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Associations of periparturient β-hydroxybutyric acid and non-esterified fatty acids blood serum concentrations with milk yield, milk composition and milk somatic cells count of intensively managed Chios dairy ewes

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ABSTRACT. This research paper addresses the hypothesis whether in dairy ewes: periparturient β-hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA) concentrations are associated with milk yield, composition and udder halves with increased somatic cell counts (SCC ≥ 0.5×10^6 cells/mL). A total of 186 Chios ewes reared under intensive system were used for this research. Serum BHBA and NEFA concentrations were measured before lambing (-30d, -15d), and BHBA concentrations after lambing (+7d, +15d, +30d, +45d). Milk samples were collected at 15, 30 and 45days in milk (DIM). Total milk yield (MY) of the first 30, 60 and 90 DIM and total fortnightly milk yield (FMY) produced from 15 to 59 DIM were recorded. Positive associations between BHBA at +7d and MY of the first 30, 60 and 90 DIM were revealed ($P < 0.001$). For every increased unit of BHBA at +15d, +30d and +45d, FMY was decreased (DIM: 15 – 29 and 30 – 44) ($P = 0.001$ and $P = 0.015$, respectively) or increased (DIM: 45 – 59) ($P < 0.001$). BHBA before lambing (-30d, -15d) affected the number of halves presented SCC ≥ 0.5×10^6 cells/mL at 15 and 30 DIM ($P = 0.011$, $P = 0.014$, $P = 0.009$, $P = 0.096$, respectively). Finally, for every increased unit of BHBA during lactation (+15d, +30d, +45d) a decrease in the concentration of milk in proteins, solids not fat and lactose was noted ($P < 0.001$). This work demonstrates the associations of periparturient blood biochemical parameters (BHBA, NEFA) with milk yield and specific milk production characteristics.

Keywords: dairy sheep, milk yield, milk composition, somatic cell count, β-hydroxybutyric acid

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ΠΕΡΙΛΗΨΗ. Στην παρούσα εργασία διερευνήθηκε εάν οι συγκεντρώσεις του β-υδροξυβουτυρικού οξέος (BHBA) και των μη εστεροποιημένων λιπαρών οξέων (NEFA), κατά την περιγεννητική περίοδο, συσχετίζονται με το ύψος της γαλακτοπαραγωγής, με τη χημική σύνθεση του γάλακτος, καθώς και με τον αριθμό των ημιμορίων του μαστικού αδένα που εμφανίζουν υψηλό αριθμό σωματικών κυττάρων (ΑΣΚ $\geq 0,5 \times 10^6$/mL). Για τις ανάγκες της έρευνας χρησιμοποιή-θηκαν 186 προβατίνες φυλής Χίου μίας εντατικής εκτροφής. Οι συγκεντρώσεις των BHBA και ΜΕΛΟ προσδιορίστηκαν πριν από τον τοκετό (-30d, -15d), ενώ μετά τον τοκετό προσδιορίστηκαν οι συγκεντρώσεις του BHBA (+7d, +15d, +30d, +45d). Δείγματα γάλακτος ελήφθησαν τη 15^η, την 30^η και την 45^η ημέρα της γαλακτικής περιόδου. Υπολογίστηκε η συνο-λική ποσότητα γάλακτος (ΣΠΓ) που παράχθηκε κατά τις πρώτες 30, 60 και 90 ημέρες της γαλακτικής περιόδου, καθώς και η ενδιάμεση ποσότητα γάλακτος (ΕΠΓ) που παράχθηκε κατά τα δεκαπενθήμερα χρονικά διαστήματα 15 – 29, 30 – 44 και 45 – 59 ημερών της γαλακτικής περιόδου. Παρουσιάστηκε θετική συσχέτιση μεταξύ της συγκέντρωσης του BHBA την ημέρα +7d με τη ΣΠΓ των πρώτων 30, 60 και 90 ημερών της γαλακτικής περιόδου ($P< 0.001$). Η αύξηση, κατά μία μονάδα, της συγκέντρωσης του BHBA στις ημέρες +15d, +30d και +45d, συσχετίστηκε με μείωση (ημέρες: 15 – 29 και 30 – 44, $P = 0,001$ και $P = 0,015$, αντίστοιχα) ή αύξηση (ημέρες: 45– 59, $P< 0,001$) της ΕΠΓ. Η συγκέντρωση του BHBA πριν από τον τοκετό (-30d, -15d) συσχετίστηκε θετικά με τον αριθμό των ημιμορίων του μαστικού αδένα που παρουσίασε ΑΣΚ $\geq 0.5 \times 10^6$/mL κατά τις ημέρες +15dκαι +30d ($P = 0,011$, $P = 0,014$ και $P = 0,009$, $P = 0,096$, αντίστοιχα). Τέλος, η αύξηση, κατά μία μονάδα, της συγκέντρωσης του BHBA στις ημέρες +15d, +30d και +45d, συσχετίστηκε με μείωση της περιεκτικότητας του γάλακτος σε πρωτεΐνες, σε στερεό υπόλειμμα άνευ λίπους και σε λακτόζη ($P< 0.001$). Η παρούσα έρευνα καταδεικνύει τη συσχέτιση συγκεκριμένων ενεργειακών παραμέτρων (BHBA, NEFA) με το ύψος της γαλακτοπα-ραγωγής, καθώς και με κάποια ποιοτικά χαρακτηριστικά του γάλακτος.

