Prepartum feeding level and body condition score affect immunological performance in grazing dairy cows during the transition period

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ABSTRACT

Precalving feeding level affects dry matter intake, postcalving energy balance, the risk of hepatic lipodosis and metabolic disease, and gene expression in liver and adipose tissue. These coincide with a higher risk of disease postpartum and, very likely, a failure to reach optimum production as well as reproductive targets. Current interpretation of the available evidence suggest that metabolic stressors affect the immune system of transition dairy cows and lead to reduced immunocompetence. The objective of the current study was to investigate the effect of precalving body condition score (BCS) and level of feeding on immunocompetence during the peripartum period. Twenty-three weeks before calving, 78 cows were allocated randomly to 1 of 6 treatment groups ($n = 13$) in a 2×3 factorial arrangement: 2 precalving BCS categories (4.0 and 5.0, based on a 10-point scale) and 3 levels of energy intake during the 3 wk preceding calving (75, 100, and 125% of estimated requirements). Blood was sampled precalving and at 1, 2 and 4 wk after calving. Cells were analyzed by flow cytometry and quantitative real-time PCR. The numbers of T helper lymphocytes (CD4+), cytotoxic T lymphocytes (CD8+), natural killer cells (CD335+), and $\gamma\delta$ T lymphocytes (WC1+) as well as their activation status [IL-2 receptor (CD25)+ cells] were highly variable between animals, but there was no evident effect of BCS, feeding level, or time. All groups presented with an increase in expression of cytokines in unstimulated blood cells in the week after calving, although this was significant only for *IFNG* in the BCS 4.0 group. Analysis of in vitro-stimulated cells allowed 2 general observations: (1) cows with high energy intake precalving (125%) had increased cytokine expres-

sion precalving, and (2) all cows had increased cytokine expression in the week after calving. The present study provides evidence that prepartum feed management can affect immunocompetence during the transition period. Considering the current results, optimally conditioned animals might benefit from a restricted precalving diet, whereas underconditioned cows can be fed to requirements.

Key words: immunity, immunocompetence, peripartum period, transition cow

INTRODUCTION

For dairy cows, transitioning from late pregnancy to early lactation poses a significant metabolic challenge (Bell, 1995; Drackley et al., 2005). These metabolic adaptations are likely the reason for immunologic changes (Goff and Horst, 1997; Ingvarsen and Moyes, 2013; Heiser et al., 2015) that contribute to an estimated 30 to 50% of dairy cows experiencing metabolic or infectious disease in the period immediately postcalving (LeBlanc, 2010). Neutrophils and their role in innate immune defense against mastitis have been a focus of previous research (Gilbert et al., 1993; Madsen et al., 2002; Hammon et al., 2006; Graugnard et al., 2012), whereas others have analyzed lymphocyte function, primarily measuring mitogen-induced lymphocyte proliferation (Kehrli et al., 1989; Lessard et al., 2004; Lacetera et al., 2005; Karcher et al., 2008; Schwarm et al., 2013; Heiser et al., 2015; Nightingale et al., 2015). From these studies, it can be concluded that immune function is modulated during the peripartum period, which likely affects susceptibility to infectious diseases after calving. However, there is a need for further analysis of the mechanisms involved and predictive biomarkers to allow identification of susceptible animals in time for preventive intervention.

To provide a broader analysis of immune function in transition cows in this study, we used flow cytometry

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and a combination of in vitro stimulation and fluorescence-based quantitative real-time PCR (qPCR) to analyze the cellular composition of peripheral blood mononuclear cell (PBMC) subsets from pasture-based dairy cows as well as the immunological performance of these cells. Immunological performance was investigated by analyzing expression of mRNA encoding for selected cytokines after in vitro stimulation. Interferon- γ (*IFNG*) is critical for innate and adaptive immune responses against viral and intracellular bacterial infections and is also a key activator of macrophages (Schroder et al., 2004). Tumor necrosis factor (*TNF*) regulates immune cells, with variable effects. Dysregulation of *IFNG* and *TNF* is associated with several diseases (Locksley et al., 2001). Interleukin-17 (*IL17*) acts similarly to *IFNG* and synergistically with *TNF*. It is produced by T lymphocytes and supports migration of monocytes and neutrophils to the site of inflammation (Jin and Dong, 2013). In contrast to these proinflammatory cytokines, IL-10 (*IL10*) is considered an antiinflammatory cytokine (Couper et al., 2008).

Despite a significant number of studies investigating the metabolic and molecular changes associated with dietary energy intake before calving (Schmitt et al., 2011; Graugnard et al., 2012; Ji et al., 2014; Akbar et al., 2015), the underlying mechanisms are still unclear. Roche et al. (2013) reported significant effects of calving BCS on cow metabolic profiles, supporting the hypothesis that the effect of precalving level of feeding is dependent on precalving BCS (Roche et al., 2015). The objective of the current study was to investigate the effect of precalving BCS and level of feeding on immunocompetence during the peripartum period with the long-term goal of developing feeding recommendations for improved immune function.

