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Effect of β -hydroxybutyric acid, parity, and body condition score on phenotype and proliferative capacity of colostral mononuclear leukocytes of high-yielding dairy cows

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ABSTRACT

In neonatal calves, the ingestion of colostrum is imperative for preventing infectious diseases. Investigations into the transfer of passive immunity of cattle have primarily focused on the importance of colostral immunoglobulins, with a recent increase in focus on understanding the role of colostral leukocytes. The main objective of the present study was to measure the influence of parity, body condition score, serum nonesterified fatty acids, and serum β -hydroxybutyrate concentrations of periparturient cows on phenotype and mitogen- and antigen-induced proliferative capacity of bovine colostral leukocytes. Holstein-Friesian cows ($n = 141$) were intramuscularly vaccinated at 60 and 30 d before the expected parturition date with a tetanus toxoid vaccine. Of these 141 animals, 28 primiparous and 72 multiparous cows were sampled immediately after parturition. Colostrum mononuclear cell populations were identified by flow cytometry using bovine cluster of differentiation markers, and the proliferative capacity of these cells was determined using a ^3H -thymidine proliferation assay. Under-conditioned cows had a significantly higher percentage of colostral macrophages than normal-conditioned animals, whereas over-conditioned cows had significantly more colostral B-lymphocytes. Serum β -hydroxybutyrate was significantly associated with higher numbers of colostral T-lymphocytes and macrophages. Heifers had significantly higher mitogen- and antigen-induced proliferation of their colostral leukocytes than third parity or older cows. In conclusion, body condition score, parity, and serum β -hydroxybutyrate concentration of periparturient high-yielding dairy cows were shown to influence the number of colostral macrophages or the

mitogen- and antigen-induced proliferation of colostral leukocytes, possibly influencing the cellular immunity of the newborn calf.

Key words: colostrum, dairy cows, leukocytes, negative energy balance

INTRODUCTION

Investigations into the transfer of passive immunity of cattle have primarily focused on the importance of colostral immunoglobulins, but currently increasing attention is being given to other humoral and cellular components in colostrum (Hagiwara et al., 2000; Donovan et al., 2007). Bovine colostral leukocytes (CL) are mainly composed of neutrophils, macrophages, and lymphocytes (Duhamel et al., 1987). Uptake of these CL through the intestinal barrier has been shown in cattle, with a preferential route of uptake through the follicle-associated epithelium of Peyer's patches (Liebler-Tenorio et al., 2002). The functional importance of bovine CL is not yet fully understood, but they seem to enhance lymphocyte responses (Reber et al., 2005; Donovan et al., 2007), increase phagocytosis and bacterial-killing ability (Riedel-Caspari and Schmidt, 1991), and stimulate humoral immune responses in the neonatal calf (Riedel-Caspari, 1993).

The concentration of colostral immunoglobulins in the bovine species is known to be affected by breed (Tyler et al., 1999), parity (Moore et al., 2005; Morin et al., 2010), yield of colostrum at first milking (Chigerwe et al., 2008; Morin et al., 2010), season (Gulliksen et al., 2008), and the interval between parturition and first milking (Moore et al., 2005; Morin et al., 2010). In contrast, few articles have described factors affecting the phenotype or proliferative capacity of lymphocytes in bovine colostrum. Moreover, when describing CL, most manuscripts emphasize the importance of CL on cow mammary gland immunity and not on adoptive immunity transfer to the newborn (Park et al., 1993;

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Shafer-Weaver et al., 1996; Ohtsuka et al., 2010). Parity (Ohtsuka et al., 2010), infection state of the udder (Nonnecke and Harp, 1985; Park et al., 1993), and vitamin E and selenium supplementation (Wuryastuti et al., 1993) have been shown to influence the number or activation state of cow and sow mammary gland leukocytes (MGL). For example, CL of multiparous cows have a higher IFN- γ -to-IL-4 ratio than heifers (Ohtsuka et al., 2010) and the proliferative response of MGL is lower in infected than uninfected mammary glands (Nonnecke and Harp, 1985; Park et al., 1993). Furthermore, the proliferative response of MGL is generally lower than that of peripheral blood mononuclear cells (PBMC; Nonnecke and Harp, 1985; Harp and Nonnecke, 1986; Park et al., 1993). Also, high in vitro concentrations of NEFA and BHBA inhibit mitogen-induced proliferative responses and diminish DNA synthesis of PBMC (Franklin et al., 1991; Lacetera et al., 2004; Thanasak et al., 2005). Whether parity, BCS, serum NEFA, and serum BHBA concentrations of periparturient cows affect the function of bovine CL in vivo has, to the best of our knowledge, not been studied. The main objective of the present study was, therefore, to measure the effect of these variables on phenotype and mitogen- and antigen-induced proliferative capacity of bovine CL.

MATERIALS AND METHODS

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Ghent (EC2008/149).