Λέξεις κλειδιά: φυλή Χίου, γαλακτοπαραγωγή, σύνθεση γάλακτος, αριθμός σωματικών κυττάρων, β-υδροξυβουτυρικό οξύ

INTRODUCTION

Milk yield, composition and somatic cells count (SCC) are of high importance in dairy sheep industry due to their contribution to milk products, determining both their quality and quantity. Dairy sheep selection over the last decades has focused towards improvement of both milk yield and composition (Baril-let et al. 2001). This led in the development of highly productive dairy breeds housed and managed under intensive, zero-grazing conditions (Milan et al. 2011, Gelasakis et al. 2012). Among the indigenous Greek sheep, Chios breed is considered to be of high milk yield and prolificacy (Gelasakis et al. 2012).

High producing dairy breeds are better adapted in intensive farming systems, where ewes can express their genetic potential (Milan et al. 2011, Gelasakis et al. 2012). However, breeds or individuals with higher potential in milk production, may confront increased

difficulty in controlling energy balance, especially during the period around parturition (Bizelis et al. 2000). Although periparturient period in dairy ewes is not precisely defined, its importance for health and productivity has been noted (Charismiadou et al. 2000, Theodorou et al. 2007, Mavrogianniand Brozos 2008, Karagiannis et al. 2014).

Data regarding associations of blood BHBA and NEFA concentrations with milk yield, milk composi-tion and SCC in dairy sheep, are scarce in the accessed literature. However, in dairy cows increased prepartum blood BHBA and NEFA concentrations were associated with milk loss across the first 120 DIM (Ospina et al. 2010, Chapinal et al. 2012). High BHBA during early lactation had a negative short-term impact on milk yield across the first 30 DIM (Duffield et al. 2009, Chapinal et al. 2012), while for longer term milk yield, contradictory results have been published (Duffield

et al. 2009, Ospina et al. 2010, Chapinal et al. 2012). Prepartum blood BHBA and NEFA concentrations have been associated with SCC (Nyman et al. 2008), while postpartum BHBA were not associated (Al-Rawashdeh 1999). Finally, elevated postpartum BHBA have been correlated with increased milk fat and decreased milk protein percentage (Miettinen and Setala 1993, Duffield et al. 2009).

Objective of this study was to investigate whether periparturient BHBA and pre-lambing NEFA concentrations are associated with milk yield, milk composition and the count of the udder halves with increased SCC during lactation, in intensively managed Chios dairy ewes.

