RESEARCH ARTICLE

Blood and Milk Beta-hydroxybutyric Acid Concentrations in Different Dairy Cattle Breeds and Association of Subclinical Ketosis with Postpartum Health Disorders, Culling Rate, Body Condition Score, Parity and Milk Production in Holstein

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Abstract: The aim of the study was to analyse beta-hydroxybutyric acid concentration in the blood (BBAC) and milk (MBAC) at postpartum week 2 (PPW2) and week 4 (PPW4) in Holstein (n=216, 8 farms), Montbeliard (n=23), Simmental (n=38) and Holstein/Montbeliard Crossbreed (n=23, BBAC only). Furthermore, the prevalence of subclinical ketosis (SCK) and its association with postpartum health disorders (PPHD), body condition score (BCS) and average daily milk yield in 90 days in milk (90DIM) were evaluated in Holstein. Holstein-Crossbreed and Montbeliard had significantly lower postpartum BBAC than others. Primiparous Montbeliard and Holstein had significantly higher MBAC at PPW2 than PPW4. Cows having BCS2 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. SCK prevalence in the blood (BBAC≥1.2 mmol/L, BSCK) and milk (MBAC=100 μmol/L, MSCK1), (MBAC≥200 μmol/L, MSCK2) and (MBAC≥100 μmol/L, MSCK1/2) 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. Holstein with SCK was more likely to develop PPHD in 90DIM. MSCK1 and MSCK1/2 did not associate milk production loss in Holstein. Holstein with BSCK and MSCK2 at PPW2 had a 6.7 kg loss of average daily milk yield in 90DIM. Culling rate was 3.7% in Holstein and MSCK2 positive Holstein at PPW2 was significantly more likely to be culled (25%, Odds:11.2, P<0.05) in 90DIM. In conclusion, cows having BCS2 and 4 at calving had higher MBAC and BBAC at PPW2 and 4. Holstein-Crossbreed and Montbeliard had much lower postpartum BBAC than Holstein and Simmental. BSCK and MSCK2 caused significantly risk factor for PPHD, culling rate and milk production in Holstein.

Keywords: Beta-hydroxybutyric acid, Holstein, Metabolic diseases, Montbeliard, Simmental, Subclinical ketosis

Farklı Süt Sığırı Irklarında Kan ve Süt Beta-hidroksibütirik Asit Düzeyleri ve Holstein'larda Subklinik Ketozisin Postpartum Hastalıklar, Sürüden Ayırma, Vücut Kondisyon Skoru, Parite ve Süt Verimi İle İlişkisi

Öz: Bu çalışmanın amacı beta-hidroksibütirik asit konsantrasyonlarını kanda (KBAK) ve sütte (SBAK) Holstein (n=216, 8 çiftlik), Montbeliard (n=23), Simmental (n=38) ve Holstein- Montbeliard melezlerinde (HMM, n=23, yalnız KBAK) postpartum 2. ve 4. haftada (PPH2 ve PPH4) ölçmektir. Ayrıca, subklinik ketozis (SK) prevalansı ile irtibatlı doğum sonrası ilk 90 gündeki postpartum metabolik hastalıklar (PPMH), vücut kondisyon skoru (VKS) ve günlük ortalama süt verimi Holstein ırkında değerlendirilmiştir. HMM ve Montbeliard'larda diğerlerine göre anlamlı olarak daha düşük KBAK oranı tespit edilmiştir. Primiparous Montbeliard ve Holstein ırklarında SBAK PPH2'de PPH4'e göre önemli oranda yüksek çıkmıştır. VKS'si doğumda 2 veya 4 olan hayvanlar PPH2 ve 4'de anlamlı derecede daha yüksek SBAK'a sahip oldukları görülmüştür ve bu hayvanlarda metritis ve çoklu hastalık görülme oranı yüksek çıkmıştır. Doğumda VKS4'e sahip Holstein'lar PPH2 ve 4'te daha yüksek KBAK'a sahiplerdi. Holstein'larda SK prevalansı PPH2'de kanda (KBAK≥1.2 mmol/L, KSK) ve sütte [SBAK=100 μmol/L (SSK1), SBAK≥200 μmol/L (SSK2), SBAK≥100 μmol/L (SSK1/2)] sırasıyla 8.3, 11.8, 5.8 ve 17.3% ve PPH4'de sırasıyla 4.7, 4.9, 6.9 ve 11.9% olarak tespit edilmiştir. SK pozitif olan Holstein ırkında, doğum sonrası ilk 90 günde PPMH gelişme oranı anlamlı derecede risk oluşturmuştur. SSK1'in ve SSK1/2'nin süt verimi ile ilişkisi tespit edilmemiştir. PPH2'de hem KSK hem de SSK2'ye sahip Holstein'larda, ilk 90 günde günlük ortalama 6.7 kg süt verimi kaybı görülmüştür. Doğum sonrası ilk 90 günde sürüden ayırma oranı Holstein ırkında %3.7 oranında görülmüş ve bunlardan PPH2'de SSK2 pozitif olanlar anlamlı derecede yüksek riskli çıkmışlardır (Odds 11.2, %25, P<0.05). Sonuç olarak, doğumda VKS2 ve 4'e sahip hayvanlarda PPH2 ve 4'te SBAK ve KBAK düzeylerinin daha yüksek olduğu gözlenmiştir. Montbeliard ve HMM'lerin postpartum KBAK'ları Holstein ve Simmental'lerden daha düşük çıkmıştır. KSK ve SSK2, Holstein'larda PPMH, sürüden ayırma ve süt verimi için önemli bir risk oluşturmuştur.