Herds, Animals, and Study Design

An observational cross-sectional study design was used. Holstein-Friesian cows (n = 141) were randomly selected from 17 commercial dairy herds in Flanders (Belgium; Figure 1). The herds had, on average, 59 lactating cows (range 36 to 95), and an average 305-d milk yield of 8,485 kg (range = 4,938–10,756 kg). All 141 animals were intramuscularly vaccinated at 60 and 30 d before the expected parturition date with 2 mL of a tetanus toxoid vaccine (Tetapur, Merial Limited, Duluth, GA). The vaccine was chosen because tetanus is not a disease commonly vaccinated against in Belgium and because tetanus toxoid is a well-known strong inducer of humoral and cellular immunity. A pretest performed on 3 vaccinated cows (versus 3 non-vaccinated cows) showed the vaccine to perform well in terms of eliciting a strong cellular immune response. Of those 141 cows, 41 cows were excluded from the study due to various reasons: culling (n = 6), abortion (n = 3), missed parturitions (n = 22), leaking of colostrum prepartum (n = 3), or parturition before administration of booster vaccination (n = 7; Figure 1).

Data and Sample Collection

To isolate colostrum mononuclear cells (CMC), colostrum (360 mL) was sampled aseptically during the first milking postpartum (90 mL from each individual quarter, composite sample). Following colostrum collection, the SCC was determined using a DeLaval

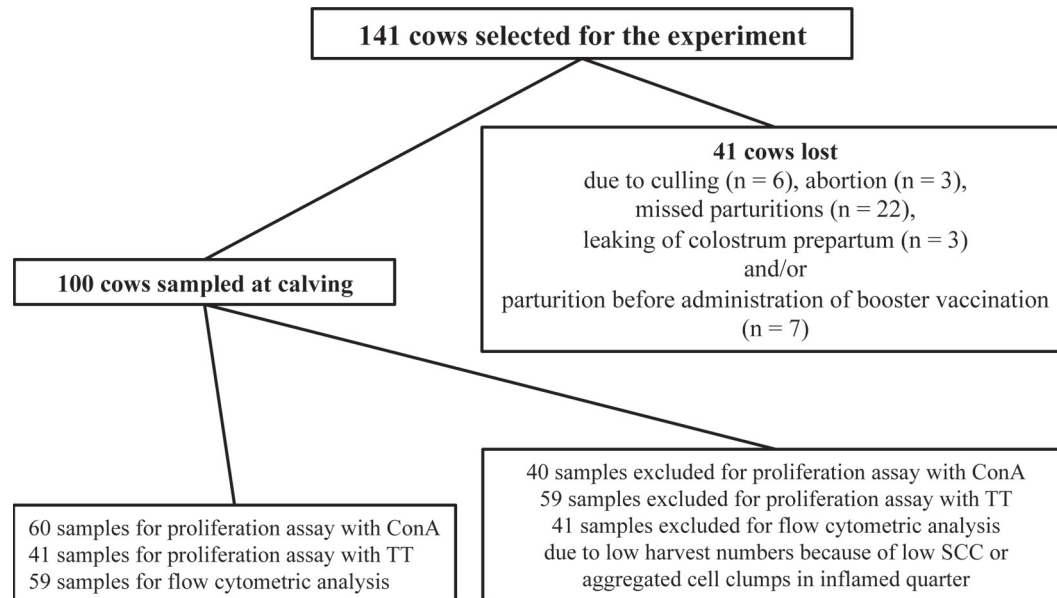


Figure 1. Flow of study subjects throughout the trial. ConA = concanavalin A; TT = tetanus toxoid.

cell counter (DeLaval, Tumba, Sweden). All colostrum samples were processed within 24 h after collection and stored on ice.

Parity and BCS were recorded at the moment of parturition. Cows were divided in 3 classes based on their parity: (1) heifers ($n = 28$), (2) second parity ($n = 39$), and (3) third parity or older ($n = 33$). Body condition score was recorded by the same person immediately after parturition. Body condition score was expressed on a 5-point scale with 0.25 increments as described by Edmonson et al. (1989). Cows were divided in 3 classes based on their BCS: (1) under-conditioned, <3 ; (2) normal condition, 3 to 3.5; and (3) over-conditioned, >3.5 .

To determine NEFA and BHBA serum concentrations, all cows were blood-sampled immediately postpartum. Animals were tethered in headlocks and were approached by the researcher as calmly as possible to avoid any stress. Immediately after parturition, blood samples were taken from the coccygeal vein via (a) 2 tubes containing EDTA as an anticoagulant (Venosafe, Terumo Europe NV, Leuven, Belgium) to isolate PBMC as described below and (b) 1 silicone-coated tube (Venosafe) to quantify NEFA and BHBA. All samples except the EDTA tubes were centrifuged ($2,460 \times g$, 20 min, 7°C) immediately after collection; serum was subsequently collected and frozen (-80°C) until further analysis. Both NEFA and BHBA were analyzed using an automated colorimetric analyzer (Konelab 20 XT Clinical Chemistry Analyzer, Thermo Scientific Inc., Waltham, MA) at the Animal Health Service Flanders (Drogen, Belgium). All herd visits were performed between July 2009 and May 2011.