MATERIALS AND METHODS

1. Animals
The study was performed in an intensively managed purebred Chios dairy sheep farm. Two hundred clinically healthy pregnant ewes were initially enrolled: 40/200 ewe-lambs (pregnant animals that were going to lamb for the 1st time), 40/200 primiparous ewes (animals that had already lambed at the start of the study and would lamb again for 2nd time during the study) and 120/200 multiparous ewes (animals with more than 2 lambings at the start of the study). The average litter size of the 200 ewes was 1.98 ± 0.83. Ninety-four out of the 200 ewes lambed in November and 106/200 in January. In both occasions, lambings took place within 10 days, due to -previously applied- estrus synchronization programs.

During the first week of lactation, 14 ewes were excluded from the study due to very low milk yield (in average < 0.65 L/d). Therefore, data from the remaining 186 ewes were finally taken into account for the statistical analysis. Moreover, for various reasons, 9/186 ewes were removed from the study between the 7th and the 45th DIM and 10/186 between 45th and 90th DIM. Data from the last 19 ewes were thereafter not included in the statistical analysis.

2. Housing and nutrition
All animals were kept indoors and fed with a controlled ration throughout the year. Dry period lasted approximately two months. The ewes were fed according to National Research Counsil (1985) recommendations.

During the first month of the dry period, each ewe received daily a ration containing 0.5 kg alfalfa hay and 0.5 kg concentrate mixture (corn 52%, barley 7%, wheat 8%, soybean 15%, wheat bran 6%, fat 2%, vitamins and minerals 10%) and grass hay on *ad libitum* basis. During the second month of the dry period, an additional 0.3 kg of concentrate mixture, plus 10 gr of sodium-propionate were added. After lambing, ewes received a total mixed ration consisting of 1.1 kg corn silage, 1 kg alfalfa hay and 1.55 kg of a mixture of concentrate feed (corn 35.5%, barley 22%, wheat 8%, soybean 22%, wheat bran 9%, fat 1%, vitamins and minerals 2.5%) per day and grass hay on *ad libitum* basis.

3. Blood sampling and analysis
Blood samples, for serum BHBA and NEFA measurements, were collected 30 ± 3 (-30d) and 15 ± 3 (-15d) days before the expected lambing (0d), as well as at 7 (+7d), 15 (+15d), 30 (+30d) and 45 (+45d) days after lambing, by jugular vein puncture from each animal into 10 mL plain glass tubes without anticoagulant (BD Vacutainer®, Plymouth, United Kingdom). The sampling procedure has been described by Oetzel (2004). Blood serum was separated by centrifugation (1600 x g for 15 minutes), transferred into plastic vials and frozen at -20ºC until assay. BHBA concentrations were measured at -30d, -15d, +7d, +15d, +30d and +45d, while NEFA at -30d and -15d.

Serum BHBA concentrations were analyzed using the D-β-HB-dehydrogenase method (Williamson and Mellanby 1974). Serum NEFA concentrations were assayed using a commercially available spectrophotometric analytic kit (NEFA kit, WAKO Chemicals GmbH, Neuss, Germany).Thirty-one blood samples collected from ewes at -30d (19 for NEFA and 12 for BHBA) were not analyzed due to technical problems at processing.

4. Milk yield recording, milk sampling and analysis
Milking of ewes started 3 days after lambing, when lambs were removed from their dams. Ewes were milked three times per day and individual milk yield was electronically recorded on a daily basis (ALPRO™ software, DeLaval, USA, North Congress Ave. Kansas City Missouri, 64153). Total milk yield (MY) produced during the first 15, 30, 45, 60 and 90 DIM and total

fortnightly milk yield (FMY) produced from 15 to 29, 30 to 44 and 45 to 59 DIM were recorded.

Individual milk samples were collected aseptically from each mammary half of all ewes prior to the morning milking, based on standard sampling protocols (Fthenakis 1994). Milk samples from halves with clinical mastitis (defined as presence of milk clots or abnormal mammary discharge) were excluded from the study. In total, three milk samplings were performed in each ewe, on 15, 30 and 45 DIM. Samples were maintained at 4°C during transportation to the laboratory.

