Blood and Milk Beta-Hydroxybutyric Acid Concentrations and Association of Subclinical Ketosis With Postpartum Health Disorders, Culling Rate, Body Condition Score, Parity and Milk Production in Holstein, Simmental, Montbeliard and Holstein-Crossbreed

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Abstract

A total of 300 dairy cows were randomly enrolled from 11 dairy farms in Turkey. The beta-hydroxybutyric acid concentration (BAC) was tested in the blood (BBAC) and individual milk (MBAC) samples at postpartum week 2 (PPW2) and week 4 (PPW4) for the detection of subclinical ketosis (SCK) in Holstein, Montbeliard, Simmental and Holstein-Crossbred (HC; only BBAC). The prevalence of BSCK (BBAC ≥ 1.2 mmol/L), MSCK1 (MBAC = 100 µmol/L), MSCK2 (MBAC ≥ 200 µmol/L) and MSCK1/2 (≥ 100 µmol/L) was 8.3, 11.8, 5.8 and 17.3% at PPW2 and 4.7, 4.9, 6.9 and 11.9% at PPW4 in Holstein respectively. SCK was not observed in Simmental and HC. The prevalence of BSCK and MSCK1 at PPW2 were 4.3 and 43.5% in Montbeliard respectively. Primiparous Montbeliard and Holstein had significantly higher MBAC at PPW2 than PPW4. Overall, HC and Montbeliard had significantly lower BBAC. Cows having body condition scores 2 and 4 at calving had higher MBAC at PPW2 and 4 that was associated significantly with metritis and multiple diseases. Holstein with BCS4 at calving had higher BBAC at PPW2 and 4. Holstein with SCK was more likely to develop postpartum metabolic health disorders (PPHD) in 90 days in milk (90DIM). MSCK1 did not associate milk production loss in Montbeliard and Holstein. Holstein with both BSCK and MSCK2 at PPW2 had a 6.7 kg average daily milk yield loss in 90DIM. Conclusively, SCK was not observed in Simmental and HC, and MSCK1 didn't cause PPHD and milk yield loss in Montbeliard. BSCK and MSCK2 created a significant risk for PPHD and milk production loss in Holstein.

Introduction

Dairy cows must orchestrate the metabolic challenges during the transition from dry-period to early lactation to support milk production with an adequate glucose supply. These critical production stages can result in several postpartum (PP) metabolic disorders if dairy cows do not overcome negative energy balance (NEB) due to reduced dry matter intake and other complications (Baumgard et al. 2017, Overton et al. 2017, Deniz et al. 2020). NEB is the main reason during the transition period and can negatively affect milk production due to subclinical ketosis (SCK) (Dohoo and Martin 1984; McArt et al. 2012), metabolic and reproduction parameters (Whitaker et al. 1983; Uyarlar et al. 2018; Deniz et al. 2020) and farm profitability through decreased milk production and increased risk of metabolic diseases (McArt et al. 2015; Raboisson et al. 2015; Benedet et al. 2019; Deniz et al. 2020). Increased demand for milk consumption resulted in increased annual milk production per cow from ca. 2.000 kg to 10.300 kg worldwide (Baumgard et al. 2017). The dairy cattle population transformed from indigenous low milk yielding breed to high milk yielding dairy cows (HYDC) from 1991 to 2019 which simultaneously resulted in increased milk production per cow from 1.4 ton to 3.1 ton and annually from 8.6 Mio ton to 20.7 Mio ton respectively in Turkey (Aksoy et al. 2021). But, this brought problems of metabolic and reproductive diseases such as ketosis, displaced abomasum resulting in early culling (Aksoy et al. 2021). SCK is associated with hyperketonemia (HK) or hyperlactatemia (HL) in the absence of clinical ketosis signs and is a common disease for HYDC. Beta-hydroxybutyric acid concentration (BAC) in the blood (BBAC) or milk (MBAC) is one of the most tested ketone bodies among others as such acetone and acetoacetic acid in recent years (Suthar et al. 2013; Benedet et al. 2019; Deniz et al. 2020). Testing of BBAC (Suthar et al. 2013; Şentürk et al. 2016; Uyarlar et al. 2018; Benedet et al. 2019; Brunner et al. 2019;) and MBAC (Berge

and Vertenten 2013; Denis-Robichaud et al. 2014; Santschi et al. 2016) indicate the NEB, that can result in clinical ketosis (CK) and SCK in early lactation. Thus, HK became an economically relevant postpartum metabolic problem in terms of its impact on farm profitability, especially in Holstein dairy farming (McArt et al. 2015; Raboisson et al. 2015; Mostert et al. 2017; Deniz et al. 2020), however, there are not enough papers published about Montbeliard, Simmental and Holstein-Crossbred. Various studies in Holstein revealed a prevalence of 21.8% (Suthar et al. 2013) and 24% (Brunner et al. 2019) worldwide if tested in the blood, in which a cut-off level for BBAC ≥ 1.2 mmol/L was taken. Studies from Turkey reported that the prevalence was 11.2% (Suthar et al. 2013) and 19.4% (Şentürk et al. 2016) with the same BAC threshold. Recent studies showed that checking of MBAC found large acceptance by using Fourier-Transform Infrared (FTIR) Spectrometry (Santschi et al. 2016) or milk ketone strips (Berge and Vertenten 2013; Benedet et al. 2019). Overall, an average prevalence of 39% in Holstein was reported by using milk ketone strips in European countries. This rate was 22.6% by using FTIR in Canada (Berge and Vertenten 2013). The cut-off value of MBAC for the definition of SCK was varying among the studies. Few papers used MBAC to define SCK prevalence. Ranges of MBAC to classify cows with suspect of HL (0.15 to 0.19 mIntroductionmol/l) or positive HL (≥ 0.20 mmol/l) were reported by the review work of Benedet et al. (2019) and others (Melendez et al. 2006; Berge and Vertenten 2013). The relationship between BBAC and MBAC and milk production was studied in Holstein cows (Duffield et al. 2009; Denis-Robichaud et al. 2014), in Finnish dairy cows (Miettinen and Setala 1993). To our knowledge, many of the studies about the prevalence of SCK were conducted via a blood test. Few studies reported the correlation between BBAC and MBAC and the prevalence of SCK defined by different cut-off values in milk, as well as its association with PPHD, body condition score (BCS), parity and culling in different dairy cow breeds. The majority of the studies about the prevalence of SCK and its association with PPHD were conducted in Holstein dairy farms, there is a lack of researches conducted in various breeds worldwide and Turkey. Montbeliard and Simmental were classified in the same family (Averdunk 2002, Felius et al. 2014) and there are not enough papers about BBAC/MBAC in different parities and the relationship of SCK with other PPHD and milk production in these breeds. The objective of the present study was to analyse BBAC and MBAC at the two most important postpartum time points such as postpartum week 2 and 4 (PPW2 and PPW4) and accordingly the prevalence and association of SCK with PPHD, BCS, parity and milk production in Holstein, Montbeliard, Simmental and Holstein-Crossbred in Turkey.

Materials And Methods

Animals and grouping

This is a randomized field study. The study was conducted in 4 provinces (İzmir, Aydın, Muğla and Denizli) of Turkey. Three hundred lactating cows in 11 integrated dairy cattle farms consisting Holstein (n = 216, farm 1 to 8), Simmental (n = 38, farm 9 and 11), Montbeliard (n = 23, farm 1) and Holstein-Crossbred (HC) (HolsteinxMontbeliard, n = 23, farm 10) were enrolled for the study. Cows in each farm were randomly chosen in turns based on the earliest parturition date, roughly 10 days before calving without selection criteria. Average number of lactating cows was roughly 300 at the start of the study in all farms and the rage of dry period for cows was 50–60 days in all farms. Parity groups were created as

primaparous (Prim) and multiparous (Mul) due to association of SCK with the parity (Duffield 2000; Brunner et al. 2018). Furthermore, groups were created for the definition of SCK in the blood (BSCK) and milk (MSCK1, MSCK2 and MSCK1/2) based on different and appropriate cut-points of MBAC. Combined prevalence groups such as BSCK/MSCK positive and BSCK or MSCK positive both at postpartum week 2 and 4 were created to observe their effects on the average daily milk yield (ADMY). Out of total 11 farms and 300 dairies, 10 farms had an automatic milking system, milk yield recording data base, and complete milk yield recording for study animals were enrolled in the milk production analysis. Total 259 dairy cows that were Holstein (n = 206, farm 1 to 8), Simmental (n = 33, farm 9 and 10) and Montbeliard (n = 20, farm 1) were allocated in the respective breed group for the milk production analysis in association with SCK prevalence.

Animal feeding

All farms had a professional self-ration program and cows were fed a ration according to the production cycle, energy, mineral and other nutrients requirements (dry period, close-up, early lactation). Water was served ad libitum. As a standard protocol for the controlling of milk fever, an anionic feeding program was initiated in the majority of farms except farms 6, 7 and 8. For anionic feeding, ammonium sulphate and calcium chloride were added to the ration at the last 21 days of gestation. Farm feeding strategy and ration have not been changed or specifically prepared for this study throughout the study period.

Beta-hydroxybutyric acid analysis and definition of SCK in the blood and milk

BBAC was analysed in the individual whole blood samples collected from the coccygeal vein by a practical cow-side analyser (Medtrust Wellionvet Belua, Med Trust Handelsges.m.b.H., Austria) at PPW2 and PPW4. The accuracy of this device was tested before (Khol et al. 2019). MBAC was analysed at the same times like blood test in 50 ml of freshly taken individual milk samples (within 5 minutes after collection) with milk-test-strips (Ketotest, Elanco). Ketotest milk strips were tested and confirmed for their sensitivity in the milk before (Carrier et al. 2004). According to the manufacturer instruction, these test strips analysed semiquantatively MBAC and showed different colours indicating 0, 50, 100, 200, 500, and 1000 µmol MBAC per L milk. MBAC could not be tested in HC (farm 10) due to a technical problem. Due to other technical issues, 169 and 137 test results of MBAC were accepted, recorded and analysed in the present study at PPW2 and at PPW4 respectively. BBAC could not be tested in four animals (Holstein n = 3, Simmental n = 1) at PPW4. SCK without clinical signs of ketosis (e.g. constipation, anorexia, rumen dysfunction, reduced rumination) was defined by a cut-off point of BBAC ≥ 1.2 mmol/L (BSCK) in the blood (Suthar et al. 2013; Brunner et al. 2018) and MBAC = 100 µmol/L (MSCK1), MBAC ≥ 200 µmol/L (MSCK2) and MBAC ≥ 100 µmol/L (MSCK1/2) in the milk as recommended by the test kits manufacturer and others (Melendez et al. 2006; Berge and Vertenten 2013; Denis-Robichaud 2014; Benedet et al. 2019).

