) concentration (panels A and B in kg/d; panels C and D in %), by parity (○ 1, ▲ 2, and ● 3+), and DIM (6–60 DIM), based on n = 5,017,004 milk samples obtained from n = 3,102,201 cow lactations. Final model included effects of parity ($P < 0.001$), DIM ($P < 0.001$), and a parity × DIM interaction ($P < 0.001$). Horizontal lines represent average value for each parity (1, solid; 2, dotted; and 3+, dashed).

Milk Yield

Most studies presenting an association of hyperketonemia or HYKL with dairy cow production outcomes considered either blood or milk (HYKL) BHB concentration. Of interest was the possibility of mACE determination suggesting an independent ($HYKL_{ACE}$) or possible additive association with mBHB compared with elevated mBHB only.

Associations of elevated blood or milk BHB with daily MY are not always unequivocal. Several studies have presented a decrease in MY in early lactation with either elevated blood BHB (Duffield et al., 2009; Chapinal et al., 2012; McArt et al., 2012) or mBHB concentrations (Santschi et al., 2016; Chandler et al., 2018; Klein et al., 2020). However, others have shown an increase in daily MY in hyperketonemic or HYKL cows. Similar to our results, Bach et al. (2019) showed that MY was increased by 2.1 kg/d for cows above the cut point for predicted mBHB (0.14 ± 0.01 mmol/L). Rathbun et al. (2017) reported that hyperketonemic-positive cows (blood BHB >1.2 mmol/L) in the first 30 d postpartum had increased MY.

A common belief holds that cows that experience excessive negative energy balance are probably higher milk producers (Duffield et al., 2009) and ketotic cows are commonly among the highest producers in the herd. Current study results might confirm this theory, but only for cows with elevated BHB ($HYKL_{BHB}$), within 6 to 18 and 6 to 38 DIM, respectively, for multiparous and primiparous cows. Cows belonging to the subpopulations $HYKL_{ACEBHB}$ and $HYKL_{ACE}$ were not consistent with this pattern. It is worth noting that the lowest daily MY was almost always found in $HYKL_{ACEBHB}$ cows, which might additionally confirm the negative association of elevated mACE with milk yield. Although the performance of the $HYKL_{ACEBHB}$ subpopulation was worse than that of the $HYKL_{ACE}$ cows, we had interest in this latter subpopulation as it relates to cows that would potentially not be diagnosed using a ketone meter, unlike those in the $HYKL_{ACEBHB}$ subpopulation. Average daily MY differences between $HYKL_{BHB}$ and $HYKL_{ACEBHB}$ cows, however, showed lower MY in $HYKL_{ACEBHB}$ cows, possibly suggesting a different metabolic status of these cows compared with those with only elevated mBHB. Elevated ACE in the body may reflect a special metabolic status of a cow, and the reason for it needs to be studied.

Bergman (1971) indicates that ACE is produced by irreversible spontaneous decarboxylation (5% per hour) of acetoacetate, suggesting that production of this ketone body is required relative to understanding the effects of ACE on metabolism. Important questions to be raised are why elevated ACE in the body happens to some cows, but not to others, as well why it is more common among primiparous cows. However, it is worth noting here that BHB can be used for energy and fatty acid synthesis, as well as in the mammary glands (Ungerfeld et al., 2019), and blood or milk BHB concentration below threshold may indicate low production or a high metabolism rate, or both. However, the ACE content below threshold may predominantly indicate low production only. According to Luick et al. (1967), ACE metabolism is predominantly glucogenic but is quantitatively unimportant in both normal and ketotic cows. Because cows with elevated content of mACE, both without and with elevated mBHB, produced less milk, this may mean that the pathways of ACE metabolism are different than those described by Luick et al. (1967) and need to be studied.