Anahtar sözcükler: Beta-hidoksibütirik asit, Holstein, Metabolik hastalık, Montbeliard, Simmental, Subklinik ketozis
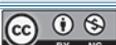
INTRODUCTION

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 metabolic disorders if dairy cows do not overcome the negative energy balance (NEB) due to reduced dry matter intake and other complications [1-3]. The NEB is the main reason during the transition period and can negatively affect milk production due to subclinical ketosis (SCK) [4], metabolic and reproduction parameters [3,5,6] and farm profitability through decreased milk production and increased risk of metabolic diseases [3,7-9]. Increased demand for milk consumption resulted in increased annual milk production per cow from roughly 2.000 kg to 10.300 kg worldwide [1]. The dairy cattle population transformed from indigenous low milk yielding breed to high milk yielding dairy breeds from 1991 to 2019 which 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 [10]. However, this brought problems of metabolic and reproductive diseases such as ketosis, displaced abomasum resulting in early culling [10]. SCK was associated with increased level of beta-hydroxybutyric acid (BHBA) also called hyperketonemia or hyperketolactia in the absence of clinical ketosis signs and it is a common disease for high milk yielding dairy cows. 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 [3,9,11]. Testing of BBAC [9,11-13] and MBAC [14-16] indicate the NEB, that can result in clinical ketosis and SCK in early lactation. Thus, hyperketonemia became an economically relevant postpartum metabolic problem in terms of its impact on farm profitability, especially in Holstein dairy farming [3,7,8,17], however, there are not enough papers published about Montbeliard, Simmental and Holstein-Crossbreed. Various studies in Holstein revealed a prevalence of 21.8% [11] and 24% [13] 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% [11] and 19.4% [12] with the same BAC threshold. Recent studies showed that checking of MBAC found large acceptance by using Fourier-Transform Infrared Spectrometry [16] or milk ketone strips [9,14]. A prevalence of 39% in Holstein was reported by using milk ketone strips in European countries. This rate was 22.6% by using Fourier-Transform Infrared in Canada [14]. The cut-off value of MBAC for the definition of SCK varied among the studies. Few papers used MBAC to define SCK prevalence. Ranges of MBAC to classify cows with suspect of hyperketolactia (0.15 to 0.19 mmol/L) or positive hyperketolactia (≥0.20 mmol/L) were reported by Benedet et al.[9] and others [14,18].

The relationship between BBAC and MBAC and milk production was studied in Holstein cows [15,19]. Most of the studies about the prevalance of SCK were cunducted via a blood test only. A 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, culling rate, body condition score (BCS) and parity. The majority of the studies about the prevalence of SCK and its association with PPHD were conducted in Holstein dairy farms. Montbeliard and Simmental were classified in the same family [20,21] and there are no comparative analysis about BBAC and MBAC in different parities compared to Holstein. Although there was a correlation between hyperketonemia and hyperketolactia [22], there is also a lack of research conducted for the comparison of BAC in the blood and milk in various breeds at most critical time points postpartum week 2 and 4 (PPW2 and PPW4) worldwide. The aim of the present study was to compare the relationship of BBAC and MBAC with parity and BCS at two different postpartum time points in Holstein, Simmental, Montbeliard and Holstein-Crossbreed dairies. Furthermore, the present study aimed to analyse the prevalence of SCK defined with hyperketonemia and hyperketolactia at two most important time points such as PPW2 and PPW4 and its associations with PPHD, culling, BCS, parity and milk production in Holstein.

MATERIAL AND METHODS

Ethical Statement

The study was approved by the Animal Experiments Local Ethics Committee of University of Erciyes (EÜHADYEK) with the ethical approval number of 15.05.2019/05/19-113.

Animals and Grouping

This is a randomized field study. The study was conducted in 4 provinces (İzmir, Aydın, Muğla and Denizli) of Turkey. The present study was conducted between September 2019 and March 2020. Three hundred lactating cows in 11 integrated dairy cattle farms consisting of Holstein (n=216, farm 1 to 8), Simmental (n=38, farm 9 and 11), Montbeliard (n=23, farm 1) and Holstein-Crossbreed (HC) (HolsteinxMontbeliard, n=23, farm 10) were enrolled for the study. Parity groups were created as primiparous (PRP) and multiparous (MUL) due to association of SCK with the parity [13,22]. Furthermore, groups were created for the definition of SCK in the blood (BSCK) and milk (MSCK1, MSCK2 and MSCK1/2) based on appropriate cut-points of BBAC and 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) in Holstein. Farms (n=8) that had an automatic milking system, milk yield recording database, and complete milk

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yield recording for study animals (n=206 Holstein) were enrolled in the milk production analysis.

Animal Feeding

All farms had a professional self-ration program and, cows were fed with a ration according to the production cycle, energy, mineral and other nutrients requirements (dry period, close-up, early lactation). Water was served *ad libitum*. Farm feeding strategy and ration have not been changed or specifically prepared for this study and throughout the research period. Cows in the study farms fed with a ration consisting of maize silage, hay, alfalfa, concentrated milk feed, maize flake, cottonseed, limestone, soy sauce, vitamin and mineral premix that had the metabolic energy of 41.77 to 54.30 Mcal/day for a cow in early lactation.

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 [23] (Medtrust Wellionvet Belua, Med Trust Handelsges.m.b.H., Austria) at PPW2 and PPW4. Semi-quantitative MBAC was analysed at the same times like blood test in 50 mL of freshly taken individual milk samples (within 5 min after collection) with milk-test-strips [24] (Ketotest, Elanco). 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 [10,13] 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 [9,14,15,18].