Body condition scores and postpartum health checks

BCS controls were performed according to the recommendations by Edmonson et al. (1989) based on a scale from 1 to 5 (where 1 = emaciated to 5 = extremely fat) at calving (postpartum day 0: PP0), PP day 30

(PP30) and PP day 60 (PP60). Groups for BCS < 2.5 (BCS1), BCS ≥ 2.5-<3.5 (BCS2), BCS ≥ 3.5 to < 4.0 (BCS3) and BCS ≥ 4.0 (BCS4) were set up. Cows were classified as fat (BCS ≥ 4), thin (BCS2), normal (BCS3) or emaciated BCS ≤ 2.5. The difference of BCS relative to calving was accepted as the body condition loss or gain (Heuer et al. 1999). All study cows were daily monitored and evaluated from the clinical health point of view, any single or multiple diseases or culling were registered immediately in 90 days in milk (90DIM). Study animals were specifically checked and monitored for retained placenta (RP), displaced abomasum (DA), metritis, mastitis, cystic ovarian (CO), lameness, clinical ketosis (CK), milk fever or combined multiple diseases (MD, more than 1 disease) in 90DIM because they were most prevalent PPHD associated with SCK which were reported in the literature (Whitaker et al. 1983; Duffield 2000; McArt et al. 2015; Raboisson et al. 2015; Uyarlar et al. 2018; Deniz et al. 2020).

Milk yield recording

The daily milk yield of each cow (n = 259) was recorded automatically in the study farms (n = 10) where various automated milking system was set and continuously recorded in a data base. Cows were milked two times a day. Milk yield was taken directly from computerized farm database. ADMY, average weekly and monthly milk yield of all breeds were calculated in 90DIM accordingly.

Statistical analysis

Statistical analyses were performed using the SPSS (version 22) software and the results were evaluated for α = 0.05. The normality of the data was evaluated by Kolmogorov-Smirnov and Shapiro-Wilks tests. The nonparametric tests (Mann-Whitney, Wilcoxon Signed Ranks, Kruskal-Wallis, Friedman) were used for statistical analysis because of the non-normality of the data and small sample sizes. Arithmetic mean (m), standard error (se) or minimum and maximum values were presented as descriptive statistics for BBAC, MBAC, BCS, parity and ADMY where it was necessary. Prevalence of BSCK and MSCK1, MSCK2 and MSCK1/2 was presented as numeric, positive, negative and % in study animals. In order to evaluate the disease incidences and dependency of BHBA between PPW2 and PPW4 in animal breed groups, Fisher's exact test was used. Incidence of the PPHD in the groups was presented as a percentage. Odds ratio (OR) was determined for each of the diseases (for those with sufficient data for computation) in the groups. Pearson correlation coefficients were calculated between BBAC/BSCK and MBAC/MSCK at PPW2 and PPW4 and between PPW2 and PPW4 for BBAC/BSCK and MBAC/MSCK. The data for BBAC and MBAC were analysed by Wilcoxon Signed Ranks test to compare PPW2 and PPW4. Mann-Whitney Test was used to compare the BBAC and MBAC between the animal breeds. However, Kruskal-Wallis test was initiated for the analysis of average LN between breed groups. Average daily milk production of the breeds including subgroups (PRP, MUL, with SCK and without SCK) were analysed using one-way analysis between the breeds, as well as Friedman test between SCK positive and negative animals. Mann-Whitney-U test is used to compare the daily, weekly, and monthly milk production between the groups and subgroups.

Results

Body condition scores, parity, blood and milk beta-hydroxybutyric acid concentrations

The averages of BCS, BBAC and MBAC in the study cows were presented in Table 1. All primiparous and multiparous cows have lost significantly BCS at PP30 and PP60 compared to calving ($p < 0.01$), except for primiparous Simmental. There was a significant difference between breeds. BCS1 was not observed at calving in breeds. BCS2, BCS3 and BCS4 were detected in Holstein by 47%, 41%, and 8.6% at calving respectively. The average parity of Holstein, Montbeliard, Simmental and HC was 2.93 ± 0.11 (n = 37 primiparous, n = 179 multiparous), 3.09 ± 0.31 (n = 5 primiparous, n = 18 multiparous), 2.26 ± 1.03 (n = 9 primiparous, n = 29 multiparous), 2.04 ± 1.15 (n = 11 primiparous, n = 12 multiparous) respectively. The average parity of Simmental and HC were significantly lower ($p < 0.01$) than Montbeliard and Holstein. Figures 1 and 2 present BBAC and MBAC for different BCSs at calving. The average BBAC of Holstein at PPW2 or 4 was significantly ($p < 0.05$) higher if they got BCS4 at calving. Holstein cows having BCS4 or BCS2 at calving had significantly higher MBAC at PPW2 compared to BCS3 groups ($p < 0.01$). The significantly high MBAC at PPW4 was observed in Holstein cows having BCS2 at calving. Holstein having significantly high BBAC at PPW2 had BCS4 at PP30 and PP60. Significantly high BBAC at PPW2 was observed in Simmental cows having BCS4 at PP30 ($p < 0.01$). Correlation coefficients between BBAC and MBAC were $r = 0.60$ and $r = 0.86$ ($p < 0.05$) at PPW2 and PPW4, it was $r = 0.36$ and $r = 0.14$ ($p > 0.05$) in Holstein and Montbeliard cows respectively. Correlation coefficients for BBAC and MBAC were $r = 0.45$ and $r = 0.75$ ($p < 0.05$) between PPW2 and PPW4 in Holstein respectively. No significant correlation was found in other breeds.

Prevalence of subclinical ketosis detected in the blood (BSCK) and milk (MSCK)

BSCK was not detected in Simmental (farm 9, 11) and Holstein-Crossbred (farm 10) neither at PPW2 nor PPW4. BSCK was detected in Holstein farms (farms 2, 3, 5, 6, 7 and 8) at a rate of 8.3 and 4.7% at PPW2 and 4 respectively. The difference between PPW2 and PPW4 was significant ($p < 0.01$). Holstein farms 1 and 4 were negative for BSCK. Out of 23 Montbeliard cows, 1 primiparous cow with BCS4 at calving showed BSCK at PPW2 (4.3%) only, but that cow became negative at PPW4. The descriptive data about the prevalence of BSCK, the parity and BCSs were presented in Tables 2 and 3 for Holstein cows. Primiparous Holstein that was tested positive for BSCK at PPW2 and 4, lost significantly ($p < 0.05$) BCS at PP60. The correlation coefficient was $r = 0.34$ ($p > 0.05$) for BSCK between PPW2 and PPW4 in Holstein. No significant correlation was found in other breeds. The prevalence of MSCK in Holstein cows was presented in Table 2. The highest prevalence was observed in the MSCK1/2 group in Holstein, which was 17.3 and 11.9% at PPW2 and 4 respectively. The difference between PPW2 and 4 was significant ($p < 0.01$). MSCK1 and MSCK2 prevalence were 4.9, 5.8% and 5.8, 6.9% at PPW2 and 4 in Holstein respectively. The difference between PPW2 and PPW4 was significant ($p < 0.01$) in MSCK2. MSCK2 prevalence was negative in Holstein farms 1, 3 and 4. There were more MSCK2 positive multiparous Holstein at PPW4 compared to primiparous. No significant difference was found in BCSs of MSCK2 positive multiparous Holstein cows between calving, PP30 and PP60. However, all primiparous cows tested positive for MSCK lost BCS between calving, PP30 and PP60. Correlation coefficients were $r = 0.26$ and $r = 0.48$ ($p > 0.05$) for MSCK1 and MSCK2 between PPW2 and PPW4 in Holstein respectively. It was

not applicable in other breeds. MSCK1 incidence in Montbeliard was 43.5% at PPW2, but all cows became negative at PPW4. MSCK1 or 2 in Simmental cows (farm 9 and 11) and MSCK2 in Montbeliard were negative. The combined prevalence of BSCK/MSCK1 and BSCK/MSCK2 was 4.0 and 2.0% at PPW4 in Holstein cows respectively. The prevalence of BSCK/MSCK1/2 was 8.6 and 4.0% at PPW2 and PPW4 in Holstein respectively. The difference between PPW2 and 4 was significant ($p < 0.01$). No combined prevalence was observed in Montbeliard and Simmental cows. It was not applicable for Holstein-Crossbred. The percentage of BSCK, MSCK1 and MSCK2 positive cases at both PPW2 and PPW4 were 2.3, 5.9 and 3.0% in Holstein respectively. No correlation between PPW2 and 4 was existed ($p > 0.05$).