In all HYKL subpopulations, HYKL was associated more negatively with MY (as percentage of MY of NKL cows) in multiparous than in primiparous cows. Similar results were found for hyperketonemic (Ospina et al., 2010; Chapinal et al., 2012) and for HYKL cows (Santschi et al., 2016). Reasons for such observed differences among parities need further research. Observed differences in MY between NKL and any HYKL cows increased with increasing weeks of lactation. Our study was limited to 60 DIM, but the data presented in Figure 2 indicate a potential increasing loss of milk production beyond 60 DIM, suggesting that a reduction in MY associated with HYKL in relation to NKL could be greater in the next weeks. The same tendency for increasing difference between negative and positive cows with increasing DIM was shown by Santschi et al. (2016); however, cows were classified as positive or negative by mBHB concentration exclusively. In contrast, cows developing hyperketonemia in the first week postpartum had a lower milk production than cows that developed hyperketonemia after the first week of lactation, even if cows had the same blood BHB concentration (Duffield et al., 2009; Chapinal et al., 2012; McArt et al., 2012). Reasons for differences between effect of timing of diagnosis using blood or milk on MY (the earlier the diagnosis with blood or the later diagnosis with milk, the lower the milk yield) also require further study.

Milk Composition

Hyperketonemia has been associated with greater MF content (see review by Benedet et al., 2019), resulting from fat mobilization, which is associated with a negative energy balance (Drackley, 1999). Results obtained in our study, referring to HYKL cows, are consistent with these well-documented facts. Also, Santschi et al. (2016) observed higher MF content in cows with

elevated mBHB. However, we found that HYKL subpopulations differed in MF content, with the greatest content found in $HYKL_{ACEBHB}$ cow milk samples followed by $HYKL_{BHB}$ cows. Compared with NKL cows, MF content was about 20% to 30% and 10% greater in milk samples of $HYKL_{ACEBHB}$ and $HYKL_{BHB}$ cows, respectively. Although the increment of MF content for milk samples of $HYKL_{ACEBHB}$ and $HYKL_{BHB}$ in relation to NKL cows seems to be quite stable throughout the whole period studied, a certain increase in MF content of $HYKL_{ACEBHB}$ in the second and third week of lactation could be seen. Also, Duffield et al. (2009) showed a higher MF content in hyperketonemic cows in the second compared with the first week of lactation. Similar to Chandler et al. (2018), we found that differences between HYKL ($HYKL_{ACEBHB}$ and $HYKL_{BHB}$) and NKL cows in MF content were independent of parity. In contrast, MF content in $HYKL_{ACE}$ cow milk samples changed apparently during the analyzed period of early lactation. In all parities studied, the MF content of these cows was greater than NKL cows within the first 3 to 4 wk, and then it was lowered with increasing DIM, almost reaching levels equal to those of NKL cows. This was especially apparent in milk samples of second-lactation cows. Although in this study we have shown that the MF content of ACE alone HYKL cows behaves differently than that of $HYKL_{ACEBHB}$ or $HYKL_{BHB}$ cows, we have no explanation for this observation, and it requires further studies. It is well known that BHB is involved in the synthesis of fatty acids in milk fat (Ungerfeld et al., 2019), but the reason for the increase in MF when mACE is increased is unknown. The question also remains open: why do some cows have elevated mACE without elevated mBHB?

Decreasing MFY with increasing DIM of $HYKL_{ACEBHB}$ or $HYKL_{BHB}$ cows in relation to NKL cows mostly reflects the higher MF content in HYKL cows. Although in the second month of lactation, especially among older cows, MFY of $HYKL_{ACE}$ cows was lower than in NKL cows, it was also related to MF content.