Body Condition Scores and Postpartum Health Checks

BCS controls were performed according to the recommendations by Edmonson et al.[25] based on a scale from 1 to 5 at calving (postpartum day 0), postpartum day 30 (PP30) and 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. The difference of BCS relative to calving was the body condition loss or gain [26]. Cows were monitored and evaluated daily from the clinical health point of view for PPHD, any single or multiple diseases or culling were registered immediately in 90 days in milk (90DIM). They were specifically checked for retained placenta, displaced abomasum, metritis, mastitis, cystic ovarian, lameness, clinical ketosis, milk fever or combined multiple diseases (more than 1 disease) in 90DIM because they were most prevalent PPHD associated with SCK [3,5-8,22].

Milk Yield Recording

The daily milk yield of Holstein (n=206) was recorded automatically in the study farms (n=8) where various automated milking system was set and continuously recorded in a data base. Milk yield was taken directly from computerized farm database.

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. Mann-Whitney-U, Wilcoxon Signed Ranks and 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. Prevalence of BSCK and MSCK1, MSCK2 and MSCK1/2 was presented as numeric, positive, negative and % in Holstein. In order to evaluate the disease incidences and dependency of BHBA between PPW2 and PPW4, Fisher's exact test was used. Incidence of the PPHD was presented as a percentage. The odds ratio was determined for each of the diseases (for those with sufficient data for computation) in SCK 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. BBAC and MBAC were analysed by Wilcoxon Signed Ranks test to compare PPW2 with PPW4. However, Kruskal-Wallis test was used for the analysis between breed groups. BBAC and MBAC between each breed group were compared with Mann-Whitney-U Test. The ADMY was analysed using Mann-Whitney-U test between SCK positive and negative cows, including subgroups. Repeated measure ANOVA and Friedman tests were initiated for the analysis of milk yield between calving and postpartum 12 weeks.

RESULTS

BBAC, MBAC, BCS and Parity in Different Dairies

The averages of BCS, BBAC and MBAC in the study cows were presented in *Table 1*. All cows lost BCS at PP30 and PP60 compared to calving BCS, except for PRP Simmental. BCS1 was not observed at calving. 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 PRP, n=179 MUL), 3.09±0.31 (n=5 PRP, n=18 MUL), 2.26±1.03 (n=9 PRP, n=29 MUL), 2.04±1.15 (n=11 PRP, n=12 MUL) respectively. The average parity of Simmental and HC was significantly lower (P<0.01) than Montbeliard and Holstein. *Fig. 1* and *Fig. 2* present BBAC and MBAC for different BCSs at calving. Calving-BCS determined significantly BBAC and MBAC at PPW2 and PPW4. Holstein having significantly high BBAC at PPW2 had BCS4 at PP30 and PP60. Significantly high BBAC at PPW2 was observed in Simmental having BCS4 at PP30 (P<0.01). Correlation coefficients (data not shown in

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 PRP and MUL Holstein, Montbeliard, Simmental and Holstein Crossbreed (HC)**