MATERIALS AND METHODS

Animals were sourced from an experiment undertaken at Scott Farm (Hamilton, New Zealand; 37°46'S 175°18'E) between January and October 2013 that was reported by Roche et al. (2015). After passing a clinical examination, 150 cows were enrolled in the experiment on January 21, 2013. Cows were allocated randomly to 1 of 6 treatment groups (13 cows per group) in a 2 $\times$ 3 factorial arrangement: 2 precalving BCS categories (4.0 and 5.0; based on a 10-point scale, where 1 is emaciated and 10 obese, **B4** and **B5**, respectively; Roche et al., 2004) and 3 levels of energy intake during the 3 wk preceding calving (75, 100, and 125% of estimated requirements, **F75**, **F100**, and **F125**, respectively; Roche et al., 2005). Although cow allocation to treatment was random, groups were assessed to ensure they represented typical New Zealand dairy cows from

a management and genetic perspective and were balanced for age, breed, BCS at the time of enrollment, and expected calving date. Age at enrollment was 4.0 $\pm$ 1.4 yr (mean $\pm$ SD). To generate the 2 BCS treatment groups before the end of lactation, feeding levels were manipulated starting on February 1 (205 DIM). Therefore, 3 groups of cows were assigned to gain, maintain, or lose BCS. All animals grazed an allowance of fresh pasture and were supplemented with pasture silage, corn silage, and palm kernel expeller. From 23 $\pm$ 5.6 d precalving, cows were offered fresh pasture at 75, 100, or 125% of their estimated ME requirements (Roche et al., 2005). Further details of the approach to establish treatment groups are described elsewhere (Roche et al., 2015). The mean calving date was July 11 $\pm$ 10.2 d. All animal manipulations were in accordance with the New Zealand Animal Welfare Act (1999) and had approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

Blood Samples

Blood was sampled on multiple days via coccygeal venipuncture at approximately 0800 h. To allow analysis of immunocompetence for a period starting before calving and covering an extended time after calving (4 wk) while maintaining feasibility and considering animal welfare, samples were taken at 4 time points. One sample each per time point (and 13 animals per treatment group) was taken at 1 to 17 d before calving (**Pre**), 3 to 11 d (wk +1), 12 to 18 d (wk +2), and 25 to 35 d (wk +4) after calving. Blood was collected into evacuated blood tubes containing lithium heparin as an anticoagulant (Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ). Following a procedure that showed good results in a previous study (Heiser et al., 2015), samples were shipped over night at ambient temperature to the Hopkirk Research Institute (Palmerston North, New Zealand) for further processing.

Phenotypic Analysis by Flow Cytometry

Peripheral blood leukocyte populations were analyzed using flow cytometry. The following antibodies were used: natural killer (**NK**) cells were identified using bovine CD335 (A488 conjugated, clone AKS1; Storset et al., 2004); $\gamma\delta$ -T lymphocytes were identified using anti-WC1 [fluorescein isothiocyanate (**FITC**)-conjugated, clone CC15; Clevers et al., 1990]; T helper cells were identified using bovine CD4 (AlexaFluor 647-conjugated, clone CC8; Bensaid and Hadam, 1991); cytotoxic T lymphocytes (**CTL**) were identified using bovine CD8 (FITC-conjugated, clone CC63; MacHugh and Sopp, 1991); cells expressing the IL-2 receptor (CD25) were

identified using bovine CD25 (phycoerythrin-conjugated, clone ILA111; Naessens et al., 1992). All antibodies were from AbD Serotec/Bio-Rad (Hercules, CA).

For analysis, 5 μ L each of various antibodies were transferred into FACS (fluorescence-activated cell sorter) tubes, and 200 μ L of whole blood was added and incubated for 20 min at room temperature in the dark. Lysis of red blood cells was performed by adding 2 mL of distilled water for no more than 2 min. Subsequently, 200 μ L of 10 $\times$ PBS was added while vortexing the tubes. Samples were then centrifuged and washed twice with PBS, both at 300 $\times g$ for 7 min at room temperature. Finally, cell pellets were resuspended in 200 μ L of PBS and analyzed using a FACSCalibur cell sorter (Becton Dickinson, Franklin Lakes, NJ). A gate for leukocytes was drawn containing lymphocytes and monocytes based on their forward and sideward scatter characteristics. For each cell type, the percentage of cells within this gate expressing the surface marker was determined. Activated cells were defined as the percentage CD25-positive cells for each of the cell types.