Isolation of PBMC

The protocol was modified from a previously developed protocol (Goddeeris et al., 1986). Fifteen milliliters of EDTA-blood was diluted into an equal volume of Alsever's solution (Sigma-Aldrich, Diegem, Belgium) and layered over 20 mL of Lymphoprep (specific gravity = 1.077; Axis-Shield, Oslo, Norway) in 50-mL conical tubes (BD Falcon, Becton Dickinson, Franklin Lakes, NJ) and centrifuged (30 min, $900 \times g$, 18°C). Cells at the interface were aspirated, transferred to new 50-mL conical tubes and washed with ice-cold Alsever's solution, containing 1% fetal bovine serum (Greiner Bio One, Wommel, Belgium; 10 min at $450 \times g$ followed by 10 min at $300 \times g$, 4°C). The resulting cell pellets were resuspended in 1 mL of RPMI 1640 medium (Sigma-Aldrich), containing 10% FBS, 2 mM L-glutamine (Gibco, Life Technologies, Carlsbad, CA), 50 $\mu\text{g}/\text{mL}$ of gentamicin (Sigma-Aldrich), and 5×10^{-5} M 2-mercaptoethanol (Merck, Darmstadt, Germany;

hereafter named culture medium). The isolated PBMC were counted in a Neubauer counting chamber using eosin-nigrosin.

Isolation of CMC

Isolation of CMC was performed as described in Meganck et al. (2014). Briefly, diluted colostrum samples were centrifuged ($600 \times g$, 15 min, 18°C), the cream layer was removed, and cell pellets were washed twice. Cell pellets were then resuspended and over-layered onto 20 mL of Ficoll-Paque premium (GE Healthcare, Barrington, IL) followed by centrifugation ($900 \times g$, 30 min, 18°C). Cells at the interface were aspirated and washed twice (4°C). The resulting cell pellets were resuspended in 1 mL of culture medium. The isolated CMC were counted in a Neubauer counting chamber using eosin-nigrosin. Due to a poor yield of CMC, data of only 60 animals could be used to perform the proliferation assay with concanavalin A (**ConA**), of 41 animals to perform the proliferation assay with tetanus toxoid (**TT**), and of 59 animals to perform the flow cytometric assay (Figure 1). These relatively low yields were caused by low SCC or aggregated cell clumps in case of clinical mastitis.

Leukocyte Phenotypic Analysis by Flow Cytometry

The flow cytometric analysis was performed as described in Meganck et al. (2014). Monoclonal antibodies used were mouse anti-bovine cluster of differentiation (**CD**) 21 (MCA1424G, AbDSerotec, Puchheim, Germany) for B-lymphocytes; mouse anti-bovine CD2 (BAQ95A, VMRD, Pullman, WA), expressed on T-lymphocytes and NK-cells; and mouse anti-bovine CD14 (MM61A, VMRD) for monocytes or macrophages. Alexa Fluor 647-conjugated donkey anti-mouse IgG (H+L; Invitrogen, Life Technologies) was used as secondary antibody. Propidium iodide (Sigma-Aldrich) was used as a marker for dead cells. Ten thousand events per sample were acquired and relative numbers of cells positive for propidium iodide, CD2, CD21, and CD14 determined with FACSDiva Software (Becton Dickinson). Gating of the cells was performed, based on cell size (forward scatter), cellular granularity (side scatter), and fluorescence (FL-2 and FL-5 channels).

Cellular Immune Response by ^3H -Thymidine Proliferation Assay

Cells were cultured in plain medium as a negative control for background proliferation, in the presence of 5 $\mu\text{g}/\text{mL}$ of ConA (Sigma-Aldrich) as a positive control for mitogen-induced proliferation, or in the presence of

various concentrations of purified TT (kindly provided by Merial Limited) for antigen-induced proliferation. The optimal stimulating concentration was used for further analysis (10 flocculation units/mL = 33.6 µg/mL). All wells contained 2×10^5 cells in a final volume of 200 µL and all added substances were performed in triplicate. Cells were incubated for 3 (medium, ConA) or 5 d (TT) at 37°C in a humidified atmosphere containing 5% CO₂. Cells were pulse-labeled for 18 h with 1 µCi of ³H-thymidine per well and harvested on glass fiber filters (Whatman, GE Healthcare), then radioactivity was measured using a Beckman β-scintillation counter (Beckman, Suarlée, Belgium). Data were acquired as counts per minute (CPM). Mitogen-induced proliferation in CMC is reported as CPM of CMC stimulated with ConA (CPM_{CMCConA}) and antigen-induced proliferation in CMC is reported as CPM of CMC stimulated with TT (CPM_{CMCTT}).