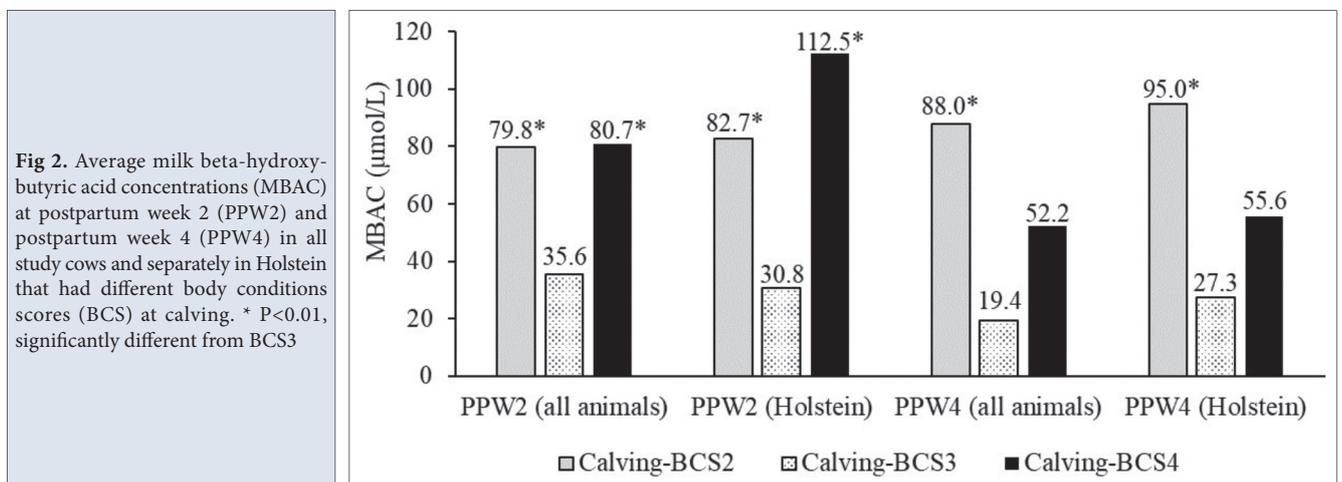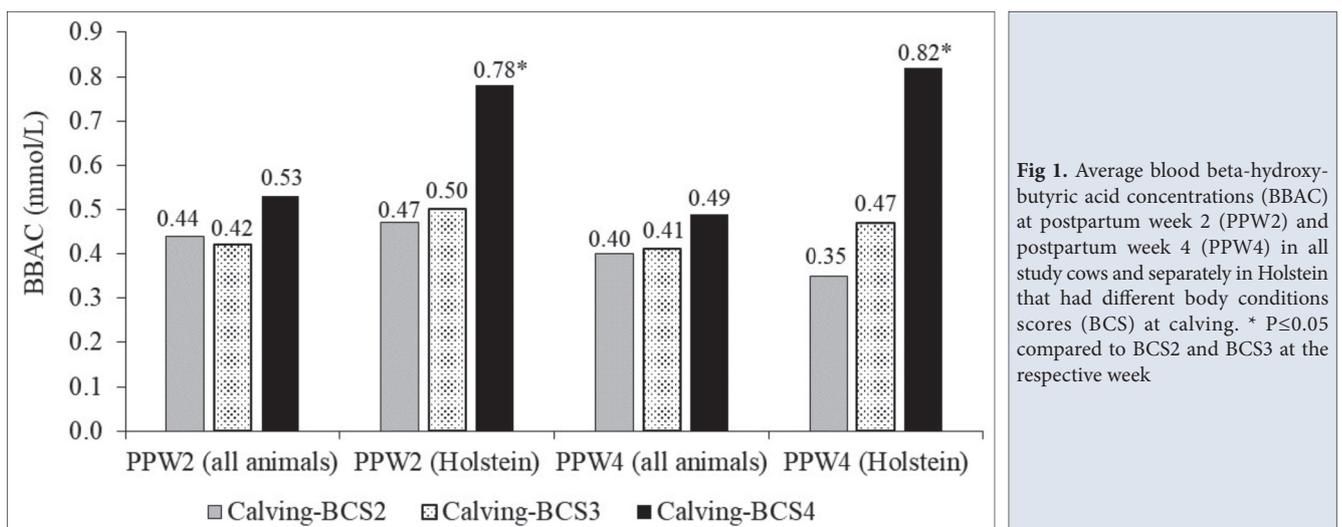| Cows	Groups	All Breeds	Holstein	Montbeliard	Simmental	HC	P (1)
All parities	BCS-calving	3.42±0.03[1]	3.35±0.03[a,1]	3.52±0.04[b,1]	3.59±0.10[b,1]	3.61±0.08[b,1]	0.00
	BCS-PP30	2.96±0.02[2]	2.94±0.03[a,2]	2.80±0.04[a,2]	3.24±0.08[b,2]	2.83±0.09[a,2]	0.00
	BCS-PP60	2.96±0.03[2]	2.88±0.03[a,3]	2.88±0.02[a,2]	3.41±0.09[b,1]	2.87±0.06[a,2]	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[a]	50.00±0.00[a]	-	0.45
	PPW4-MBAC	55.63±9.72	67.33±13.41[a]	8.70±4.04[b]	50.00±0.00[a]	-	0.00
	P(3)	0.00	0.03	0.00	1.00	-	-
PRP	BCS-calving	3.51±0.05[1]	3.48±0.06[a,1]	3.60±0.10[a,1]	3.56±0.19[a,1]	3.50±0.12[a,1]	0.76
	BCS-PP30	2.98±0.05[2]	2.98±0.05[a,2]	2.87±0.12[a,2]	3.17±0.17[a,1]	2.86±0.14[a,2]	0.44
	BCS-PP60	2.97±0.06[2]	2.92±0.07[a,2]	2.90±0.05[a,2]	3.33±0.17[b,1]	2.86±0.10[a,2]	0.03
	P(2)	0.00	0.00	0.01	0.08	0.00	-
	PPW2-BBAC	0.57±0.07	0.75±0.17[a]	0.52±0.30[b]	0.50±0.06[b]	0.03±0.02[c]	0.00
	PPW4-BBAC	0.40±0.07	0.51±0.12[a]	0.44±0.10[a]	0.42±0.08[a]	0.01±0.03[b]	0.00
	P(3)	0.04	0.08	1.00	0.20	0.16	-
	PPW2-MBAC	91.67±28.85	101.85±39.63[a]	70.00±20.00[a]	50.00±0.00[a]	-	0.57
	PPW4-MBAC	43.33±16.56	50.00±23.40[a]	10.00±10.00[a]	50.00±0.00[a]	-	0.10
	P(3)	0.00	0.03	0.06	1.00	-	-
MUL	BCS-calving	3.39±0.03[1]	3.32±0.03[a,1]	3.50±0.04[a,1]	3.60±0.12[b,1]	3.71±0.11[b,1]	0.00
	BCS-PP30	2.95±0.03[2]	2.93±0.03[a,2]	2.78±0.05[b,2]	3.27±0.10[c,2]	2.79±0.11[b,2]	0.00
	BCS-PP60	2.95±0.03[2]	2.87±0.04[a,3]	2.88±0.02[a,2]	3.45±0.11[b,2]	2.88±0.07[a,2]	0.00
	P(2)	0.00	0.00	0.00	0.00	0.00	-
	PPW2-BBAC	0.45±0.04	0.50±0.04[a]	0.27±0.03[b]	0.43±0.05[a]	0.03±0.03[c]	0.00
	PPW4-BBAC	0.42±0.03	0.45±0.04[a]	0.29±0.03[b]	0.45-0.04[a]	0.03±0.05[c]	0.00
	P(3)	0.68	0.36	0.41	0.57	1.00	-
	PPW2-MBAC	55.86±8.44	58.04±10.79[a]	47.22±11.05[a]	50.00±0.00[a]	-	0.56
	PPW4-MBAC	58.93±11.51	71.88±15.81[a]	8.33±4.52[b]	50.00±0.00[a]	-	0.00
	P(3)	0.01	0.21	0.01	1.00	-	-

PRP: *primiparous cows;* **MUL:** *multiparous cows;* * *MBAC was not tested in Holstein-Crossbreed (Holstein/Montbeliard);* **P(1):** *Kruskal-Wallis Test between breeds;* [a,b,c] *different letters refer significant difference (P≤0.05) 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 (P≤0.05) in the same column between BCS-calving, BCS-PP30 and BCS-PP60;* **P(3):** *Wilcoxon Sig. Ranks test between PPW2 and PPW4*

tables) between BBAC and MBAC were r=0.60 and r=0.86 (P<0.05) at PPW2 and it was r=0.36 and r=0.14 (P>0.05) at PPW4 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 BSCK and MSCK in Holstein

