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Lymphocyte Functions in Overconditioned Cows Around Parturition*

N. Lacetera, D. Scalia, U. Bernabucci, B. Ronchi, D. Pirazzi, and A. Nardone
Dipartimento di Produzioni Animali, Università della Tuscia, 01100 Viterbo, Italy

ABSTRACT

The objective of the study was to evaluate the relationships between body condition and lymphocyte functions in periparturient dairy cows. Thirty days before expected calving, 21 Holstein cows were categorized as thin (n = 6), medium (n = 8), or overconditioned (n = 7) based on body condition score (BCS). Blood samples were collected on 21, 14, 7, and 3 d before calving and on d 3, 7, 14, 21, 28, and 35 after parturition. An aliquot of blood was used to determine plasma nonesterified fatty acids (NEFA) and glucose. At 14 and 7 d before, and 14 and 35 d after calving, a second aliquot of blood was used to assess peripheral blood mononuclear cell (PBMC) functions: DNA synthesis, immunoglobulin (Ig) M, and interferon-gamma (IFN-γ) secretion after mitogen stimulation. During the experiment, all 21 cows showed a decline in BCS. Overconditioned cows lost significantly more BCS than thin cows. After calving, overconditioned cows had higher plasma NEFA compared with thin and medium cows. Conversely, plasma glucose never differed between the 3 categories of cows. Regardless of BCS, DNA synthesis and IgM secretions were significantly lower in PBMC isolated on 7 d before calving compared with those recorded 14 and 35 d after parturition. Conversely, PBMC from the 21 cows did not show any change of IFN-γ secretion during the experimental period. Taking into consideration the BCS categories, PBMC isolated from overconditioned cows presented lower IgM secretion compared with thin cows on d 14 and 35 after calving. Furthermore, PBMC isolated from overconditioned cows secreted less IFN-γ compared with thin and medium cows on d 7 before calving. The DNA synthesis of PBMC stimulated with the 3 mitogens did not differ between the 3 categories of cows. In conclusion, immunodepression occurring in cows around calving would be particularly evident in overconditioned cows.
(**Key words:** periparturient cow, body condition score, lymphocyte function)

Abbreviation key: BrdU = 5-bromo-2′-deoxyuridine, **ECM** = enriched culture medium, **PBMC** = peripheral blood mononuclear cells, **PWM** = pokeweed mitogen.

INTRODUCTION

Several authors report that periparturient dairy cows are characterized by immunodepression (Mallard et al., 1998; Kehrli et al., 1999). Other authors specified that immunoresponsiveness decreases gradually in the prepartum period and reaches its minimum expression immediately before calving (Saad et al., 1989; Wagter et al., 1996). In dairy cows, the periparturient period is also characterized by profound endocrine and metabolic changes (Goff and Horst, 1997), which, according to some authors, may partially explain immunosuppression (Kaneene et al., 1997; Lacetera et al., 2004a).

With regard to lymphocyte functions, a series of studies (Targowski and Klucinski, 1983; Franklin et al., 1991; Suriyasathaporn et al., 1999) tested the hypothesis that hyperketonemia may in part explain the immunosuppression in the peripartum period. However, those studies provided conflicting results. Negative effects of ketones on functional activities of neutrophils have instead been reported in sheep and cows (Hoeben et al., 1997, 1999, 2000; Sartorelli et al., 1999, 2000; Suriyasathaporn et al., 1999). In particular, Hoeben et al. (2000) described negative relationships between neutrophil functions and plasma concentrations of BHBA and NEFA, and indicated plasma concentrations of these metabolites as possible diagnostic markers of impaired neutrophil function around calving. In a previous study carried out in ewes, we found negative relationships between cellular and humoral immunity and plasma concentrations of NEFA or BHBA (Lacetera et al., 2001). Furthermore, we found that addition of NEFA to culture medium was responsible for alteration of lymphocyte functions in sheep and cows (Lacetera et al., 2002, 2004a).

Previous studies carried out in other species demonstrated that obesity and weight loss are associated with dysfunction of the immune system (Samartín and Chandra, 2001). With regard to cows, no data have been found on relationships between obesity or overconditioning and lymphocyte functions, whereas overconditioning was associated with reduction of reproductive efficiency (Gil-

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Corresponding author: Nicola Lacetera; e-mail: nicgio@unitus.it.
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Table 1. Ingredients and nutrients composition of diets fed during the experimental period (DM basis).

Ingredients (%)	Far-off dry cows	Close-up dry cows	Lactation cows
Corn silage	14.4	20.0	28.0
Triticale-grass silage	2.9	4.0	5.6
Alfalfa hay	5.2	7.3	10.2
Rye-grass hay	51.8	33.0	6.1
Corn ground	6.2	8.6	12.2
Oat ground	7.2	10.0	14.1
Soybean meal	5.5	7.5	10.7
Cotton seed	3.4	4.8	6.5
Dry beet pulp	2.6	3.6	5.1
Buffer[1]	0.5	0.7	0.9
Vitamin-mineral premix[2]	0.3	0.5	0.6
Nutrient composition			
NE_L, Mcal/kg	1.29	1.41	1.62
Crude protein, %	12.70	13.70	15.60
NDF, %	43.10	40.00	36.40

[1]A mixture of 33.3% $CaCO_3$, 31.7 $Ca_3(PO_4)_2$, 16.7% MgO, 16.6% $NaHCO_3$, and 1.7% $ZnSO_4$.