Statistical Analysis

The distribution of all dependent variables was checked for normality and eventually data were transformed to approximate the Gaussian distribution. All CPM data and flow cytometric data for colostrum were therefore log-transformed before further analysis. Descriptive statistics were done using PROC MEANS and PROC FREQ of SAS v9.4 (SAS Institute Inc., Cary, NC).

To determine the associations between parity, body condition of cows, NEFA and BHBA serum concentrations, and CPM_{CMCConA}, CPM_{CMCTT}, percentage of CD2⁺ CMC, percentage of CD21⁺ CMC, and percentage of CD14⁺ CMC, linear regression models with parity, BCS, BHBA, and NEFA concentrations measured immediately postpartum as independent variables of main interest were tested (PROC GLIMMIX). Herd was tested as a random factor, but the covariance parameter estimate was negligible and further inclusion of the variable herd was not needed. Initially, separate associations were tested between the outcome variables CPM_{CMCConA}, CPM_{CMCTT}, CD2⁺ CMC, and CD14⁺ CMC and all independent variables mentioned above. Statistical significance in these univariate univariable models was assessed at $P < 0.20$. Second, Pearson cor-

relation coefficients were calculated among the significant independent variables using the CORR procedure in SAS v9.4 to check for multicollinearity. If 2 independent variables had a correlation coefficient ≥ 0.6 , only one was selected for further analysis. In the third step, a univariate multivariable or univariable model was fit. Statistical significance was assessed at $P < 0.05$.

To compare CPM of CMC and PBMC, and to identify differences between leukocyte phenotype of CMC and PBMC, separate one-way ANOVA tests were performed in SAS v9.4 for CD2⁺ and CD14⁺ cells and for TT-induced proliferation and separate nonparametric ANOVA tests (Wilcoxon signed-rank test) were performed for the CD21⁺ cells and for the ConA-induced proliferation. Significance was assessed at $P < 0.05$.

RESULTS

Descriptive Statistics

Descriptive statistics on SCC and isolation of PBMC and CMC can be found in Table 1. Descriptive statistics for the leukocyte phenotypic analysis by flow cytometry and for the cellular immune response by ³H-thymidine proliferation assay can be found in Table 2.

Leukocyte Phenotypic Analysis by Flow Cytometry

Only BHBA was significantly associated with the percentage of CD2⁺ CMC. The higher the BHBA serum concentration, the higher ($P = 0.049$) the percentage of CD2⁺ CMC (Table 3). Only BCS was significantly associated with the percentage of CD21⁺ CMC. Over-conditioned cows (BCS >3.5) had significantly ($P = 0.006$) more CD21⁺ CMC than normal-conditioned cows: 4.04 vs. 1.37%, respectively (BCS 3–3.5; Table 4).

A first reduction based on the univariable associations revealed all 4 variables (parity, BCS, NEFA, and BHBA) to be associated with CD14⁺ CMC. None of the parameters were correlated with each other ($r = -0.06-0.13$; $P \geq 0.218$). In the final multivariable model, under-conditioned cows (BCS <3) had more ($P = 0.008$) CD14⁺ CMC than normal-conditioned animals (BCS 3–3.5): 40.25 vs. 25.88%, respectively (Table 5, Figure 2). Also, the higher the BHBA serum concentra-

Table 1. Descriptive statistics on SCC and isolation of peripheral blood mononuclear cells (PBMC) and colostrum mononuclear cells (CMC) based on 100 cows

Variable	Mean	Median	IQR ¹
Isolated PBMC (cells/mL of blood)	7.7×10^6	4.3×10^6	$1.5 \times 10^6-9.7 \times 10^6$
Colostrum SCC (cells/mL of colostrum)	1.2×10^6	6.9×10^5	$2.1 \times 10^5-1.3 \times 10^6$
Isolated CMC (cells/mL of colostrum)	1.2×10^5	3.2×10^4	$6.3 \times 10^3-1.3 \times 10^5$

¹IQR = interquartile range.

Table 2. Descriptive statistics for the phenotypic analysis by flow cytometry for the cellular immune response by the ³H-thymidine proliferation assay

Protocol	Peripheral blood mononuclear cells				Colostrum mononuclear cells			
	No. of samples	Mean	Median	IQR ¹	No. of samples	Mean	Median	IQR
Flow cytometry ² (%)								
CD2 ⁺	65	32 ^a	33	26–41	49	16 ^b	10	3–23
CD21 ⁺	56	25 ^a	26	20–28	41	2.0 ^b	0.9	0.3–2.5
CD14 ⁺	72	15 ^a	14	9–20	59	28 ^b	24	11–43
PI ⁺	74	2 ^a	1	0–2	58	19 ^b	18	12–24
Proliferation (CPM ³ × 10 ³)								
Medium	90	6 ^a	1	0–3	60	15 ^b	4	1–14
Concanavalin A	90	321 ^a	346	176–425	60	86 ^b	15	6–56
Tetanus toxoid	51	15	1	0–14	41	22	4	0–14

^{a,b}Values within a row with different superscript letters are significantly different ($P < 0.01$).