Culling and postpartum health disorders

The culling rate was 3.7% among Holstein cows in 90DIM. None of culled Holstein cows had BSCK at PPW2 or 4. Holstein cows that were positive for MSCK1, MSCK1/2 and MSCK2 at PPW2 created a likelihood of 6.3, 12.5 and 25% for culling risk respectively. MSCK2 positive cows were significantly more likely (OR:11.20, $p < 0.05$) to be culled than even MSCK1/2 (OR:3.46). MSCK1 did not create a significant risk for culling. The average BCS of culled Holstein was normal (BCS3) at calving, but a significantly BCS loss was observed at PP30 (BCS1). The difference between BCSs of culled Holstein (2.48 ± 0.20) and non-culled Holstein (2.95 ± 0.03) was significant at PP30 ($p = 0.026$). The average parity of culled Holstein was 3.62 (one primiparous, 7 multiparous). Mastitis (n = 1), metritis (n = 1) and displaced abomasum (n = 1), MD (n = 1, 12%), lameness (n = 2, 25%) were observed in culled Holstein. No associations were observed in other breeds between culling and SCK. The incidence of PPHD and its association with SCK in Holstein was presented in Table 3. Simmental and HC were not positive for SCK and no severe PPHD was observed in Montbeliard cows that were positive for BSK and MSCK1 at PPW2. MSCK1 did not correlate with PPHD in Holstein. CK, DA, metritis, mastitis, lameness and multiple diseases were observed moderately and, in some cases, significantly higher in Holstein cows that were positive for BSCK, MSCK1/2 and MSCK2 at PPW2 or 4 (Table 3). BSCK and MSCK2 positive Holstein at PPW2 or 4 were more likely to developing CK (OR: 15.4, $p < 0.05$). These cows had significantly higher average BCS at PP30 and moderately lower average parity compared to cows not having CK. DA was detected in 10% of Holstein cows that were positive for BSCK at PPW4, however, this did not create a highly significant risk ($p = 0.09$) (Table 3). Metritis was one of the most frequently observed PPHD in study cows (Montbeliard n = 1, Holstein n = 15). Holstein that was positive for SCK had frequently metritis cases, however, the incidence (25%) was most remarkable in MSCK1/2 positive Holstein at PPW4 ($p = 0.06$, OR: 4.48). Average BBAC at PPW2 and MBAC at PPW4 were significantly ($p < 0.05$ and < 0.01) higher in cows having metritis (Fig. 3). Those cows had also a moderate significantly ($p = 0.07$) lower BCS (2.81) at PP60 than other cows. Out of 300 study cows, 42 Holstein (14%), 2 Montbeliard (0.6%) and 1 Simmental cow had mastitis in 90DIM. Cows with mastitis had significantly ($p < 0.05$) lower average BCS at calving (3.23) and higher average parity (3.38 ± 0.27). There was a significant difference ($p = 0.015$) between BCS2 (having 24% of mastitis) and other BCS groups concerning the existence of mastitis. However, Holstein cows that were positive for MSCK1/2 at PPW4, were more likely to have mastitis (33.3%, $p < 0.05$, OR: 5.08) (Table 3). Ten percent of all study cows had lameness in 90DIM. The majority of them were multiparous (average parity was 3.29 ± 0.31, $p = 0.06$). The BBAC of cows with lameness was moderately high but not

significant (p = 0.09) at PPW4 compared to cows without lameness. Holstein cows had frequently lameness in all SCK groups, especially cows having BSCK at PPW4 had a moderate significantly higher incidence of lameness (30%, p = 0.06, OR: 4.25). The incidence of CO was 3% among all study cows (Holstein n = 8, and Simmental n = 1), no significant relation was found between BBAC and MBAC. Sixteen among all study cows (Montbeliard n = 1, Holstein n = 15) had multiple diseases. They have got constantly higher BBAC and MBAC at PPW2 and 4, thus MBAC at PPW4 was significantly higher ($p < 0.05$) in cows having multiple diseases (Fig. 3). These cows had also significantly lower BCS at PP60 (2.73 ± 0.08) compared to cows without multiple diseases (2.97 ± 0.03). Holstein cows with positive BSCK, MSCK1/2 and MSCK2 had multiple diseases at certain rates. But, Holstein having had positive MSCK1/2 at PPW4 showed an incidence of 25% multiple diseases (OR: 4.5, p = 0.06) (Table 3).

Association of BSCK and MSCK with the average daily milk yield

The ADMY was compared between SCK positive and negative cows in 90DIM and presented in Tables 4, 5 and 6, Figs. 4 and 5 in Holstein cows only. Simmental cows (all were SCK negative) was disregarded in the milk yield analysis concerning SCK to prevent the dilution of the data. ADMY of all Simmental was 26.80 ± 0.80 kg in 90DIM. The average monthly milk yield of Simmental cows were 26.27 ± 0.87, 27.10 ± 0.66, 27.36 ± 0.85 kg in the first, second and third month after calving respectively (data was not shown in tables). There was no significant effect ($p > 0.05$) of high MSCK1 prevalence in Montbeliard at PPW2 on ADMY (positive: 38.45 ± 2.17 kg; negative: 35.47 ± 2.14 kg) in 90DIM. Montbeliard had in an average of 31.91 ± 1.45, 37.17 ± 1.91 and 40.45 ± 1.65 kg milk yield in the first, second and third month after calving respectively, and the first-month milk yield was significantly different (p = 0.00) (data was not shown in tables). Holstein had a significantly lower average monthly milk yield in the first month compared to the second and third months after calving, however, there was a difference between SCK positive and negative cows (Table 4). Average daily, weekly and monthly milk production of BSCK and MSCK2 negative Holstein had always an upwards trend throughout 12 weeks postpartum in comparison to positive cows (Table 4, 5, 6 and Figs. 4 and 5). The difference in ADMY between SCK negative and positive cows was significant at weeks 10th, 11th and 12th (Figs. 4 and 5). ADMY of Holstein cows having negative BSCK at PPW2 and PPW4 looked higher than cows having positive BSCK, however, the difference was not statistically significant. However, MSCK2 positive Holstein cows at PPW2 had moderate significantly (p = 0.07) lower ADMY (Table 4). ADMY was significantly different in Holstein that was in combined prevalence groups of BSCK and MSCK2, and roughly 5.4 kg and 4 kg higher ADMY was observed in SCK negative cows than positive cows respectively (Table 5). If Holstein cows were positive both for BSCK and MSCK2 at PPW2, ADMY was 6.7 kg less than negative cows, which was significant ($p \leq 0.05$) (Table 5). MSCK1 and MSCK1/2 in Holstein did not have a significant effect on average daily, weekly and monthly milk yield in 90 DIM, even no effect was observed in the combined prevalence groups. Therefore the data was not presented in the tables. ADMY of PRP Holstein that was negative for BSCK (40.59 ± 1.45 kg) at PPW2 was significantly higher ($p \leq 0.05$) than those with positive BSCK (33.55 ± 2.79 kg) in 90 DIM, which meant an average 7 kg milk yield loos per day (Table 4). Besides, PRP Holstein was negative for BSCK at PPW2, had a much higher milk yield in the second and third months postpartum (Table 6). The average monthly milk yield of all Holstein and PRP Holstein, that were negative

for BSCK or MSCK2 at PPW2, was markedly higher at second and third month postpartum, thus the difference was significant ($p < 0.05$) at 2nd month after calving, and moderately significant ($p = 0.07$) at third month after calving (Table 6).

Discussion

The present study reported a lower prevalence of BSCK in Holstein than the previous studies by Suthar et al. (2013) (21.8% in European countries, 11.2% in Turkey), 24% worldwide (Brunner et al. 2019), 19.4% average in 3 regions in Turkey (Şentürk et al. 2016), although the same cut-off point of BBAC for BSCK was used in all these studies. Even, BSCK prevalence was reported 12% (Başbuğ et al. 2014) and 9.7% (Şahal et al. 2017) using the lower threshold level of BBAC (≥ 1.0 mmol/L) in Turkey. Other studies defined BSCK by a threshold level of BBAC ≥ 0.95 mmol/L (Ribeiro et al. 2013), ≥ 1.0 mmol/L (Whitaker et al. 1983; Szelényi et al. 2015) and ≥ 1.4 mmol/L (Carrier et al. 2004; Duffield et al. 2009; Denis-Robichaud et al. 2014; Rodriguez-Jimenez et al. 2018). But, the cut-point of BBAC ≥ 1.20 mmol/L for BSCK definition was found the most acceptance which was much related to PPHD and milk production loss (Suthar et al. 2013; McArt et al. 2015; Raboisson et al. 2015; Benedet et al. 2019; Brunner et al. 2019; Deniz et al. 2020). Therefore, the present study used also this cut-point of BBAC. The reason for this discrepancy can be that no BSCK was detected in two Holstein and two Simmental farms and 1 Holstein-Crossbred farm. Thus, this might indicate that there is no NEB developing in postpartum cows in these farms which resulted in a lower prevalence. Overall, significantly higher BSCK prevalence in Holstein and BBAC and MBAC in primiparous cows were detected at PPW2 compared to PPW4 in the present study. This resulted in the reduced number of SCK positive cases at PPW4, especially in Holstein cows. This was in compromise with other studies, which reported that the first two weeks postpartum are the most prevalent and critical time points for SCK in Holstein dairy cows (Duffield 2000; Carrier et al. 2004; Suthar et al. 2013; Brunner et al. 2019). Most BSCK positive cows were multiparous (2/3) and 1/3 were primiparous cows. This was consistent with other studies (Jordan and Fourdraine 1993; Duffield et al. 1997; Vanholder et al. 2015; Brunner et al. 2019). However, there are also not consistent reports in Holstein (Steen et al. 1996; Carrier et al. 2004), in Jersey (Chandler et al. 2018). As observed in the present study, primiparous Holstein needs better intense care in early lactation to overcome NEB and adapt to the first lactation in the early period. More positive milk ketone tests were observed in the first month compared to the second month of lactation and the peak prevalence of HK occurred in the third and fourth week of lactation this result was slightly close to the present study (Dohoo and Martin 1984). Serum BHBA was tested in the dry period and early lactation in Holstein and the highest BAC and most prevalent SCK was observed at < 65 DIM (Duffield et al. 1997). It was also reported that the incidence and prevalence of SCK in lactating dairy Holstein was around 30% and the most prevalent time was around the 2nd week of the lactation and it decreased to around 5% at the fourth week of the lactation (Khol et al. 2019). Similarly, Carrier et al. (2004) reported a higher prevalence at PPW1 and 2 than PPW3 and thereafter. It seems to be that BBAC testing should be done earlier but not later than PPW2. Therefore, the test points of the present study set for BAC analysis at PPW2 and PPW4 in the blood and milk is consistent with most of the previously reported studies. On the other side, the present study reported differently from others an