SCK prevalence analysis was not performed in the breeds other than Holstein, none of Simmental and HC cows have exceed the cut-point of BHBA for SCK definition. Out of 23 Montbeliard cows, 1 PRP Montbeliard with BCS4 at

calving showed BSCK at PPW2 only, but that cow became negative at PPW4. BSCK was detected in Holstein farms (farms 2, 3, 5, 6, 7 and 8) 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. The descriptive data about the prevalence of BSCK and MSCK, their relation with the parity and BCSs were presented in *Table 2* for Holstein. PRP Holstein with BSCK at PPW2 and 4, lost significantly (P<0.05) BCS at PP60. The difference between PPW2 and PPW4 was significant (P<0.01) in MSCK1/2 and MSCK2 prevalence. MSCK2 prevalence was negative in Holstein farms 1, 3 and 4. No significant difference was found in BCSs of MSCK2

Fig 1. Average blood beta-hydroxy-butyric 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

Fig 2. Average milk beta-hydroxy-butyric 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

positive MUL Holstein cows between calving, PP30 and PP60. However, all PRP cows with positive MSCK lost BCS between calving, PP30 and PP60. 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). 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 for BSCK and MSCK was found between PPW2 and 4 (P>0.05). The correlation coefficient was r=0.34 (P>0.05) for BSCK between PPW2 and PPW4 in Holstein. Correlation coefficients were r=0.26 and r=0.48 (P>0.05) for MSCK1 and MSCK2 between PPW2 and PPW4 in Holstein respectively.

Postpartum Health Disorders and Culling in Holstein

The incidence of PPHD except culling rate and its association with SCK were presented in Holstein in *Table 3*. MSCK1 did not significantly correlate with PPHD in Holstein and therefore it was not presented. The overall culling rate was 3.7% among Holstein in 90DIM. Holstein 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 (odds ratio: 11.20, P<0.05) to be culled than even MSCK1/2 (odds ratio: 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 PRP, 7 MUL). Mastitis (n=1), metritis (n=1) and displaced abomasum (n=1), multiple diseases (n=1, 12%), lameness (n=2, 25%) were observed in culled Holstein. Clinical ketosis, displaced abomasum, metritis, mastitis, lameness and multiple diseases were observed moderately and, in some cases, significantly higher in Holstein that were positive for BSCK, MSCK1/2 and MSCK2 at PPW2 or 4 *(Table 3)*. Displaced abomasum and cystic ovarian incidence did not correlate significantly with SCK in Holstein. BSCK and MSCK2 positive Holstein at PPW2

Table 2. *Descriptive statistic about the prevalence of subclinical ketosis (SCK) at PPW2 and PPW4 in PRP and MUL Holstein 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 (%)	PRP/MUL	%	Calving	PP30	PP60
BSCK-PPW2	216	8.3	PRP	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	PRP	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	PRP	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	PRP	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	PRP	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	PRP	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	PRP	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	PRP	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-hydroxybutyric acid concentration ≥1.20 mmol/L;* **MSCK1:** *milk beta-hydroxybutyric acid concentration = 100 µmol/L;* **MSCK2:** *milk beta-hydroxybutyric acid concentration ≥200 µmol/L;* **MSCK1/2:** *milk beta-hydroxybutyric acid concentration ≥100 µmol/L;* **PRP:** *primiparous;* **MUL:** *multiparous;* **PPW2:** *postpartum week 2;* **PPW4:** *postpartum week 4*

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

SCK group	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	30.0[6]	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[1]	16.7	0.0	0.0	25.0[7]
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;* [5,6,7] *P=0.06;* **PPW2:** *postpartum week 2;* **PPW4:** *postpartum week 4;* **BSCK:** *blood beta-hydroxybutyric acid concentration (BAC) ≥1.20 mmol/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:** *cystic ovarian;* **MD:** *multiple diseases. No significant relation was found between MSCK1 and PPDH*

or 4 were more likely to developing clinical ketosis (odds ratio: 15.4, P<0.05). Holstein with positive SCK had frequently metritis, however, the incidence was most remarkable in MSCK1/2 positive Holstein at PPW4 (P=0.06, odds ratio: 4.48). Those cows had also a moderate

significantly (P=0.07) lower BCS (2.81) at PP60 than other cows. Holstein with MSCK1/2 at PPW4, were more likely to have mastitis (odds ratio: 5.08, P<0.05) *(Table 3)*. Holstein with BSCK at PPW4 had a moderate significantly high incidence of lameness (odd ratio: 4.25, P=0.06).
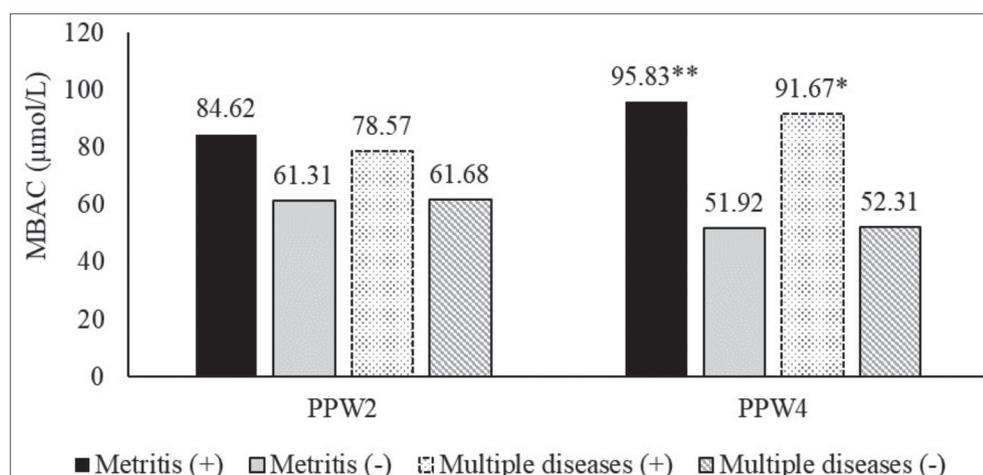
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Fig 3. Average milk beta-hydroxy-butyric acid concentrations (MBAC) at postpartum week 2 (PPW2) and postpartum week 4 (PPW4) in cows having metritis and multiple diseases throughout the study period. Based on Mann-Whitney Test; * P≤0.05 versus multiple disease negative, ** P<0.01 versus metritis negative