¹IQR = interquartile range.

²CD2⁺ = cluster of differentiation 2, recognizes T-lymphocytes; CD21⁺ = cluster of differentiation 21, recognizes B-lymphocytes; CD14⁺ = cluster of differentiation 14, recognizes macrophages/monocytes; PI = propidium iodide, recognizes dead cells.

³CPM = counts per minute.

tion, the higher the percentage of CD14⁺ CMC ($P = 0.023$; Table 5). On average, lymphocyte percentages were lower ($P < 0.001$) in colostrum compared with blood, whereas the opposite was found for macrophages or monocytes (Table 2).

Cellular Immune Response by ³H-thymidine Proliferation Assay

A first reduction based on the univariable associations and highly correlated variables revealed only 1 variable (parity) to be associated with CPM_{CMCC_{ConA}}. Heifers had significantly higher CMC mitogen-induced proliferation than second ($P = 0.049$) or third parity or older cows: 1.9×10^5 vs. 1.3×10^4 vs. 7.4×10^4 CPM, respectively ($P = 0.006$; Figure 3, Table 6).

A first reduction based on the univariable associations revealed only 1 variable (parity) to be associated with CPM_{CMCTT}. Heifers had higher ($P = 0.045$) CMC antigen-induced proliferation than third parity or older cows: 4.4×10^4 vs. 1.3×10^4 CPM (Figure 4, Table 6).

The mean background proliferation in PBMC was lower ($P < 0.001$) than that in CMC (Table 2). The mean PBMC mitogen-induced proliferation was higher ($P < 0.001$) than that of the CMC (Table 2). The mean

antigen-induced proliferation for the PBMC was practically equal ($P = 0.382$) to that of the CMC (Table 2).

DISCUSSION

The main objective of the present study was to measure the effect of parity, BCS, serum NEFA, and serum BHBA concentrations on phenotype and mitogen- and antigen-induced proliferative capacity of bovine CL. Fat cows have large amounts of adipose tissue, and in times of energy deficiency this over-condition is linked with adverse health effects (Goff, 2006). Blood mononuclear leukocytes isolated from over-conditioned cows secreted less IFN- γ than those of thin and medium cows 1 wk before the expected parturition date. In the present study, BCS of periparturient cows had a significant effect on the number of B-lymphocytes and macrophages resident in colostrum. The proposed causal relationship of Collard et al. (2000), that thin cows or cows in severe negative energy balance may be more susceptible to infection, could explain our findings.

High-yielding dairy cows are known to have an impaired immunity in early lactation compared with the

Table 3. Final univariate univariable model for the log-transformed percentage of colostrum CD2⁺ leukocytes using data of 48 animals

Predictor	Estimate (log%)	No. of samples	SE	P-value
Intercept	0.89	48	0.59	0.137
BHBA	1.57	48	0.78	0.049

Table 4. Final univariate univariable model for the log-transformed percentage of colostrum CD21⁺ leukocytes using data of 35 animals

Predictor	Estimate (log%)	No. of samples	SE	P-value
Intercept	-0.08	48	0.23	0.726
BCS ¹				
<3	0.19	6	0.49	0.695
3–3.5	Reference	21		
>3.5	1.30	8	0.44	0.006

¹Measured at the day of parturition.

Table 5. Final univariate multivariable model for the log-transformed percentage of colostrum macrophages (CMC_{CD14}) using data of 57 animals

Predictor	Estimate (log%)	No. of samples	SE	P-value
Intercept	2.21	57	0.31	<0.001
BHBA ¹	0.96	57	0.41	0.023
BCS ¹				0.028
<3	0.85	8	0.31	0.008
3-3.5	Reference	39		
>3.5	0.22	10	0.28	0.439

¹Measured on the day of parturition.

mid- to late lactation period, and are therefore more susceptible to all kinds of infections during the periparturient period (Ishikawa, 1987; Kehrli et al., 1989a,b). This impaired immunity explains the relatively low CMC mitogen- and antigen-induced proliferation found in the present and other studies (Concha et al., 1980; Archambault et al., 1988a; Kehrli et al., 1989a). In humans, increased plasma NEFA and oxidative stress are well known factors effecting systemic inflammation (Calder, 2008; Wood et al., 2009). Although neutrophils isolated from cows with high BHBA levels had a decreased respiratory burst activity (Opsomer et al., 1998;

Hoeben et al., 2000; Lacetera et al., 2005), the present study could not show a significant effect of BHBA nor NEFA on mitogen- and antigen-induced proliferation in CMC. The latter might be (partly) explained by the fact that the number of animals with high values, and thus expected to suffer from negative energy balance, was almost negligible. Conversely, serum BHBA was positively associated with colostrum T-lymphocytes and macrophages. The latter results comply with the theory of the impaired immunity of cows caused by ketosis.