SCC were measured by the Fossomatic method (Gonzalo et al. 1993), using the Fossomatic® 9000 (A/S N. Foss Electric, Hillerød, Denmark). Samples with $\geq 0.5 \times 10^6$ cells/mL were considered indicative of inflammation (Berthelot et al. 2005). Finally, proportional (%) milk composition (fat, protein, lactose, solids-not-fat) was determined by using automated midrange infrared spectroscopy (MilkoScan FT 120, Foss Electric, Hillerød, Denmark). The analysis for the milk components was based only on the halves that presented SCC below the threshold of 0.5×10^6 cells/mL. This was decided because milk composition is affected by subclinical mastitis (Olives et al. 2013).

5. Statistical analysis

Univariate analysis was carried out by descriptive statistics and results were expressed as mean (*M*), standard deviation (*SD*), median (*Mdn*), minimum (*min*) and maximum (*max*) at the examined times (days) before and after parturition. In all tests statistical significance was declared at $P < 0.05$. The examination of the relationship between BHBA (-30d, -15d, +7d) and NEFA concentrations (-30d, -15d) with MY during 30, 60 and 90 DIM, as well as average milk SCC of each udder half on DIM 15 and 30 was performed through the non-parametric Spearman's correlation coefficient. The Generalized Linear Models (GLM) under Poisson distribution were used to evaluate the effect of BHBA and NEFA concentrations before lambing (-30d,-15d) on the number of the halves with SCC $\geq 0.5 \times 10^6$ cells/mL at 15 and 30 DIM. Regarding post-lambing period, the Generalized Linear Mixed Models (GLMM), under Poisson distribution, were used to evaluate the effect of BHBA concentrations after lambing (+15d, +30d and +45d) and Time (days) on the number of the halves with increased SCC ($\geq 0.5 \times 10^6$ cells/mL) at 15, 30

and 45 DIM. In addition, Linear mixed effects (LME) modeling (Pinheiro and Bates, 2006) was used to assess the effect of BHBA concentrations on milk components (fat, protein, solids-not-fat, lactose) at +15d, +30d, and +45d and on FMY during the 15-days intervals (15 – 29, 30 – 44 and 45– 59 DIM, respectively). All statistical analyses were conducted using the statistical language R (R Core Team, 2013) and the function lme from package nlme (Pinheiro and Bates 2006).

RESULTS

1. Associations of periparturient BHBA and pre-lambing NEFA serum concentrations with milk yield

The descriptive statistics for BHBA (days: -30d, -15d, +7d, +15d, +30d, +45d) and NEFA (days: -30d, -15d) serum concentrations, as well as MY (15, 30, 45, 60 and 90 DIM) are shown in Table 1, respectively; BHBA concentrations are also depicted in Figure 1.

The Spearman's correlation coefficient revealed a statistically significant and weak positive correlation between BHBA serum concentration at -30d and MY produced during the first 30 DIM ($r_s = 0.185$, *N*=155, P = 0.021). Moreover, BHBA concentration at +7d was significantly positive correlated with MY produced during the first 30 ($r_s = 0.339$, *N*=182, $P < 0.001$), 60 ($r_s = 0.401$, *N*=171, $P < 0.001$) and 90 DIM ($r_s = 0.396$, *N*=167, $P < 0.001$). In contrast, there was not detected any statistically significant correlation between NEFA serum concentrations before lambing (-30d,-15d) and MY for any of the studied time periods.