Hyperketonemia has always been associated with lower MP content (see review by Benedet et al., 2019), and the same association was observed in the current study. Lower MP may result from reduced microbial protein yield or repartitioning of amino acids to support gluconeogenesis due to energy deficit (Osorio et al., 2016). In our study, except in the very first DIM for cows in lactations 1 and $\geq$3, HYKL decreased MP content by 2% to 5%. A decrease in MP content was more apparent among primiparous cows, especially in $HYKL_{ACEBHB}$ and $HYKL_{BHB}$ cows. Lower MP content found in HYKL primiparous cows is consistent with results obtained by others (Santschi et al., 2016; Chandler et al., 2018). This is likely a consequence of greater requirements for protein in support of growth in younger cows. Changes in MPY resulted to a large extent from changes in MY. Compared with NKL cows, HYKL cows had lower ML content, except $HYKL_{ACE}$ cows in the second month. Similarly, Santschi et al. (2016) reported lower ML content in HYKL-positive cows. Contrary to Santschi et al. (2016), we did not find a clear effect of parity on the decrease in ML content in relation to healthy cows. The largest decline in ML content during the first 2 to 3 wk might have resulted from insufficient blood glucose at the very early stage of lactation (Drackley, 1999). We do not have an explanation for why a decrease in ML content lasts only to 35 to 40 DIM in $HYKL_{ACE}$, whereas it lasts the whole study period in $HYKL_{ACEBHB}$ and $HYKL_{BHB}$ cows.

Energy-corrected milk yield has been reported to increase in HYK cows (Santschi et al., 2016; Rathbun et al., 2017). In our study, HYKL cows produced more ECM than healthy cows, but this refers only to $HYKL_{BHB}$ cows in all parities and to $HYKL_{ACEBHB}$ in first-lactation cows. If HYKL comes from elevated mACE ($HYKL_{ACEBHB}$ or $HYKL_{ACE}$), HYKL cows produced more ECM than NKL cows only in the first 2 to 4 wk ($HYKL_{ACE}$) and 4 to 6 wk ($HYKL_{ACEBHB}$) of lactation in cows in lactation 2 and $\geq$3. Reasons for such trends, as well as for the fact that ECM of cows from the $HYKL_{ACE}$ subpopulation was always lowest among hyperketolactic cows, require further investigation. Additionally, the cause of declining ECM relative to healthy cows as DIM increases, requires further study. Rathbun et al. (2017) found that the greatest differences in ECM between HYK and healthy cows occurred in the first week of lactation.

Summarizing the HYKL association with milk composition in relation to NKL cows, it is clearly seen that HYKL, similar to hyperketonemia, is associated with increased MF content but decreased MP and ML contents. However, these effects, also considering parity or DIM, depend very much on the HYKL subpopulation. The reason for differences among HYKL subpopulations needs further investigation. Among HYKL groups, those addressing mACE, either alone or with mBHB, seem to be the most intriguing.

Association Between mACE or mBHB and Milk Yield

By using multilevel linear regression modeling (model 2), we predicted daily MY based on mBHB or mACE concentrations accounting for parity and DIM. To our knowledge, this is the first such modeling derived from population data with over 5 million milk samples. Klein et al. (2020) reported on a prediction model based on milk samples obtained from the first test-day of about 62,000 cows, also considering parity and DIM. Average

MY loss associated with each 0.01 mmol/L increase in mACE or mBHB differed among parities, the lowest being among primiparous cows. The reason for such trends needs further investigation, as does determining the cause of MY losses associated with an increase in mACE or mBHB with progressing DIM, irrespective of parity. Such changes, along with progressive DIM, might encourage discussion on the use of a single threshold value for either mACE or mBHB, regardless of the day of lactation. It is worth noting that the lowering effect of progressing DIM is much more pronounced in mBHB than in mACE. Similarly, a more pronounced effect of mBHB than mACE on milk production decline was also reported by Klein et al. (2020), and the reason of such trends remains unknown.

CONCLUSIONS

In this study, we found that hyperketolactia is associated with altered production outcomes of dairy cows during the first 60 d of lactation. Such effects depend on parity and DIM. Moreover, we identified different subpopulations of hyperketolactic cows that differ in production outcomes, with both elevated mACE and mBHB hyperketolactia ($HYKL_{ACEBHB}$) associated with the most impaired performance. Given that both ACE and BHB are directly derived from acetoacetate through the ketogenesis pathway, this is of interest, as these different subpopulations may represent unique metabolic states. Further research is needed to ascertain biochemical defects and health or reproduction effects of HYKL from elevated ACE, alone or in combination with BHB, in early-lactating dairy cows.

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