Parity was shown to have a significant effect on the mitogen- and antigen-induced proliferation of CMC. Older cows had lower mitogen- and antigen-induced CPM values than heifers. Studies investigating the effect of aging on immune function in humans have indicated a diminished role of type 1 cytokines, such as IFN- γ and IL-2, which promote cell-mediated immunity (Alberti et al., 2006). This is in line with the results of the current study. Ohtsuka et al. (2010) found 2 contradictory phenomena: activation of colostrum lymphocytes versus suppression of the systemic type 1 reaction in periparturient healthy cows. Cows in parity ≥ 4 suffered from more profound periparturient impairment of neutrophil function than younger cows (Gilbert et al., 1993), again confirming the present results.

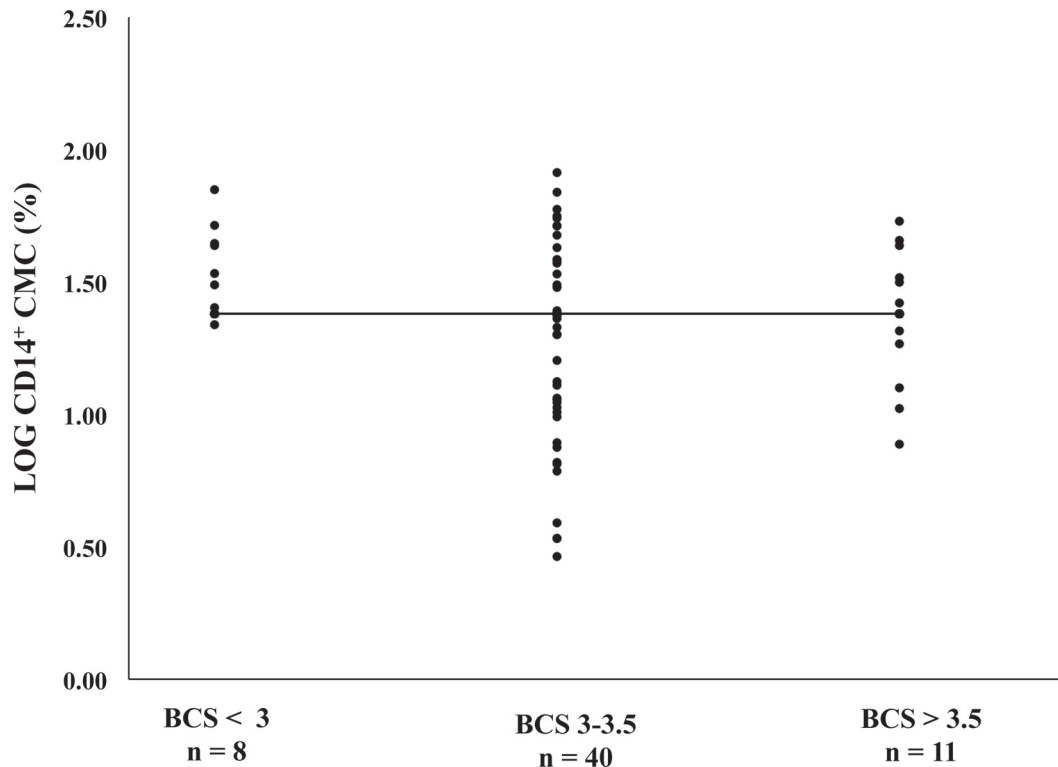


Figure 2. Under-conditioned cows (BCS <3) had significantly more colostrum macrophages than normal conditioned cows (BCS 3-3.5; $P = 0.008$). Results are represented as the raw logarithmic unmodeled data with the solid black line denoting the median value. CD14⁺ = cluster of differentiation 14; CMC = colostrum mononuclear cells.

Table 6. Final univariate univariable model for the log-transformed counts per minute of colostrum mononuclear cells stimulated with the mitogen concanavalin A (data of 60 animals) or tetanus toxoid (data of 41 animals)

Predictor	Concanavalin A				Tetanus toxoid			
	Estimate (logCPM ¹)	No. of samples	SE	<i>P</i> -value	Estimate (logCPM)	n	SE	<i>P</i> -value
Intercept	10.89		0.47	<0.001	9.10	41	0.62	<0.001
Parity				0.019				0.104
Heifer	Reference	16			Reference	14		
2nd parity	-1.25	27	0.59	0.049	-1.35	17	0.83	0.113
≥3rd parity	-1.97	17	0.67	0.006	-1.98	10	0.96	0.045

¹CPM = counts per minute.