individual BBAC/BSCK and MBAC/MSCK at PPW2 and PPW4 exactly, while many of studies reported at not an exact time point postpartum such as 3–16 DIM (McArt et al. 2012), dry period – >149 DIM (Gustafsson et al. 1993), postpartum week 1-week 2 (Duffield et al. 2009), day 30 and 60 post-calving ((Gustafsson et al. 1993), 2–15 DIM (Suthar et al. 2013) and 0–21 day postpartum (Brunner et al. 2019), 7–15 day postpartum (Şahal et al. 2017). Fat Holstein at calving had significantly higher BBAC at PPW2 and 4 and MBAC at PPW2 in the present study. Interestingly, all thin cows and thin Holstein at calving had higher MBAC at PPW4. The majority of cows lost BCS in PP30 and 60 compared to calving BCS. This meant cows suffered from NEB in the early lactation. Fatty cow syndrome was already reported as a reason for ketosis development in Holstein (Seifi et al. 2011; Roche et al. 2018) and was also reported cows with a moderate or fat BCS (≥ 4) at prepartum period have a higher risk of developing SCK and CK than thin cows (BCS ≤ 3.0) (Vanholder et al. 2015). Primiparous Holstein that was positive for BSCK or MSCK has lost BCS within 60 days postpartum. Probably, these cows had fat mobilisation due to NEB to compensate energy requirement for the first milk production in that farms (Duffield et al. 2009). The reported prevalence of MSCK1, MSCK2 and MSCK1/2 in the present study was different due to different cut-points of MBAC. The cut-off value of MBAC for the definition of MSCK in the milk is also varying among the studies. Ranges of MBAC to classify cows with suspect SCK (150 to 190 µmol/L) or positive SCK (≥ 200 µmolL) have been recently reported (Benedet et al. 2019). Some of the studies reported the prevalence of SCK based on cut-off point MBAC ≥ 100 µmol/L using the milk test strips (Berge and Vertenten 2013). MBAC ≥ 200 µmol/L was recommended to define cows having severe and positive ketosis (Benedet et al. 2019) and used also in the previous studies (Melendez et al. 2006; Denis-Robichaud et al. 2014). This is in line with the present study. Overall, Berge and Vertenten (2013) reported an average prevalence of 39% by using milk ketone strips at cut-point of MBAC ≥ 100 µmol/L in European countries, while this rate was 22.6% by using FTIS (Fourier-Transform Infrared) in Canada (Santschi et al. 2016). Both MSCK1 and MSCK2 prevalence rate reported by the present study was low compared to the literature although it was tested at two different postpartum time points. MBAC of multiparous cows increased significantly at PPW4 compared to PPW2 in the present study. In a compromise with this, MSCK2 positive Holstein cows were more likely to develop CK, although other PPHD such as metritis, mastitis and multiple diseases were observed, but without significant OR. SCK was frequently associated with PPHD in Holstein (Suthar et al. 2013; McArt et al. 2015; Raboisson et al. 2015; Brunner et al. 2019; Deniz et al. 2020). The results of the present study revealed that BSCK positive Holstein cows at PPW2 were more likely to develop CK. If they were positive at PPW4, CK, DA and lameness were seen much frequently in Holstein cows. Transformation of SCK into CK was frequently reported with a risk factor between 4 – 10.5 times likelihood (Duffield et al. 2009; Seifı et al. 2011; Overton et al. 2017). The present study showed BSCK diagnosed at PPW4 cause a risk for CK, DA and lameness. BSCK and MSCK1/2 positive Holstein at PPW4 had a nonsignificant incidence of DA, because DA was overall low (1%) in all study cows. DA is the most frequently detected metabolic disorder related to SCK in the first weeks after calving with a high-risk factor (4–13.6 times likely) (LeBlanc et al. 2005; Duffield et al. 2009; Seifi et al. 2011; Suthar et al. 2013). Unfilled rumen due to reduced appetite at calving is possibly one of the reasons for DA that creates a large abdominal space (McGuffey 2017). Mastitis was found in all SCK positive multiparous Holstein in the present study, however, it created 5 times higher risk and 33.3% of MSCK1/2

positive cows had mastitis. Overall, all cows that had mastitis (15% out of 300) have got significantly lower BCS (thin) at calving. Suthar et al. (2013) did not found mastitis in BSCK positive Holstein in European countries. It was reported 4.2% of mastitis incidence in Holstein with BSCK in Turkey (Uyarlar 2018). Brunner et al. (2019) found 3.4% mastitis incidence and Rabiosson et al. (2015) reported a 1.6 times higher risk for mastitis in BSCK positive Holstein. Kremer et al. (1993) stated that cows with BBAC > 1.4 mmol/L were more susceptible to severe mastitis in an experimental *E.coli* study. Metritis was present in SCK positive Holstein in the present study, but it created 4.5 times the high risk for MSCK1/2 positive Holstein at PPW4 (25%). A metritis incidence by 25% and 5.3% was reported in a study in Turkey, and worldwide in BSCK positive Holstein cows respectively (Uyarlar 2018; Brunner et al. 2019). Higher risk for metritis as 3.3, 1.7 and > 4.0 times likelihood was reported by others (Duffield et al. 2009; Suthar et al. 2013; Overton et al. 2017). The early PPHD such as RP and milk fever as well as cystic ovarian were not found in the present study concerning SCK incidence. It wasn't established a cut-point of BBAC for this early PPHD (Suthar et al. 2013). Others reported risks for RP (4.7 times or 4.8%) in association with BSCK (Seifi et al. 2011; Brunner et al. 2019). CO associated with BSCK was observed at a rate of 13.5% (Jordan and Fourdraine 1993) and 5.6 times more likely (Dohoo and Martin 1984) in dairy cows. Lameness was found in much higher incidence (30%) and significant risk (4.3 times likely) in BSCK positive Holstein in the present study. A 1.8 times higher risk and 1.8% incidence of lameness have been reported in BSCK positive Holstein (Suthar et al. 2013; Brunner et al. 2019). It wasn't reported lameness in SCK or CK positive Holstein in Turkey (Uyarlar et al. 2018). The culling rate in MSCK1/2 and MSCK2 positive cows were significantly high and they were 3.4 and 11.4 times more likely to be culled within 90 DIM. Culled cows and MSCK1/2 positive cows at PPW4 had also multiple diseases at a rate of 25%. It was reported that the culling rate was 26.4% in BSCK and 36% in CK positive cows, besides 5.6% of BSCK positive cows had multiple diseases (metritis/mastitis) in Turkey (Uyarlar et al. 2018). This is in line with the present study, however, the present study found and investigated additionally culling rates in MSCK positive cows. The results of the present study indicated a reduced milk yield in BSCK and MSCK2 positive Holstein cows which is consistent with the finding of previously reported studies (Dohoo and Martin 1984; Gustafsson et al. 1993; Miettinen and Setala 1993; Duffield et al. 1997; Duffield et al. 2009; McArt et al. 2012; Raboisson et al. 2015). Holstein cows, that were tested positive for BSCK at PPW2 or 4 and positive for both BSCK and MSCK2 at PPW2 had constantly lower average weekly and monthly milk yield, and these cows had significantly ADMY losses of ca. 4 kg and 6.7 kg in 90 DIM respectively. Previous studies reported the association between reduced daily milk yield and BSCK incidence in Holstein (Duffield et al. 2009; McArt et al. 2012; Raboisson et al. 2015). Increasing BBAC above 1.0 mmol/L during PPW2 was associated with progressively less 305-d milk yield (Duffield et al. 2009). A linear negative effect of BBAC beginning at 1.2 mmol/L at PPW1 on 305 days of milk production was observed (Duffield et al. 2009). This elevated BBAC in the first week postpartum can result in milk yield losses up to 1,281 kg over 305 milking days and calculated milk yield losses due to SCK was reported as 328 kg (Gustafsson et al. 1993), 305–427 kg (Dohoo and Martin 1984). This can result in economic losses throughout the production cycle (McArt et al. 2015; Raboisson et al. 2015; Mostert et al. 2017), e.g in an average of USD 200–290 per cow (Deniz et al. 2020). BSCK associated with high BBAC at PPW2 resulted in significantly lower milk yield in PRP Holstein in the present study. Similar trend was observed in MSCK2 positive PRP