[2]Contained per kilogram: 6,000,000 IU of vitamin A; 600,000 IU of vitamin D_3; 7000 mg of vitamin E; 5000 mg of vitamin PP; 300 mg of vitamin B_1; 100 mg of vitamin B_2; 10,000 mg of choline chloride; 2 mg of vitamin B_{12}; 10,000 mg of Fe; 2,500 mg of Cu; 20,000 mg of Mn; 100 mg of Mo; 100 mg of Co; 800 mg of I; 50,000 mg of Zn; and 100 mg of Se.

lund et al., 2001) or alteration of liver functions (Drackley et al., 2001).

The present ex vivo study was carried out to evaluate the relationships between body condition and lymphocyte functions in periparturient dairy cows.

MATERIALS AND METHODS

Animals

The study was carried out on 21 multiparous healthy Holstein cows in a commercial herd, which were selected according to BCS on d 30 before calving. Body condition score was established according to ADAS (1986) using a 5-point scale. Six cows were categorized as thin (BCS < 2.5), 8 as medium ($2.6 <$ BCS < 3.5), and 7 as overconditioned (BCS ≥ 3.5). The 3 groups were homogeneous for parity, and examination of the herd books indicated that none of the selected cows had suffered from reproductive or health problems previously. The BCS was then established weekly until the 35th day after parturition. Cows were fed diets (for dry and lactating cows, Table 1) consisting of a base ration fed as a TMR given daily at 0930 h and offered ad libitum to achieve 5 to 10% refusals. The close-up diet was offered starting 10 d before the expected calving.

Measurements, Samplings, and Laboratory Analyses

Feeds were sampled and analyzed. Dry matter was determined by forced-air oven drying at 65°C to constant weight. Crude protein was determined by macro-Kjeldahl method (AOAC, 1984). Ether extract and ash were determined according to AOAC methods (AOAC, 1984). Neutral detergent fiber was analyzed according to the method described by Goering and Van Soest (1970).

Blood samples were collected on 21, 14, 7, and 3 d prepartum (before expected calving), and on d 3, 7, 14, 21, 28, and 35 after parturition via jugular venipuncture, using evacuated glass tubes coated with sodium heparin. An aliquot of blood was used to determine NEFA (NEFA-C kit; Wako Fine Chemical Industries USA, Inc., Dallas, TX) and glucose (Instrumentation Laboratory, Lexington, MA). Fourteen and 7 d before, and 14 and 35 d after calving, a second aliquot of blood was used to assess DNA synthesis, IgM, and IFN-γ secretion in peripheral blood mononuclear cells (**PBMC**) stimulated with mitogens.

The DNA synthesis was evaluated as already described (Lacetera et al., 2001). Peripheral blood mononuclear cells were isolated by density gradient centrifugation. Blood diluted in RPMI-1640 medium containing 25 m*M* HEPES (Sigma, Milano, Italy) was layered over Ficoll-Paque PLUS (APB, Milano, Italy) and centrifuged ($600 \times g$ for 45 min at 20°C). The mononuclear cell band was recovered and washed twice in PBS using centrifugation ($400 \times g$ for 10 min at 4°C). Residual red blood cells were eliminated by hypotonic shock treatment using redistilled water. The PBMC recovery and viability were determined by hemocytometer count using the trypan blue exclusion method. Viability of PBMC typically exceeded 90%. After isolation, PBMC were resuspended at a concentration of 1×10^6 cells/mL of RPMI-1640 enriched culture medium (**ECM**). The ECM comprised RPMI-1640 containing 25 m*M* HEPES, 10% heat-inactivated fetal bovine serum, 2 m*M* L-glutamine, 100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B/mL (Sigma). Triplicate cultures were assayed using 96-well tissue culture plates. Each well contained 1×10^5 mononuclear cells in 100 μL of ECM. Control wells contained 100 μL of PBMC suspension without mitogens. Additional control wells were used that contained 100 μL of ECM without cells or 100 μL of PBMC suspension without the pyrimidine analog 5-bromo-2′-deoxyuridine (**BrdU**). An optimal concentration of phytohemagglutinin (2.5 μg/mL), pokeweed mitogen (**PWM**, 1 μg/mL), or concanavalin A (2.5 μg/mL) (Sigma) was added to plates. Plates were incubated in an atmosphere of 95% air and 5% CO_2 for 48 h at 39°C. Afterwards, 100 μM BrdU in 10 μL of RPMI-1640 was added to each well, and plates were incubated for an additional 18 h. The DNA synthesis was quantified by an ELISA assay (Lacetera et al., 2002). The assay was performed with a commercial kit (APB), and was based on measurement of BrdU incorporated during DNA synthesis in prolifer-

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