With regard to the post-lambing period, the LME models fitted on the raw data revealed significant violations of the homogeneity of variance assumption and for this reason the measurements (BHBA and FMY) were logarithmically transformed using the natural logarithm. The final model indicated statistically significant main effects of both BHBA ($P = 0.042$) and Time ($P < 0.001$) on the FMY, and a significant interaction term (BHBA × Time) ($P < 0.001$). The parameters of the model are presented in Table 2. Interpreting the LME model with the interaction effect, the coefficient of BHBA at +15d ($\beta = -0.188$, $P = 0.001$) indicates that one percentage change in BHBA results in a 0.188 % decrease for FMY of the next 15-days period (15 – 29 DIM). Similarly, for one percentage change in BHBA at +30d, a

Table 1. Descriptive statistics for serum β-hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA) concentrations during the study (days: -30d, -15d, +7d, +15d, +30d, +45d), for body condition score (BCS) (-30d, 0d, +30d), for total milk yield (MY) of ewes during the first 15, 30, 45, 60 and 90 days in milk and for the ewes according to the count of halves (0, 1 or 2 halves) that found with milk somatic cells count (SCC) $\geq 0.5\times10^6$ cells/mL (days: +15d, +30d, +45d).

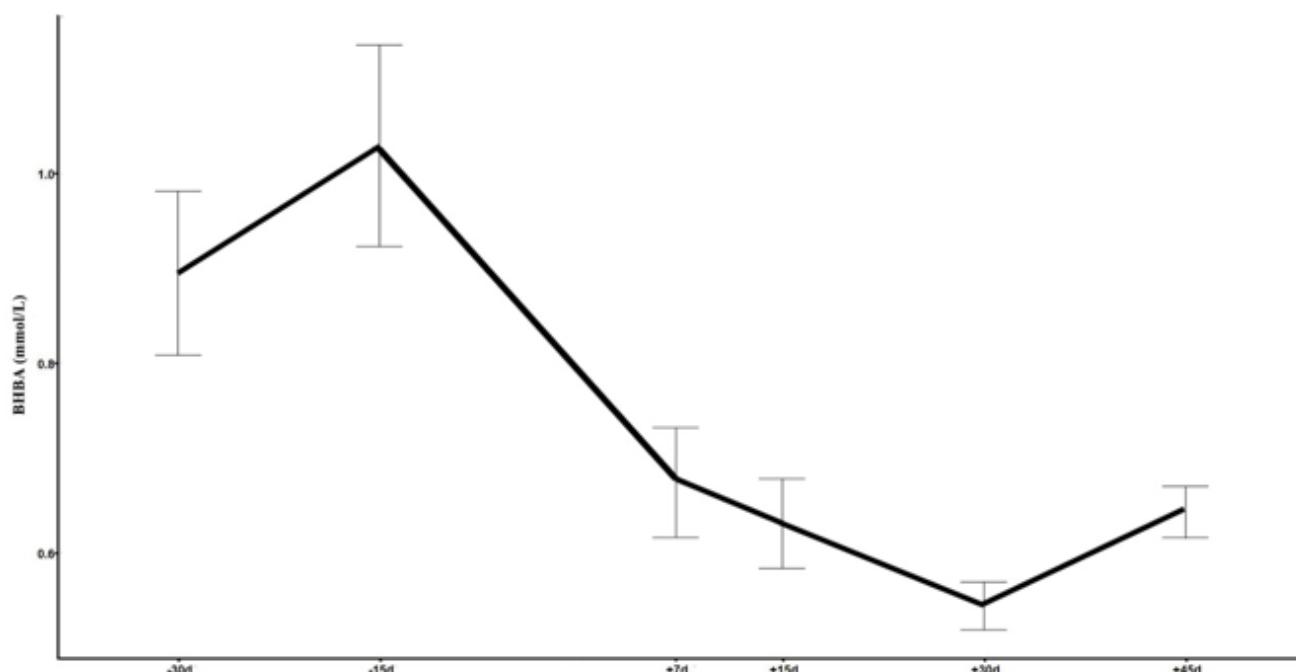|		Days (before/after lambing)								
		-30d	-15d	0d	+7d	+15d	+30d	+45d	+60d	+90d
N (ewes)		155	186	186	186	185	182	177	171	167
BHBA (mmol/L)	*Mean (± SD[+])*	0.89 (±0.55)	1.03 (±0.74)	-	0.67 (±0.40)	0.63 (±0.33)	0.54 (±0.17)	0.64 (±0.18)	-	-
	Median [min, max]	0.76 [0.35, 4.40]	0.78 [0.44, 4.60]	-	0.61 [0.28, 5.20]	0.58 [0.27, 4.00]	0.54 [0.26, 1.60]	0.61 [0.23, 1.10]	-	-
NEFA (mmol/L)	*Mean (± SD[+])*	0,43 (±0.37)	0,44 (±0.43)	-	-	-	-	-	-	-
	Median [min, max]	0.31 [0.005, 2.00]	0.30 [0.005, 2.30]	-	-	-	-	-	-	-
BCS (scale 1–5)	*Mean (± SD[+])*	2.87 (±0.58)	-	2.36 (±0.69)	-	-	2.35 (±0.60)	-	-	-
Total Milk Yield (L)	*Mean (± SD[+])*	-	-	-	-	26.89 (±9.54)	60.59 (±18.65)	98.34 (±27.33)	135.87 (±34.72)	202.36 (±47.39)
	Median [min, max]	-	-	-	-	26.00 [7.00, 58.00]	60.00 [20.00, 109.00]	98.00 [39.00, 168.00]	133.00 [50.00, 237.00]	199.00 [100.00, 344.00]
Count of halves with SCC ≥ 0.5 x 10^6 cells/mL	*0*	-	-	-	-	148 (80%)	148 (81.3%)	145 (82%)	-	-
	1	-	-	-	-	28 (15.1%)	28 (15.4%)	28 (15.8%)	-	-
	2	-	-	-	-	9 (4.9%)	6 (3.3%)	4 (2.2%)	-	-