Holstein, but the number of animals was not enough to do comparison. Probably these PRP cows suffered a poor adaptive response to the onset of the first lactation and the resulting NEB (Duffield et al. 2009). A little difference in HK incidence was observed between PRP and MUL cows (Steen et al. 1996). Chandler et al. (2018) found more prevalent HK in PRP Jersey than MUL Jersey cows. A very small difference in ketosis prevalence between PRP and MUL cows was found in Ayrshire and Friesian cows (Kauppinen 1983). A similar prevalence rate of SCK was reported in PRP and MUL Holstein at PPW1, but it was higher at PPW2 and after PPW3 in MUL Holstein, even there was no SCK at and after PPW3 in PRP Holstein (Carrier et al. 2004). This might lead to the point that PRP Holstein needs more intense care in early lactation to overcome NEB and adapt to the first lactation. However, this was not observed at milk yield between MSCK1 positive and negative Holstein and Montbeliard. The lower threshold level of MBAC (≥ 100 µmol/L) for MSCK1 definition at PPW2 or PPW4 did not significantly affect ADMY in Holstein and Montbeliard in the present study. Previous studies (Dohoo and Martin 1984; Gustafsson et al. 1993) reported the association between MSCK and ADMY in Holstein in contrast to the present study. On the other side, a low correlation between BBAC and MBAC (Denis-Robichaud et al. 2014) was also observed but it was moderate in the present study in Holstein, no correlation was found in other breeds, especially, in terms of changes of BAC between PPW2 and PPW4. In contrast, no significant correlation was found between BSCK and MSCK neither at PPW2 and PPW4. This was controversy to the results of BBAC and MBAC. The reason might be due to the lower sensitivity of milk BHBA test strips compared to cow-side blood BHBA analysers (Carrier et al. 2004). Semiquantitative determination of MBAC which bases on the colour indication for BAC might affect the results. It was stated that concentrations of milk and blood BAC were poorly correlated with the concentrations of ketone bodies and the use of milk strips overestimated the concentrations of BAC in the milk (Enjalbert et al. 2001). The lack of relationship between MBAC and BBAC observed by Andersson (1984) suggested that milk BAC could be of low value for the detection of SCK, so that few authors presented a critical cut-off point for MBAC. Geishauser et al. (1988) reported that the correlation coefficient between BBAC and MBAC were from 0.00 to 0.87. BHBA can be utilised by the mammary gland for fatty acids synthesis and converted to butyrate (Dodds et al. 1981; Duffield 2000) that is why MBAC is only 10–15% of BBAC, possibly because of the ketone body's role in fat metabolism in the udder (Andersson 1984). These fluctuations in MBAC in contrast to BBAC may be a reason for the difference of BSCK from MSCK1 and MSCK2 in the present study. However, the prevalence of MSCK in Holstein was often reported higher than the prevalence of BSCK (Berge and Vertenten 2013; Benedet et al. 2019). In the present study, once the cut-off point of MBAC for the definition of SCK was increased to MBAC ≥ 200 µmol/L (MSCK2), a negative effect was observed on the daily weekly and monthly milk production, although there was no negative effect of MSCK1 in Holstein and Montbeliard. The cut-point of MBAC ≥ 200 µmol/L for SCK definition was already recommended (Melendez et al. 2006; Denis-Robichaud et al. 2014). This is in line with the results of the present study because MSCK2 had a detrimental effect on ADMY than MSCK1 and MSCK1/2. Nevertheless, the specificity and sensitivity of the milk test strips used in the present study were confirmed both for BAC (100 µmol/L and ≥ 200 µmol/L) to be useful in cows (Carrier et al. 2004), there are still possibilities to observe around 3–5% false positive and false negative cases, which need to be taken into account by interpreting the milk results. That was the reason why both blood and milk tests were performed for the detection of BSCK and MSCK

in the present study. In the present study, BSCK and MSCK were not detected in Simmental and Holstein-Crossbreed cows, overall BBAC and MBAC were much lower in those animals and Montbeliard. Montbeliard showed a low incidence of BSCK and a high incidence of MSCK1 at PPW2, but none of the positive cases had a detrimental effect on postpartum health status and ADMY. Controversially, a study reported a quite high incidence for BSCK in Simmental that were in early lactation and late pregnancy (Djoković et al. 2013). But no information and explanation was given about the parity and the reason for this high incidence. A significantly reduced milk yield (12.5% reduction) was observed in Montbeliard with BSCK in the second month of lactation, however, no details were available about the incidence rate and detection time of BSCK (Yameogo et al. 2008). On the other side, Simmental cows were mostly culled because of sterility and reproductive diseases, but Montbeliard cows were culled due to poor yield and udder problems (Zółkiewski et al. 2008). The reason for the discrepancy between the results of the studies might be management system differences and parity effect. Thus, the average parity was quite low for Holstein-Crossbred and Simmental in the present study. Calavas et al. (1996) did not report ketosis cases, besides many other metabolic diseases in 8 Montbeliard herds, which were monitored clinically throughout 3 years. SCK reduced milk production in the early lactation of Finnish Ayrshire and Holstein Friesen (Miettinen and Setala 1993). Gantner et al. (2018) found that the highest prevalence risks of ketosis were observed in 20 DIM of Prim Simmental cows, parity 2 and 3 cows, while cows in parity 4 had a peak prevalence risk in 25 DIM. The French Simmental family has three strains; Pie rouge de l'Est (or French Simmental), Montbeliard and Abondance (Averdunk 2002). Thus, Montbeliard and Simmental cows were classified in the same family of French Simmental (Averdunk 2002, Felius et al. 2014) and they might show a certain extent resistance to SCK (Gantner et al. 2018), especially it can be much obvious under well-cared modern feeding and management system. Strong resistance of this dual-purpose Simmental Flechvieh breeds to mastitis was reported (Averdunk 2002). Although the number of Flechvieh cows looked small compared to Holstein for an evaluation in the present study, the resulting evidences in these breeds might show the overall trend for SCK.

Conclusion

Conclusively, BSCK and MSCK were still a herd problem causing PPHD and culling in not all but in many of the Holstein farms in Turkey. The prevalence of SCK was much higher at PPW2 than PPW4 and fat cows at calving were more likely to have high BBAC and emaciated and fat cows showed much higher MBAC that was associated with metritis and MD. BSCK and MSCK2 positive Holstein at PPW2 had an ADMY loss of 6.7 kg. Similarly, ADMY loss was 7 kg in PRP Holstein, that were positive for BSCK at PPW2. The cut-off point of MBAC ≥ 100 µmol/L for MSCK definition did not cause a significant effect on ADMY, which was overall in line with the latest statement in the literature. However, a higher cut-off point of MBAC ≥ 200 µmol/L (MSCK2) caused a reduced average daily, weekly and monthly milk yield trend that was certain extend significantly different from MSCK2 negative cows. The reason for the reduced, but not strong significantly different milk yield and OR of PPHD caused by BSK and MSCK2 in Holstein can be the low prevalence of SCK and small sample size. SCK was not observed in Simmental and HC farms, however, the high prevalence of MSCK1 at PPW2 did not affect the postpartum health status and ADMY

in Montbeliard. Simmental and related breeds might have a certain resistance to SCK, therefore SCK prevalence and its effect on ADMY and PPHD need to be investigated in much larger samples sizes in all related breeds (Flechvieh breed). PRP Holstein needs to be investigated more intensively in terms of the development of NEB and associated ADMY under current modern conditions and high expectations for milk production.

Declarations

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Tables

Table 1

Beta-hydroxybutyric acid concentrations (BAC, x ± se) in the blood (BBAC mmol/L) and milk (MBAC µmol/L) at postpartum week 2 and 4 (PPW2, PPW4) and body condition scores (BCS, x ± se) at calving, postpartum day 30 (PP30) and 60 (PP60), in primiparous and multiparous Holstein, Montbeliard, Simmental and Holstein Crossbred (HC)*

		All breeds	Holstein	Montbeliard	Simmental	HC	p(1)
All parities	BCS-calving	3.42 ± 0.03[1]	3.35 ± 0.03[a1]	3.52 ± 0.04[b1]	3.59 ± 0.10[b1]	3.61 ± 0.08[b1]	0.00
	BCS-PP30	2.96 ± 0.02[2]	2.94 ± 0.03[a2]	2.80 ± 0.04[a2]	3.24 ± 0.08[b2]	2.83 ± 0.09[a2]	0.00
	BCS-PP60	2.96 ± 0.03[2]	2.88 ± 0.03[a3]	2.88 ± 0.02[a2]	3.41 ± 0.09[b1]	2.87 ± 0.06[a2]	0.00
	p(2)	0.00	0.00	0.00	0.01	0.02	-
	PPW2-BBAC	0.48 ± 0.04	0.54 ± 0.05[a]	0.32 ± 0.07[b]	0.45 ± 0.01[a]	0.03 ± 0.04[c]	0.00
	PPW4-BBAC	0.42 ± 0.03	0.46 ± 0.04[a]	0.33 ± 0.03[b]	0.44 ± 0.04[a]	0.02 ± 0.09[c]	0.00
	p(3)	0.21	0.11	0.43	0.95	0.48	-
	PPW2-MBAC	62.98 ± 9.01	76.73 ± 14.84[a]	52.17 ± 9.68[b]	50.00 ± 0.00[b]	-	0.45
	PPW4-MBAC	55.63 ± 9.72	67.33 ± 13.41[a]	8.70 ± 4.04[b]	50.00 ± 0.00[c]	-	0.00
	p(3)	0.00	0.03	0.00	1.00	-	-
Prim	BCS-calving	3.51 ± 0.05[1]	3.48 ± 0.06[a1]	3.60 ± 0.10[a1]	3.56 ± 0.19[a1]	3.50 ± 0.12[a1]	0.76
	BCS-PP30	2.98 ± 0.05[2]	2.98 ± 0.05[a2]	2.87 ± 0.12[a2]	3.17 ± 0.17[a1]	2.86 ± 0.14[a2]	0.44
	BCS-PP60	2.97 ± 0.06[2]	2.92 ± 0.07[a2]	2.90 ± 0.05[a2]	3.33 ± 0.17[b1]	2.86 ± 0.10[a2]	0.03
	p(2)	0.00	0.00	0.01	0.08	0.00	-
	PPW2-BBAC	0.57 ± 0.07	0.75 ± 0.17	0.52 ± 0.30	0.50 ± 0.06	0.03 ± 0.02	0.00
	PPW4-BBAC	0.40 ± 0.07	0.51 ± 0.12	0.44 ± 0.10	0.42 ± 0.08	0.01 ± 0.03	0.00
	p(3)	0.04	0.08	1.00	0.20	0.16	-

		All breeds	Holstein	Montbeliard	Simmental	HC	p(1)
	PPW2-MBAC	91.67 ± 28.85	101.85 ± 39.63	70.00 ± 20.00	50.00 ± 0.00	-	0.57
	PPW4-MBAC	43.33 ± 16.56	50.00 ± 23.40	10.00 ± 10.00	50.00 ± 0.00	-	0.10
	p(3)	0.00	0.03	0.06	1.00	-	-
Mul	BCS-calving	3.39 ± 0.03[1]	3.32 ± 0.03[a1]	3.50 ± 0.04[a1]	3.60 ± 0.12[b1]	3.71 ± 0.11[b1]	0.00
	BCS-PP30	2.95 ± 0.03[2]	2.93 ± 0.03[a2]	2.78 ± 0.05[b2]	3.27 ± 0.10[c2]	2.79 ± 0.11[b2]	0.00
	BCS-PP60	2.95 ± 0.03[2]	2.87 ± 0.04[a3]	2.88 ± 0.02[a2]	3.45 ± 0.11[b2]	2.88 ± 0.07[a2]	0.00
	p(2)	0.00	0.00	0.00	0.00	0.00	-
	PPW2-BBAC	0.45 ± 0.04	0.50 ± 0.04	0.27 ± 0.03	0.43 ± 0.05	0.03 ± 0.03	0.00
	PPW4-BBAC	0.42 ± 0.03	0.45 ± 0.04	0.29 ± 0.03	0.45 – 0.04	0.03 ± 0.05	0.00
	p(3)	0.68	0.36	0.41	0.57	1.00	-
	PPW2-MBAC	55.86 ± 8.44	58.04 ± 10.79	47.22 ± 11.05	50.00 ± 0.00	-	0.56
	PPW4-MBAC	58.93 ± 11.51	71.88 ± 15.81	8.33 ± 4.52	50.00 ± 0.00	-	0.00
	p(3)	0.01	0.21	0.01	1.00	-	-