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

SCK Group	All Holstein			PRP			MUL		
	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	

PRP: primiparous; MUL: multiparous; 90DIM: 90 days in milk; NA: not applicable due to low number of cases; BSCK: blood beta-hydroxybutyric acid concentration ≥1.20 mmol/L; MSCK2: milk beta-hydroxybutyric acid concentration ≥200 µmol/L

MSCK1/2 positive Holstein at PPW4 showed an incidence of 25% multiple diseases (odds ratio: 4.5, P=0.06) *(Table 3)*. Average MBAC at PPW4 were significantly higher in cows having metritis and multiple diseases *(Fig. 3)*. BBAC at PPW2 was significantly higher in cows with metritis (P<0.05) in 90DIM (data not shown in tables).

Association of BSCK and MSCK with Average Daily Milk Yield in Holstein

The comparison of ADMY between SCK positive and negative Holstein was presented in *Table 4, 5, 6* and *Fig. 4* and *5*. The 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 Fig. 4* and *5)*. The difference between SCK negative and positive cows regarding the ADMY was significant at weeks 10th, 11th and 12th *(Fig. 4* and *5)*. MSCK2 positive Holstein cows at PPW2 had moderate significantly (P=0.07) lower ADMY *(Table 4)*. The 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≤0.05) *(Table 5)*. MSCK1 and MSCK1/2 in Holstein did not have a significant effect on average daily, weekly and monthly milk yield, even no effect was observed in the combined prevalence groups. Therefore, the data was not presented in the tables. The ADMY of PRP Holstein without BSCK at PPW2 was significantly higher (P≤0.05) than those with BSCK, which meant an average 7 kg milk yield loos per day *(Table 4)*. Holstein with a negative BSCK and MSCK2 at PPW2 had a much higher ADMY in the second and third months postpartum *(Table 6)*. BSCK negative PRP Holstein at PPW2 had 13.6 kg more ADMY in the second month of postpartum that was significantly different (P≤0.05) from the positive cows (data not shown in tables).

DISCUSSION

The present study reported a lower prevalence of BSCK in Holstein than the previous studies by other reports in European countries [11], Turkey [11,12] and worldwide [13], although the same cut-off point of BBAC for BSCK was

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: blood beta-hydroxybutyric acid concentration ≥1.20 mmol/L; MSCK2: milk beta-hydroxybutyric acid concentration ≥200 μmol/L; * 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 all Holstein 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)*

SCK Group	SCK	n	First Month	Second Month	Third Month	P[(1)]
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

*P[(1)]: difference between average milk productions of month 1, 2, 3; average milk yield of the first month is significantly lower than month 2nd and 3rd where it is applicable (P value); * P≤0.05; ** P=0.07 between SCK positive and negative groups at the respective testing month; BSCK: blood beta-hydroxybutyric acid concentration ≥1.20 mmol/L; MSCK2: milk beta-hydroxybutyric acid concentration ≥200 μmol/L*
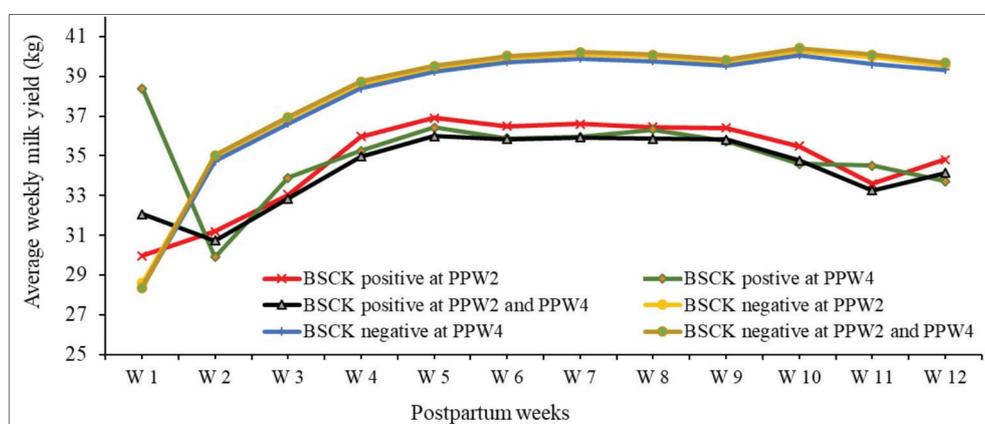