In the present study, the mean percentages of CD2⁺ and CD14⁺ CMC were comparable or lower than percentages found in previous studies (Park et al., 1992; Yang et al., 1997; Kiku et al., 2010). The mean percentage of CD21⁺ CMC was much lower than the percentage of B-lymphocytes found in bovine colostrum in other studies (Park et al., 1992; Kiku et al., 2010). Our results concerning B-lymphocytes agree with others (Taylor et al., 1994; Harp et al., 2004), as T-lymphocytes traffic selectively into bovine mammary gland secretions whereas B-lymphocytes represent a minor population as compared with peripheral blood. In general, the composition of the lymphoid compartment in the lac-

tating mammary gland differs from that in peripheral blood, as demonstrated in the present and other studies (Park et al., 1992; Wirt et al., 1992; Van Kampen et al., 1999). Furthermore, the percentages of the different CMC subpopulations depend on differences in the techniques used to identify them (Prescott and Breed, 1910; Concha et al., 1980; Nonnecke and Kehrli, 1985), the gating strategy (Ohtsuka et al., 2010), the definition of colostrum (Archambault et al., 1988a), and the health status of the udder (Kiku et al., 2010).

Cell-mediated immunity was tested *in vitro* through mitogen- and antigen-induced proliferation of lymphocytes. Several research groups reported CMC to re-

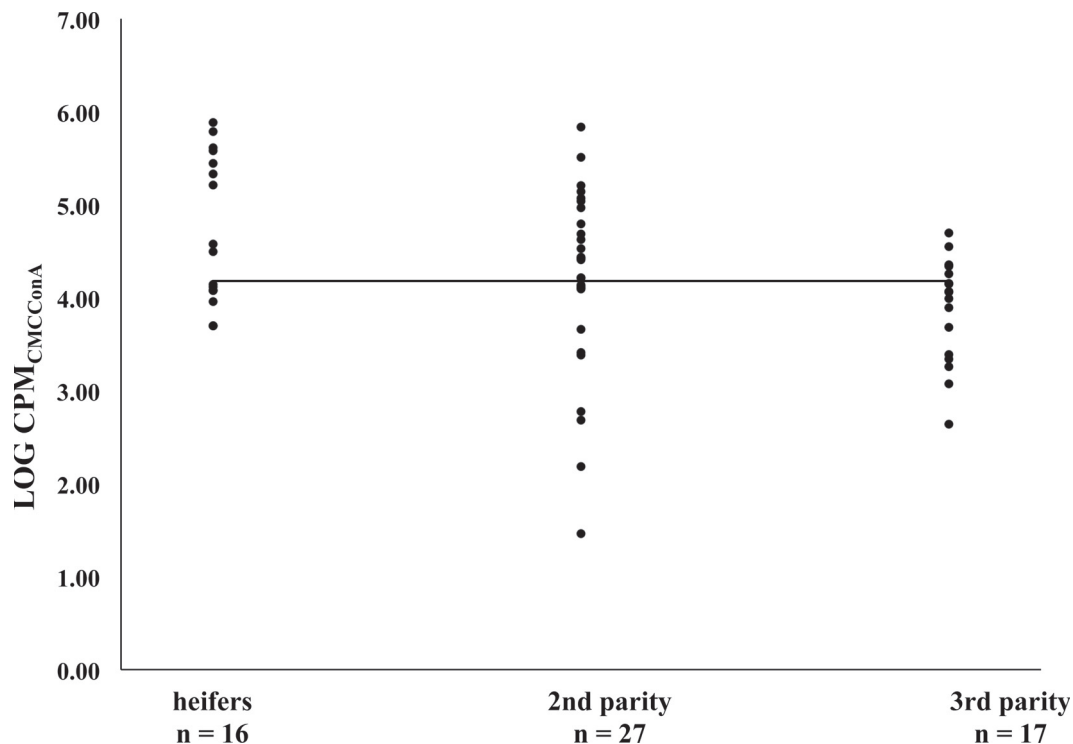


Figure 3. Heifers had significantly higher mitogen-induced proliferation of colostrum mononuclear cells (CPM_{CMCConA}) than second ($P = 0.049$) and third parity or older cows ($P = 0.006$). Results are represented as the raw logarithmic unmodeled data with the solid black line denoting the median value.