Prim: primiparous cows, Mul: multiparous cows. *: MBAC was not tested in Holstein-Crossbred (Holstein/Montbeliard). p(1): Kruskal-Wallis Test between breeds, [a,b,c]: different letters refer significant difference in the same line between breeds. p(2): Kruskal-Wallis Test between BCS check times (calving, PP30, PP60), [1,2,3]: different numbers refers significant difference in the same column between calving BCS, BCS30 and PP60. p(3): Wilcoxon Sig.Ranks test between PPW2 and PPW4

Table 2

Descriptive statistic about the prevalence of subclinical ketosis (SCK) at PPW2 and PPW4 in primiparous and multiparous Holstein cows and their body condition scores (BCS, mean, minimum and maximum) at calving, postpartum day 30 (PP30) and 60 (PP60)

Total tested			**Parity**		**BCS (mean, min and max)**		
SCK Group	n	SCK (%)	Pri/Mul	SCK (%)	Calving	PP30	PP60
BSCK-PPW2	216	8.3	Prim	27.7	3.44 (3.00–4.00)	3.10 (3.00-3.50)	2.95 (2.50–3.50)
			Mul	72.2	3.33 (2.50-4.00)	3.16 (2.50–4.50)	3.32 (2.50-5.00)
BSCK-PPW4	213	4.7	Prim	30.0	3.58 (3.00–4.00)	3.25 (3.00-3.50)	3.00 (2.50–3.50)
			Mul	70.0	3.70 (3.50-4.00)	3.43 (3.00–5.00)	3.50 (3.00–5.00)
MSCK1-PPW2	139	11.5	Prim	12.5	3.50 (3.50–3.50)	2.85 (2.70-3.00)	2.77 (2.75–2.80)
			Mul	87.5	3.35 (2.50-4.00)	2.91 (2.50–3.50)	2.87 (2.50–3.50)
MSCK1-PPW4	101	4.9	Prim	20.0	3.75 (3.75–3.75)	3.25 (3.25–3.25)	3.50 (3.50–3.50)
			Mul	80.0	3.20 (3.00–4.00)	2.88 (2.50–3.50)	2.67 (2.50-3.00)
MSCK2-PPW2	139	5.8	Prim	50.0	3.60 (3.00–4.00)	3.16 (3.00-3.50)	3.16 (3.00-3.50)
			Mul	50.0	3.25 (3.20–3.50)	3.25 (2.50-4.00)	3.40 (2.75-4.00)
MSCK2-PPW4	101	6.9	Prim	14.3	3.20 (3.20–3.20)	3.00 (3.00–3.00)	3.00 (3.00–3.00)
			Mul	85.7	3.30 (3.20–3.50)	3.36 (3.00–5.00)	3.28 (2.75-5.00)
MSCK1/2-PPW2	139	17.3	Prim	25.0	3.58 (3.50-4.00)	3.00 (2.60–3.50)	3.00 (2.75–3.50)
			Mul	75.0	3.33 (2.50–3.50)	2.98 (2.50–4.50)	2.98 (2.50–4.50)
MSCK1/2-PPW4	101	11.9	Prim	16.7	3.50 (3.20–3.75)	3.12 (3.00-3.25)	2.75 (2.50-3.00)
			Mul	83.3	3.25 (2.50-4.00)	3.18 (2.50-5.00)	3.10 (2.50-5.00)

BSCK: blood beta-hydroxybutryric acid concentration≥1.20 mmol/L, MSCK1: milk beta-hydroxybutryric acid concentration =100 µmol/L, MSCK2: milk beta-hydroxybutryric acid concentration ≥200 µmol/L, MSCK1/2: milk beta-hydroxybutryric acid concentration ≥100 µmol/L. Prim: primiparous cows, Mul: multiparous cows

Table 3
Incidences of postpartum health disorders (PPHD) in Holstein cows that were tested positive or negative for subclinical ketosis (SCK)

	SCK		PPHD (%)								
	positive/ negative	%	CK	RP	DA	Met	Mast	Lam	MF	CO	MD
BSCK-PPW2	negative	91.7	0.0	2.5	1.5	6.6	17.7	11.1	1.0	3.5	6.6
	positive	8.3	44.2[1]	0.0	0.0	5.6	11.1	22.2	0.0	0.0	5.6
BSCK-PPW4	negative	95.3	1.5	2.5	1.0	6.4	17.2	10.3	1.0	3.4	6.4
	positive	4.7	20.0[2]	0.0	10.0[4]	10	20.0	30[7]	0.0	0.0	10.0
MSCK1/2-PPW2	negative	82.7	0.9	2.6	1.7	7.0	11.3	7.8	1.7	4.3	8.7
	positive	17.3	16.7	0.0	0.0	12.5	12.5	16.7	0.0	0.0	8.3
MSCK1/2-PPW4	negative	88.1	2.2	0.0	1.1	7.9	10.1	5.6	1.1	5.6	7.9
	positive	11.9	8.3	0.0	8.3	25.0[5]	33.3[6]	16.7	0.0	0.0	25.0[8]
MSCK2-PPW2	negative	94.2	1.5	2.3	1.5	7.6	10.7	9.9	1.5	3.8	0.8
	positive	5.8	37.5[3]	0.0	0.0	12.5	25.0	0.0	0.0	0	12.5
MSCK2-PPW4	negative	93.1	2.1	0.0	2.1	9.6	11.7	7.4	1.1	5.3	9.6
	positive	6.9	14.3	0.0	0.0	14.3	28.6	0.0	0.0	0.0	14.3

[1]: $p<0.05$, [2,3]: $p<0.01$, [4]: $p=0.09$, [5,7,8]: $p=0.06$, [6]: $p<0.05$. PPW2: postpartum week 2, PPW4: postpartum week 4. BSCK: beta-hydroxybutryric acid concentration (BAC) in the blood ≥1.20 mmol/L. MSCK1: milk BAC=100 µmol/L. MSCK2: milk BAC ≥200 µmol/L. MSCK1/2: milk BAC ≥100 µmol/L. CK: clinical ketosis, RP: retained placenta, DA: displaced abomasum, Met: metritis, Mast: mastitis, Lam: lameness, MF: milk fever, CO: Cyctic ovarian, MD: multiple diseases. No significant relation was found between MSCK1 and PPDH

Table 4

Average daily milk yield of Holstein cows (x ± se, kg) with positive and negative subclinical ketosis (SCK) at postpartum week 2 or 4 (PPW2 or PPW4) in primiparous and multiparous Holstein cows in 90DIM

	All Holstein			Primiparous			Multiparous		
	Positive	Negative	p	Positive	Negative	p	Positive	Negative	p
BSCK at PPW2	34.62 ± 1.55	38.16 ± 0.65	0.10	33.55 ± 2.79	40.59 ± 1.45	0.05	35.16 ± 1.92	37.70 ± 0.71	0.33
n	18	188		6	30		12	158	
BSCK at PPW4	35.05 ± 2.19	37.92 ± 0.63	0.43	33.60 ± 3.23	39.95 ± 1.42	NA	36.24 ± 1.80	37.56 ± 0.72	0.68
n	11	192		3	33		8	159	
MSCK2 at PPW2	32.46 ± 2.48	38.02 ± 0.75	0.07	29.71 ± 0.66	37.03 ± 1.21	NA	34.11 ± 3.91	38.25 ± 0.88	0.31
n	8	126		3	23		5	103	
MSCK2 at PPW4	33.85 ± 11.87	36.70 ± 10.88	0.47	28.39	36.63 ± 1.29	NA	35.21 ± 1.66	36.72 ± 1.07	NA
n	5	97		1	21		4	76	

90DIM: 90 days in milk. NA: not applicable

Table 5

Average daily milk production of Holstein (x ± se, kg) with positive and negative subclinical ketosis (SCK) in the combined groups at postpartum week 2 or 4 (PPW2 or PPW4) in 90DIM

SCK Group	Milk Yield		p	SCK (%)	Not matching to group* (n/%)
	SCK Positive	SCK Negative			
BSCK at PPW2 or 4	34.25 ± 1.44	38.24 ± 0.65	0.05	11.6	0
n (206)	24	182			
MSCK2 at PPW2 or 4	32.68 ± 12.02	38.10 ± 10.76	0.05	7.6	0
n (tot:134)	10	124			
BSCK/MSCK2 at PPW2	31.33 ± 3.23	37.99 ± 0.79	0.05	4.5	9/6.7
n (tot: 134)	6	119			
BSCK/MSCK2 at PPW4	36.7	36.56 ± 0.85	NA	0.98	8/7.8
n (tot: 102)	1	93			
BSCK or MSCK2 at PPW2	34.74 ± 1.39	38.19 ± 0.65	0.09	8.9	0
n (tot: 134)	12	122			
BSCK or MSCK2 at PPW4	34.88 ± 1.26	38.07 ± 0.65	0.17	8.8	0
n (tot: 102)	9	93			

90DIM: 90 days in milk. NA: not applicable. BSCK: BBAC ≥1.2 mmol/L in the blood, MSCK2: ≥200 µmol/L in the milk. *: These animals cannot be allocated in the respective group because they were positive for one of SCK only

Table 6

Average daily milk production (x ± se) (kg) per month of primiparous and multiparous Holstein cows tested for positive (+) or negative (-) of subclinical ketosis (SCK) in the blood (BSCK) and milk (MSCK2) at postpartum week 2 or 4 (PPW2 or 4)