Fig 4. Average daily milk yield per week of Holstein with positive or negative subclinical ketosis (SCK) at postpartum week 2 or 4 (PPW2 or 4) in the blood (BSCK: blood beta-hydroxybutyric acid concentration ≥1.2 mmol/L). Remark: line of BSCK negative cows at 'PPW2 and PPW4' overlaps the line of BSCK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

used in all these studies. Even, BSCK prevalence was little higher using the lower cut-point of BBAC (≥1.0 mmol/L) in Turkey [27,28]. Others defined BSCK by a threshold level of BBAC ≥0.96 mmol/L [29], ≥1.0 mmol/L [5,30] and ≥1.4 mmol/L [15,19,24]. But, the cut-point of BBAC≥1.20 mmol/L for BSCK definition was found the most acceptance
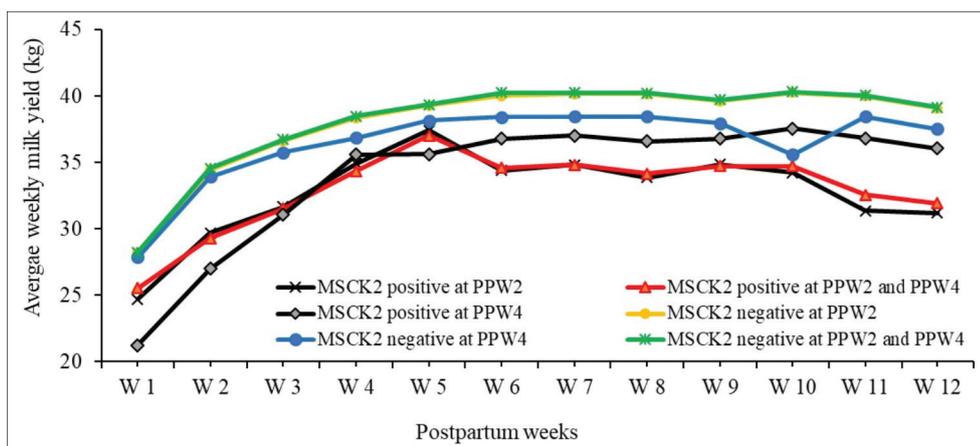
Fig 5. Average daily milk yield per week of Holstein with positive or negative subclinical ketosis (SCK) at postpartum week 2 or 4 (PPW2 or 4) in the milk (MSCK2: milk beta-hydroxybutyric acid concentration ≥200 μmol/L). Remarks: The line of MSCK2 negative cows at 'PPW2 and PPW4' overlaps the line of MSCK2 negative cows at PPW2. The line of MSCK2 positive cows at 'PPW2 and PPW4' overlaps the line of MSCK2 positive cows at PPW2

which was much related to PPHD and milk production loss [3,7-9,11,13]. Therefore, the present study used also this cut-point of BBAC. The reason for this discrepancy in the results can be that no BSCK was detected in two Holstein farms, or a seasonal difference might be that a large part of the present study was conducted in the wintertime. 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 PRP cows were detected at PPW2 compared to PPW4 in the present study. 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 cows [11,13,22-24]. Most BSCK positive cows were MUL cows. This was consistent with other studies [13,31-33]. However, there are also not consistent reports in Holstein [24,34] and in Jersey [35]. The peak prevalence of SCK occurred in the third and fourth week of lactation [4], this result was slightly close to the present study. Similarly, Carrier et al.[24] 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 was consistent with most of the previously reported studies. 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 [11,13,19,28,32,36]. Fat Holstein at calving had significantly higher BBAC at PPW2 and 4 and MBAC at PPW2 in the present study. This was in compromise with the previous reports [33,37]. Differently, the present study showed that 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. PRP Holstein with positive BSCK or MSCK lost BCS within 60 days postpartum. Probably, these cows had fat mobilisation due to NEB to compensate energy requirement for the first milk production [19]. The prevalence rate of MSCK 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 varied among the studies [9,14-16,18]. MBAC≥200 μmol/L was recommended to define cows having severe and positive ketosis [9] and used also in the previous studies [15,18]. Both MSCK1 and MSCK2 prevalence rate was low compared to the literature [14-16] although it was tested at two different postpartum time points. But, MBAC of MUL cows increased significantly at PPW4 compared to PPW2. Consequently, MSCK2 positive Holstein were more likely to develop clinical ketosis although other PPHD such as metritis, mastitis and multiple diseases were observed. SCK was frequently associated with PPHD in Holstein [3,7,8,11,13]. The present study showed that BSCK at PPW4 caused a risk for displaced abomasum and lameness without a high significant odd. BSCK positive Holstein at PPW2 and 4 were more likely to develop clinical ketosis. Transformation of SCK into clinical ketosis was frequently reported at a high risk factor and likelihood [2,19,37]. BSCK positive Holstein at PPW4 had a nonsignificant incidence of displaced abomasum, because displaced abomasum was overall low in study cows. Displaced abomasum was the most frequently detected metabolic disorder related to SCK in the first weeks after calving with a high-risk factor [11,19,37]. Mastitis incidence was frequently high in SCK positive Holstein in the present study. This finding was in line with others [6,8,13], but Suthar et al.[11] did not observe significant risk for mastitis. Differently from others, cows with mastitis have got significantly lower BCS (thin) at calving. Kremer et al.[38] stated that cows with BBAC >1.4 mmol/L were more susceptible to severe mastitis in an experimental *E. coli* study. MSCK1/2 at PPW4 created a high risk for metritis in Holstein and those cows had significantly high MBAC at PPW4. Similarly, a high metritis incidence was already reported in Turkey and worldwide [2,6,11,13,19]. The retained placenta and milk fever as well as cystic ovarian were not observed in Holstein with SCK in the present study. A cut-point of BBAC for this early PPHD wasn't established previously [11]. Others reported high risks for retained