[+]Standard deviation

Figure1. Serum β-hydroxybutiric acid (BHBA) concentrations (95% Confidence Interval) during the study (days from lambing: -30d, -15d, +7d, +15d, +30d, +45d).

Table 2. Linear mixed effects models for fortnightly milk yield (FMY) during 15 – 29, 30 – 44 and 45 – 59 days in milk (DIM) and for milk protein, solids not fat and lactose at +15, +30 and +45 days in milk, with β-hydroxybutyric acid (BHBA) (log-transformed), Time and interaction term logBHBA × Time as fixed effects.

	Estimate	SE	t	P
Model 1 [Fortnightly Milk Yield (log-transformed)**]**				
Intercept	3.350	0.040	84.202	< 0.001
BHBA (log-transformed)	-0.188	0.055	-3.420	0.001
Time: [30 – 45 DIM]	0.184	0.055	3.699	< 0.001
Time: [45 – 60 DIM]	0.206	0.045	4.559	< 0.001
logBHBA×Time: [30 – 45 DIM]	0.177	0.072	2.452	0.015
logBHBA×Time: [45 – 60 DIM]	0.295	0.078	3.805	< 0.001
Model 2 [Milk Protein (log-transformed)**]**				
Intercept	1.570	0.007	211.734	< 0.001
BHBA (log-transformed)	-0.033	0.008	-4.219	< 0.001
Time: +30d	-0.025	0.003	-7.240	< 0.001
Time: +45d	-0.030	0.005	-5.954	< 0.001
Model 3 [Milk Solids Not fat (log-transformed)**]**				
Intercept	2.383	0.004	648.033	< 0.001
BHBA (log-transformed)	-0.020	0.004	-5.070	< 0.001
Time: +30d	-0.007	0.002	-4.351	< 0.001
Time: +45d	-0.007	0.002	-2.779	0.006
Model 4 [Milk Lactose (log-transformed)**]**				
Intercept	1.654	0.004	393.612	< 0.001
BHBA (log-transformed)	-0.007	0.004	-1.727	0.085
Time: +30d	0.010	0.002	5.591	< 0.001
Time: +45d	0.013	0.002	5.488	< 0.001
Note: Time: +15d is the reference category				

decrease of 0.011 % in FMY (β = 0.177, P = 0.015) of the next 15-days period (30 – 44 DIM) was noted. In contrast, for one percentage change in BHBA at +45d, an increase of 0.107 % FMY (β = 0.295, P < 0.001) of the next 15-days period (45 – 59 DIM) was found.