				Average daily milk production (x ± se) after calving			
		SCK	n	First month	Second month	Third month	p(1)
All Holstein	BSCK at PPW2	+	18	32.77 ± 1.50	34.58 ± 2.54	35.08 ± 2.35	0.03
		-	188	34.74 ± 0.54	39.86 ± 0.76*	39.89 ± 0.77**	0.00
	MSCK2 at PPW2	+	8	30.25 ± 2.36	30.74 ± 5.12	32.92 ± 4.27	0.16
		-	126	34.42 ± 0.67	39.91 ± 0.89*	39.74 ± 0.85**	0.00
	BSCK at PPW4	+	11	33.98 ± 1.43	34.31 ± 3.86	36.01 ± 1.63	0.27
		-	192	34.58 ± 0.54	39.65 ± 0.75	39.67 ± 0.78	0.00
	MSCK2 at PPW4	+	5	28.22 ± 2.14	36.51 ± 2.67	36.81 ± 2.11	0.07
		-	97	33.61 ± 0.73	38.38 ± 1.04	38.11 ± 1.05	0.00
Primiparous	BSCK at PPW2	+	6	32.09 ± 2.50	29.72 ± 6.69	34.45 ± 3.80	0.16
		-	30	35.06 ± 1.12	43.32 ± 1.64*	43.40 ± 1.91***	0.00
	MSCK2 at PPW2	+	3	27.75 ± 1.32	21.00 ± 10.62	30.20 ± 0.07	0.22
		-	23	33.54 ± 1.21	39.28 ± 1.36	38.26 ± 1.53	0.00
	BSCK at PPW4	+	3	31.09 ± 2.76	35.97 ± 13.26	35.25 ± 5.12	0.13
		-	33	34.88 ± 1.09	42.43 ± 1.63	42.53 ± 1.86	0.00
	MSCK2 at PPW4	+	1	26.18	28.73	30.26	NA
		-	21	32.83 ± 1.12	39.02 ± 1.47	38.06 ± 1.67	0.00

				Average daily milk production (x ± se) after calving			
Multiparous	BSCK at PPW2	+	12	33.11 ± 1.94	37.01 ± 1.78	35.34 ± 3.03	0.17
		-	158	34.68 ± 0.60	39.20 ± 0.84	39.22 ± 0.83	0.00
	MSCK2 at PPW2	+	5	31.76 ± 3.68	36.58 ± 4.10	34.00 ± 6.10	0.25
		-	103	34.62 ± 0.77	40.05 ± 1.05	40.07 ± 0.99	0.00
	BSCK at PPW4	+	8	35.07 ± 1.60	37.43 ± 2.30	36.21 ± 1.82	0.68
		-	159	34.51 ± 0.61	39.08 ± 0.84	39.08 ± 0.85	0.00
	MSCK2 at PPW4	+	4	28.73 ± 2.69	38.45 ± 2.37	38.45 ± 1.72	0.10
		-	76	33.83 ± 0.87	38.21 ± 1.27	38.14 ± 1.26	0.00

$p^{(1)}$: difference between average milk productions of month 1, 2, 3; average milk yield of the first month is significantly lower than month 2^{nd} and 3^{rd} where it is applicable (p value). *: $p<0.05$, **:$p=0.07$, ***: $p=0.08$ between SCK positive and negative groups at the respective testing month. NA: not applicable because of low number of data. MSCK2: milk beta-hydroxybutryric acid concentration ≥200 µmol/L

Figures

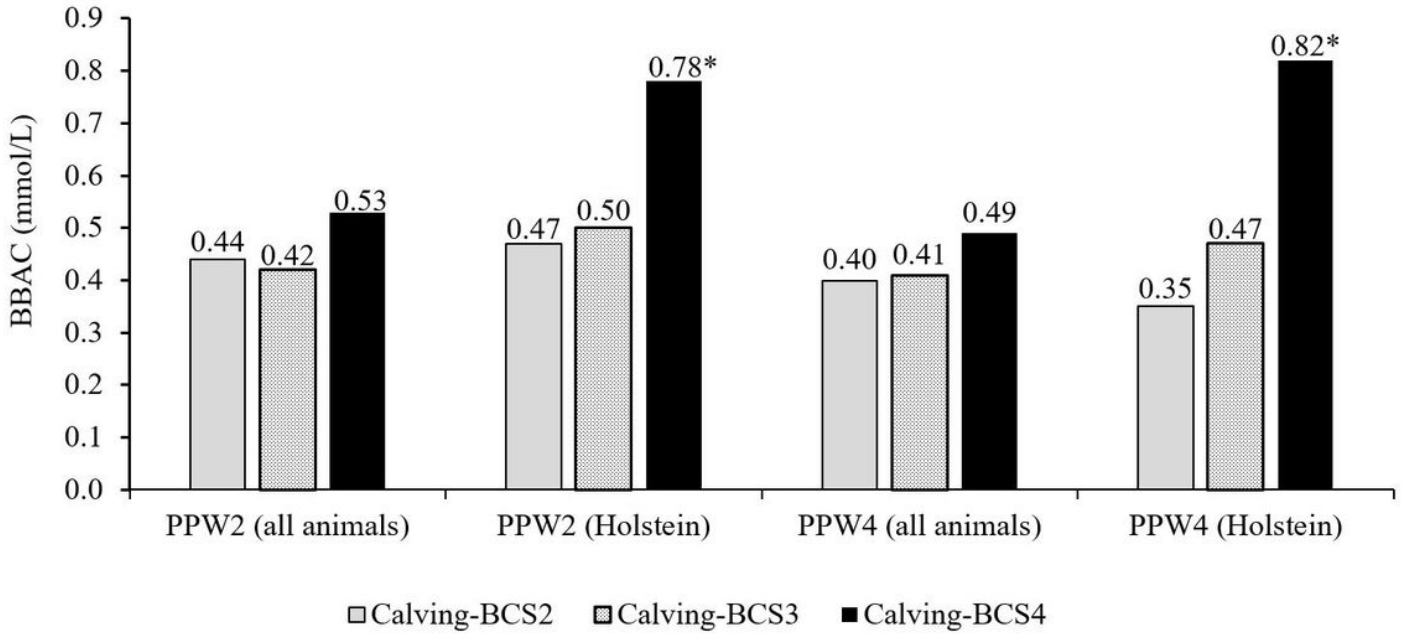

Figure 1

Average blood beta-hydroxybutyric acid concentrations (BBAC) at postpartum week 2 (PPW2) and postpartum week 4 (PPW4) in all study cows and separately in Holstein that had different body conditions scores (BCS) at calving. *:p<0.05 compared to BCS2 and BCS3 at the respective week

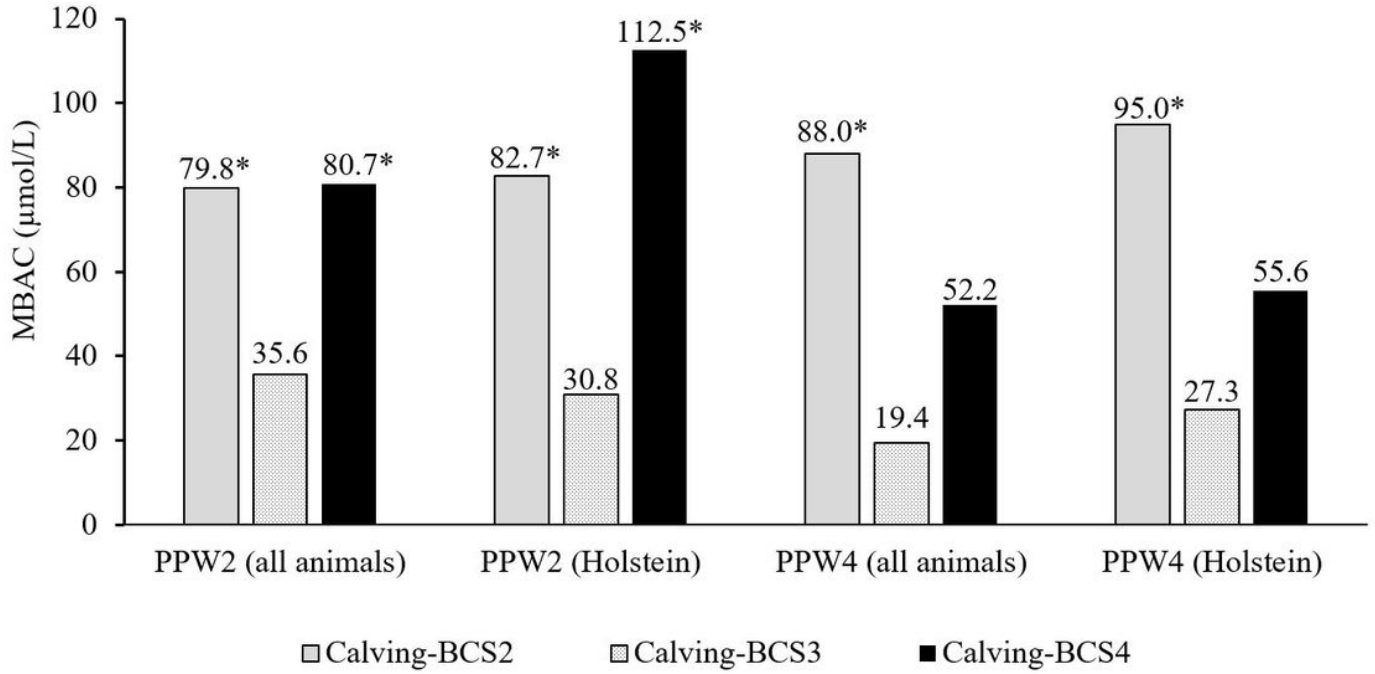

Figure 2

Average milk beta-hydroxybutyric acid concentrations (MBAC) at postpartum week 2 (PPW2) and postpartum week 4 (PPW4) in all study cows and separately in Holstein that had different body conditions scores (BCS) at calving. *:p<0.01, significantly different from BCS3