placenta in association with BSCK [13,37]. The cystic ovarian associated with BSCK was reported by previous reports [4,31]. Lameness incidence was high and created moderately significant risk in BSCK positive Holstein at PPW4. Similar findings were observed by others in BSCK positive Holstein [11,13]. Other study [6] did not report lameness in SCK or clinical ketosis positive Holstein in Turkey. MSCK1/2 and MSCK2 positive cows at PPW4 were significantly more likely to be culled and having metritis and multiple diseases. Similar findings were reported in BSCK and in clinical ketosis in Turkey previously [6]. The present study found culling rates in MSCK positive cows and a positive correlation between multiple diseases, metritis and high MBAC additionally. The present study indicated a reduced ADMY in BSCK and MSCK2 positive Holstein which was consistent with the finding of previously reported studies [4,8,19,32,36]. MSCK2 positive Holstein had 6.7 kg ADMY loss in 90DIM, it was 4 kg for cows with positive BSCK at PPW2 or 4. Increasing BBAC above 1.0 mmol/L during PPW2 was associated with progressively less milk yield [19]. A linear negative effect of BBAC beginning at 1.2 mmol/L at PPW1 was observed on milk production [19]. This can result in economic losses throughout the production cycle [7,8,17], e.g., in an average of USD 200-290 per cow [3]. Differently from other reports, BSCK at PPW2 resulted in significantly lower ADMY in PRP Holstein. 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 [19]. A little difference was observed between PRP and MUL cows in hyperketonemia incidence [34]. Chandler et al.[35] found more prevalent hyperketonemia in PRP than MUL Jersey cows. A very small difference in ketosis prevalence between PRP and MUL cows was found in Ayrshire and Friesian cows [39]. 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 ADMY between MSCK1 positive and negative Holstein. The lower threshold level of MBAC ($\geq$100 μmol/L) for MSCK1 and MSCK1/2 definition at PPW2 or PPW4 did not significantly affect ADMY in Holstein. Previous studies [4,36] reported the association between MSCK1 and reduced milk production in Holstein. A positive moderate correlation between MBAC and BBAC was observed in Holstein, but not in other breeds. Especially, the correlation was high in terms of changes of BBAC and MBAC between PPW2 and PPW4. This correlation between BBAC and MBAC was observed by others [15]. In contrast, this correlation was not confirmed between BSCK and MSCK neither at PPW2 and PPW4. The reason might be due to the lower sensitivity of milk BHBA test strips compared to cow-side blood BHBA analysers [24]. Semiquantitative

determination of MBAC which based on the colour indication for BAC might affect the results. It was stated that concentrations of milk and blood BAC were poorly correlated and the use of milk strips overestimated the concentrations of BAC in the milk [40]. The lack of relationship between MBAC and BBAC was observed by Andersson [41] and they 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. BHBA can be utilised by the mammary gland for fatty acids synthesis and converted to butyrate [22,42] that is why MBAC is only 10% to 15% of BBAC, possibly because of the ketone body's role in fat metabolism in the udder [41]. 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. But, the prevalence of MSCK in Holstein was often reported higher than the prevalence of BSCK [9,14]. Nevertheless, the specificity and sensitivity of the milk test strips used in the present study were confirmed for MBAC in cows [24], but there were 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. Low BBAC and MBAC in Montbeliard and HC compared to Holstein and Simmental was an important outcome of this study. SCK was not found in Simmental and HC cows. One Montbeliard exceeded the cut-point of BBAC at PPW2 but not at PPW4. That was irrelevant because no prevalence analysis was conducted due to the small sample number in these breeds and that was also not aimed. In contrast to the present study, a study [43] reported a high incidence of BSCK in Simmental that were in early lactation and late pregnancy. A significantly reduced milk yield was observed in Montbeliard with BSCK in the second month of lactation [44]. Simmental cows were mostly culled because of sterility and reproductive diseases, but Montbeliard cows were culled due to poor yield and udder problems [45]. 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-Crossbreed and Simmental in the present study. Gantner et al.[46] found that the highest prevalence risks of ketosis were observed in 20 DIM of PRP Simmental, parity 2 and 3 cows. The French Simmental family has three strains; Pie rouge de l'Est (or French Simmental), Montbeliard and Abondance [20]. Thus, Montbeliard and Simmental cows were classified in the same family of French Simmental [20,21] and they might show a certain extent resistance to SCK [46], especially it can be much obvious under modern management system. Strong resistance of this dual-purpose Simmental Flechvieh breeds to mastitis was reported [20]. Although the number of Flechvieh cows looked small compared to Holstein for

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a prevalence analysis in the present study, the resulting evidences about BBAC and MBAC might show the overall trend for SCK development associated to NEB in those breeds. Holstein lost BCS throughout the study period, although other breeds lost BCS at PP30 compared to calving only. Even, Simmental's average BCS at PP60 was similar to calving. This resulted for Holstein in higher BBAC at PPW2 and PPW4 than other breeds with exception of Simmental. No significant change was observed in BBAC between PPW2 and PPW4 in any breeds, however overall MBAC was much higher at PPW2 than PPW4 in PRP Holstein and Montbeliard. Taking all breeds parities into account, it can be speculated that MBAC showed the BCS losses better than BBAC. BCS losses are the result of fat mobilisation due to NEB [3].

In conclusion, BSCK and MSCK were still a herd problem causing PPHD, culling and ADMY loss in 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 multiple diseases. BSCK and MSCK2 positive Holstein at PPW2 had an ADMY loss of 6.7 kg. ADMY loss was 7 kg in PRP Holstein with BSCK at PPW2. The cut-off point of MBAC≥100 µmol/L did not cause a significant effect on ADMY. However, a higher cut-off point of MBAC≥200 µmol/L (MSCK2) caused a significantly reduced ADMY. BCS losses were better reflected with an increased MBAC at PPW2 in all related breeds. Simmental and related breeds (Flechvieh 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. PRP Holstein needs to be investigated more intensively in terms of the development of NEB and associated ADMY performance under current modern conditions and high expectations for milk production.

Availability of Data and Materials

The datasets analyzed during the current study are available from the corresponding author on a reasonable request.

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Competing Interests

Authors declare there are no conflicts of interest in the present study.

Author Contributions

Experimental design and data collection were conceived by KA, AD and ACO. Statistical analysis was conducted by SD and validated by ACO. Original draft was written by AD and KA. All authors have contributed to the revision and final proof-reading of the manuscript.

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