2. Associations of periparturient BHBA and pre-lambing NEFA concentrations with the count of the halves with increased SCC

Table 1 shows the distribution of the ewes according to the count of the halves (0, 1 or 2 halves) that found to have SCC ≥ 0.5 × 10⁶ cells/mL at 15, 30 and 45 DIM. The GLM revealed a statistically significant main effect of BHBA concentration at -30d on the count of the halves with SCC ≥ 0.5 × 10⁶ cells/mL at +15d (P = 0.011) and at +30d (P = 0.009). Concerning BHBA concentration at -15d, a statistically main positive

effect on the count of the halves with SCC ≥ 0.5 × 10⁶ cells/mL was detected at +15d (P = 0.014), while was not detected at +30d. The GLM indicated a marginally significant positive main effect of NEFA concentration at -30d on the count of the halves with SCC ≥ 0.5 × 10⁶ cells/mL at +15d (P = 0.051). On the other hand, there was not detected any main effect of NEFA concentrations at -30d on the count of the halves with increased SCC at +30d (P = 0.116) and NEFA concentrations at -15d on the count of the halves with increased SCC at +15d (P = 0.179) and at +30d. Finally, the GLMM model for the examination of the effect of BHBA concentrations and Time on the count of the halves with SCC ≥ 0.5 × 10⁶ cells/mL at 15, 30 and 45 DIM did not reveal any statistically significant main or interaction effects for the post-lambing period.

3. Associations of post-lambing BHBA concentrations with milk composition

Table 2 also presents the findings of the LME models that were fitted in order to study the effects of the covariate BHBA and factor Time on milk composition parameters. Due to violations of the homogeneity of variance assumption, both the covariate (BHBA) and the set of dependent variables (milk protein, solid not fat and lactose) were logarithmically transformed. BHBA presented a statistically significant main effect on milk protein and solid not fat parameters ($P < 0.001$) and a marginally significant effect on milk lactose ($P = 0.085$). The negative coefficients for covariate BHBA revealed a negative correlation between BHBA and milk composition parameters. Regarding the factor Time, there were noted statistically significant differences between the mean values of milk composition parameters between +15d and +30d periods and between +15d and +45d periods. In contrast, the Tukey's HSD procedure did not reveal anysignificant difference between the mean values of the examined parameters for +30d and +45d periods.

DISCUSSION

BHBA concentration 30 days before parturition was weakly positively correlated with milk yield produced until 30 DIM. It was previously reported that, blood BHBA concentration increases during late gestation and may reach its peak before or around lambing (Raoofi et al. 2013; Karagiannis et al. 2014). It is possible that the ewes with potential for higher milk production could have higher energy metabolism before lambing, expressed as increased BHBA concentrations. However, this plausible explanation requires further investigation. BHBA concentration at +7d was positively correlated with milk yield of the first 30, 60 and 90 DIM, implying that a moderate ketone metabolism in early lactation may be beneficial for achieving higher milk yield. However, a rise of BHBA concentrations at +15d had a negative short-term effect in the cumulative milk yield of the next 15-days (15 – 29 DIM). As lactation progressed (at 30 DIM), BHBA short-term effect on the FMY of 30 – 44 DIM was still negative, but weaker, while high levels of BHBA at +45d had a positive effect on FMY of the next 15-days (45 – 59 DIM). It has been shown that ewes with greater potential for increased milk yield confront a longer period of NEB

during early lactation (Bizelis et al. 2000). It could be assumed that increased BHBA concentrations during early lactation (until 30 DIM) could cause a short-term decrease in milk production. The finding that +45d BHBA concentration positively affected FMY of the next 15 days (45 – 59 DIM) probably implies that energy balance was restored or may became positive around the 45th day of lactation. Under this hypothesis, increased BHBA at the beginning of lactation may indicate a potential for high milk production during the first 90 DIM (longer-term effect), including though a possible short-term negative effect, especially when NEB is not timely restored. Relevant information correlating BHBA concentrations after lambing with short and longer-term milk yields are lacking in the accessed literature for dairy ewes.