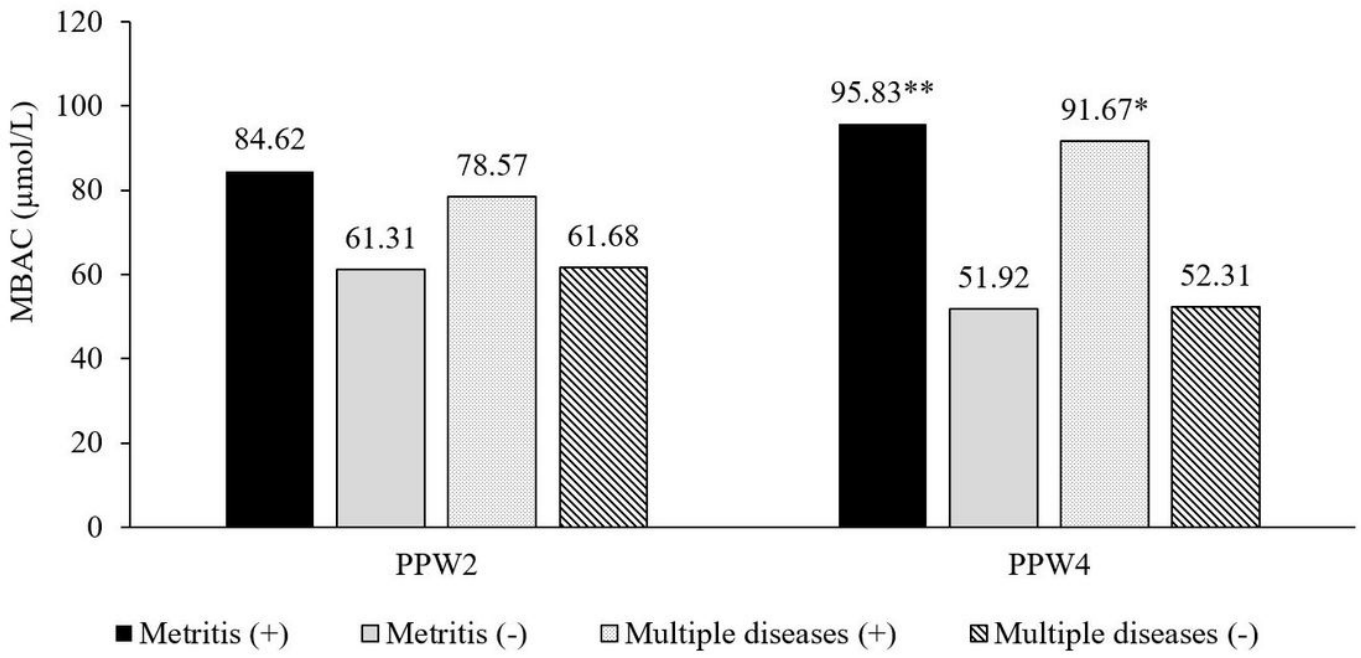

Figure 3

Average milk betahydroxybutyric acid concentrations (MBAC) at postpartum week 2 (PPW2) and postpartum week 4 (PPW4) in cows having metritis and multiple diseases throughout the study period. Based on Mann-Whitney Test; *:p<0.05 versus multiple disease negative, **:p<0.01 versus Metritis negative

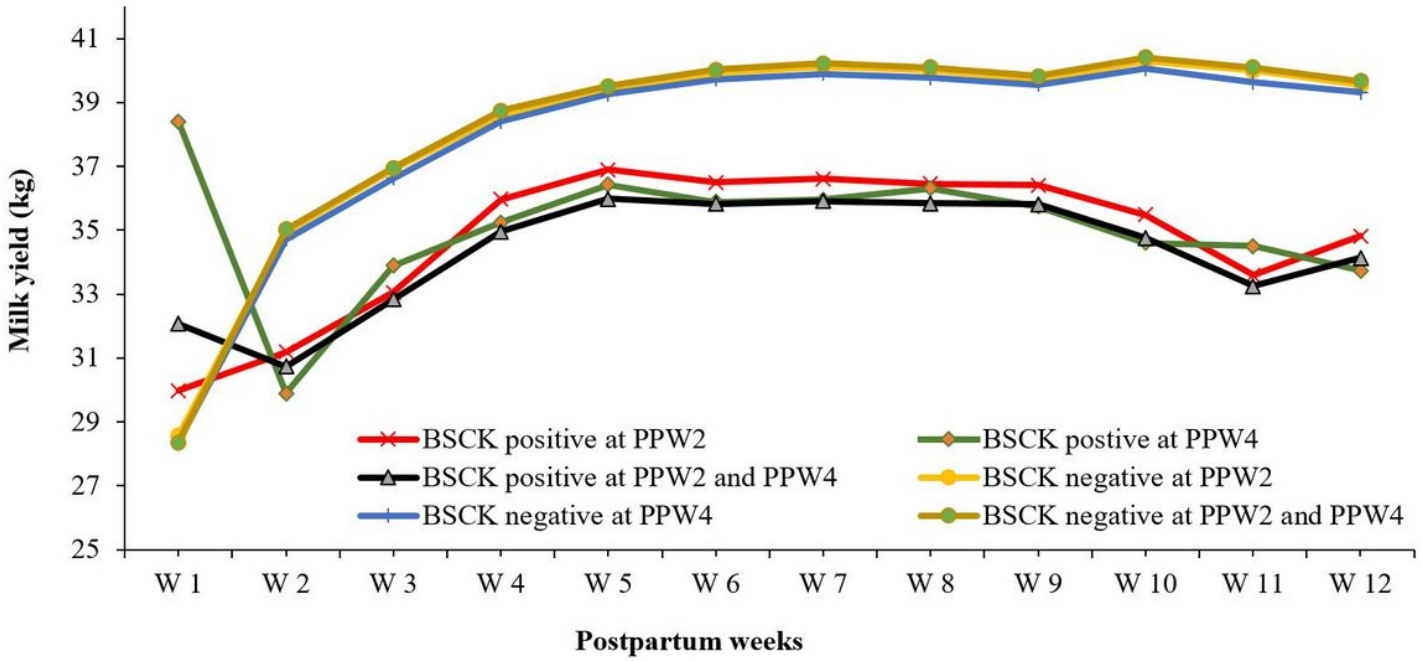

Figure 4

Average daily milk yield per week of Holstein with positive or negative subclinical ketosis (SCK) at postpartum week 2 or 4 (PPW2 or 4) in the blood (BSCK, blood betahydroxybutyric acid concentration ≥1.2 mmol/L). Remark: line of BSCK negative cows at 'PPW2 and PPW4' overlaps the line of BSCK negative cows at PPW2

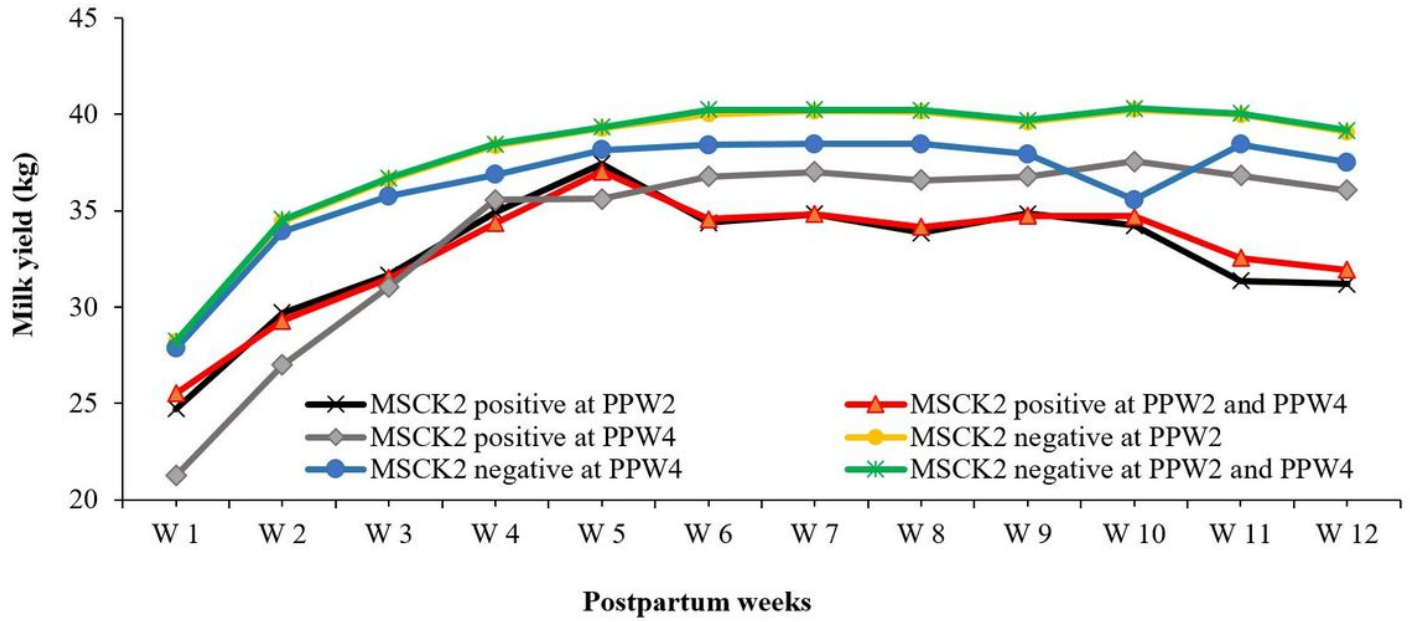

Figure 5

Average daily milk yield per week of Holstein with positive or negative subclinical ketosis (SCK) at postpartum week 2 or 4 (PPW2 or 4) in the milk (MSCK2, MBAC≥200 μmol/L). Remarks: The line of MSCK2 negative cows at ‘PPW2 and PPW4’ overlaps the line of MSCK2 negative cows at PPW2. The line of MSCK2 positive cows at ‘PPW2 and PPW4’ overlaps the line of MSCK2 positive cows at PPW2