spond to mitogens and specific antigens (Archambault et al., 1988a,b). Background CPM are generally higher in MGL than in PBMC (Parmely et al., 1976; Concha et al., 1980; Nonnecke and Kehrli, 1985), as demonstrated in the current study. Specifically for CMC, this higher background CPM might be explained by the activating effects of colostrum on leukocytes (Sugisawa et al., 2001; Reber et al., 2006). Overall, MGL, both in cattle and in humans, seem not to react as strong as PBMC when confronted in vitro with mitogens and certain antigens (Parmely et al., 1976; Nonnecke and Harp, 1985; Park et al., 1993), as demonstrated in the present study for the mitogen ConA but not for the antigen TT. However, antigen-induced proliferation strongly depends on the antigen used (Smith and Goldman, 1968; Parmely et al., 1977; Archambault et al., 1988b). Various reasons can be proposed for the lower proliferation of MGL than PBMC: fewer CD4⁺ cells producing growth promoting cytokines (Shafer-Weaver et al., 1996), a higher percentage of CD8⁺ lymphocytes that have a suppressive effect (Park et al., 1993; Shafer-Weaver et al., 1996; Asai et al., 2000), changes in other cell types such as macrophages (Goddeeris et al., 1986; Nickerson, 1989), or memory cell phenotype (Bertotto

et al., 1990; Taylor et al., 1994). Testing various antigens is needed to study the possibility of vaccinating dams to protect the newborn via adoptive cellular immunity.

CONCLUSIONS

Increasing evidence demonstrates the relevance of viable lymphocytes in bovine colostrum. Most studies have focused on CL in terms of mastitis; however, the possible important role of CL as part of the adoptive immunity transfer to newborn calves should not be overlooked. Body condition score of periparturient cows had a significant effect on the number of macrophages and B-lymphocytes resident in colostrum. Furthermore, heifers had a significantly higher mitogen- and antigen-induced proliferation of CL compared with cows of third or higher parity. Also, serum BHBA concentration was significantly associated with higher numbers of colostrum T-lymphocytes and macrophages. Further studies are warranted to deepen the knowledge concerning CL and to research possible adverse effects of negative energy balance in periparturient dairy cows on the adoptive immunity transfer to their neonatal calves.

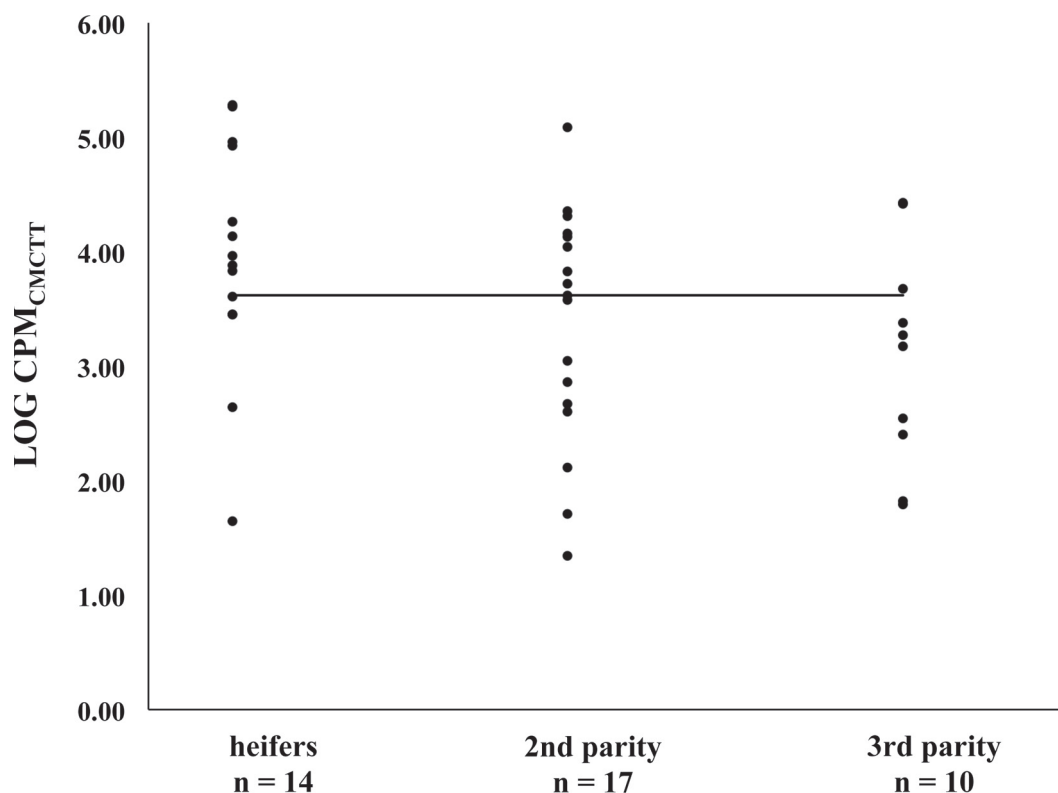


Figure 4. Heifers had significantly higher tetanus toxoid-induced proliferation of colostrum mononuclear cells (CPM_{CMCCTT}) than third parity or older cows (*P* = 0.045). Results are represented as the raw logarithmic unmodeled data with the solid black line denoting the median value.

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