High levels of BHBA and NEFA before lambing predispose to several periparturient health disorders, such as clinical mastitis (Karagiannis et al. 2014). The results of the present study indicated that increased pre-lambing BHBA (-30d, -15d) and NEFA (-30d) concentrations were positively correlated with the count of the halves with increased SCC after lambing (15 and 30 DIM). The negative effect of subclinical pregnancy toxemia and elevated BHBA and NEFA concentrations to immune function was previously documented (Sartorelli et al. 2000, Lacetera et al. 2001) and could explain the early postpartum intramammary infections and increased SCC found in the present study. Recently, Bouvier-Muller et al. (2016) outlined that after a dietary-induced energy restriction during lactation, NEFA and BHBA concentrations were higher in mastitis-susceptible ewes compared with mastitis-resistant ones, implying a genetic association between energy metabolism and mastitis susceptibility. This could explain the positive effect of increased pre-lambing BHBA and NEFA concentrations on the count of the halves with increased SCC postpartum.

In the current study, increased post-lambing BHBA did not have any effect on the count of the halves with elevated SCC. The fact that BHBA concentration during lactation was much lower compared with late gestation (Table 1), could imply that BHBA did not reach the required levels to impair the immune function, as previously described. Interestingly, no difference in SCC was reported, after inflammation by Staphylococcus-associated ligands, between ewes con-

fronting dietary-induced NEB and ewes in a positive energy balance (Bouvier-Muller et al. 2016).

After lambing, an increase in BHBA concentrations at 15, 30 and 45 DIM was associated with a decrease in milk protein and milk lactose percentage, while no association was found with milk fat. Although relevant information for dairy ewes is lacking in the accessed literature, research in dairy cows has indicated that increased milk fat content and decreased milk protein were associated with elevated postpartum BHBA concentrations (Duffield et al. 2009). The increase of BHBA concentrations after lambing was associated with the reduced milk protein percentage. It has been reported that milk protein is an indicator of energy balance (Grieve et al. 1986). The association of increased BHBA concentrations with decreased milk lactose percentage could be explained by the negative correlation between blood BHBA and glucose concentrations, demonstrated in dairy sheep (Panousis et al. 2012).

Chemical composition of milk was also affected by time after lambing. More specifically, a reduction in milk fat, protein and SNF percentage was detected between 15 and 30, and between 15 and 45 DIM. Dairy ewes reared under intensive system peak milk yield at about 27 – 45 DIM (Gootwine and Pollott 2000, Pollott and Gootwine 2000;). Therefore, the recorded reduction in milk components (fat, protein and SNF) seems reasonable, due to a dilution effect (Gonzalo et al. 1994, Ochoa-Cordero et al. 2002). However, an expected increase in milk lactose was noticed between 15 and 30, and between 15 and 45 DIM, since increasing daily milk yield (until 45 DIM) is positively correlated with lactose percentage (Ploumi et al. 1998, Ochoa-Cordero et al. 2002).

CONCLUSIONS

The present study investigated the potential associations of periparturient BHBA and NEFA concentrations with milk yield, milk composition and the count of the udder halves with increased SCC. It has been shown that BHBA concentration 30 days before lambing had a weak positive impact on milk yield of the first 30 DIM. BHBA during early lactation had a positive long-term (first 90 DIM), but a negative short-term (15 – 29 and 30 – 44 DIM) effect on milk yield. Increased BHBA concentrations before lambing were correlated with a rise in the count of the halves that presented increased SCC during lactation. Moreover, BHBA increase during the first 45 DIM was negatively associated with milk protein percentage, but not with milk fat percentage.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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