In Vitro Stimulation of Blood Samples

For in vitro stimulation, 2 mL of whole blood was transferred to a 50-mL tube. Stimulation was performed by adding 20 μ L of Staphylococcal enterotoxin B (**SEB**) and LPS (both from Sigma-Aldrich, St. Louis, MO) to a final concentration of 500 ng/mL each. For each blood sample, another 2 mL of whole blood was used without stimulation. Tubes were incubated for 22 h at 37°C in 5% CO₂. Warmed (37°C) Tris-buffered ammonium chloride lysis buffer was added to lyse red blood cells and samples were incubated for 10 min at 37°C. After centrifugation, 2 washes with PBS (300 $\times g$, 7 min, room temperature) were performed, cell pellets were resuspended in TRIzol (Invitrogen, Carlsbad, CA) and frozen at -80°C.

The Bovigam ELISA test kit for bovine *IFNG* (Prionics, Schlieren, Switzerland) was used to determine the amount of *IFNG* released into cell supernatants. The amount of *IFNG* was calculated against a standard curve prepared with recombinant *IFNG*.

RNA Preparation

Samples were retrieved from -80°C storage and quickly thawed for subsequent preparation of RNA. The phases were separated by adding 200 μ L of chloroform and vigorously shaking by hand for 15 s, followed by incubation for 2 to 3 min at room temperature. Samples were centrifuged at 12,000 $\times g$ for 15 min at 4°C. The top aqueous layer was transferred to a fresh microcentrifuge tube, and 5 μ g of RNase-free glycogen

(Invitrogen) added. After precipitation of RNA in the aqueous phase by addition of 500 μ L of 100% isopropanol and incubation at room temperature for 20 min, samples were centrifuged at 12,000 $\times g$ for 15 min at 4°C. Isopropanol was removed by decanting and briefly draining liquid from the inverted tube. The pellet was washed by adding 1 mL of 75% ethanol [ethanol in diethylpyrocarbonate (**DEPC**)-treated water] and centrifugation at 8,000 $\times g$ for 5 min at 4°C. The supernatant was removed and the RNA pellets air-dried for 5 min. Twenty microliters of DEPC-treated water was used to dissolve the RNA, and a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to determine RNA concentration and ratio of absorbance at 260 and 280 nm (A260/A280). The RNA samples were adjusted to 0.2 to 1.0 μ g/ μ L by adding DEPC-treated water and stored at -80°C.

DNase Treatment and DNA Preparation

Residual genomic DNA was removed by combining 1 μ g of RNA sample with 1 μ L of 10 $\times$ DNase I buffer, 1 μ L of DNase I (both Invitrogen), and DEPC-treated water to a total volume of 10 μ L. Samples were centrifuged at 10,000 $\times g$ for 15 min at 4°C and incubated at room temperature for 15 min. Inactivation of DNase was achieved by adding 1 μ L of 25 mM EDTA, centrifuging at 10,000 $\times g$ for 15 min at 4°C, and incubating at 65°C for 10 min.

For cDNA preparation, 10 μ L of DNase I-treated RNA was combined with 1 μ L of Oligo (dT)20 primer (at 500 μ g/mL = 0.5 μ g; Invitrogen) and 2 μ L of DEPC-treated water and incubated at 65°C for 10 min. Samples were quickly cooled and centrifuged briefly. Seven microliters of reverse transcriptase Master Mix (Roche, Basel, Switzerland) was added to each reaction, mixed gently, and centrifuged briefly. Samples were incubated at 55°C for 30 min followed by prompt inactivation of the enzymes at 80°C for 5 min. The DNA samples were stored at -20°C.

Fluorescence-Based Quantitative Real-Time PCR

For quantitative real-time (q)PCR analysis, 10 μ L of SyBr Premix Ex *Taq*II master mixture (Takara/Clontech, Mountain View, CA), 1 μ L of template cDNA, 1 μ L of each gene-specific primer (5 mM), and 7 μ L of sterile Milli-Q water (Millipore, Billerica, MA) were combined in a 20- μ L reaction mixture in duplicate. Primer sequences for *U1*, *IFNG*, *TNF*, *IL17*, and *IL10* were published previously (Heiser et al., 2015). A Rotor-Gene 6000 real-time PCR system (Corbett Life Science, Mortlake, NSW, Australia) was used and the cycle number at which the various transcripts be-

come detectable, referred to as threshold cycle (**Ct**), with threshold 0.05. The first 5 cycles were eliminated using Rotor-Gene 6000 series software 7.0 (Corbett Life Science). The threshold for detection was set to 32 Ct; anything above that was defined as “not expressed.” The mean Ct value of duplicate reactions was used for calculations. For analysis of unstimulated cells, expression levels were calculated as ΔCT ; that is, Ct of gene of interest minus Ct of reference gene (*U1*). For analysis of in vitro-stimulated cells, $\Delta\Delta\text{CT}$ was calculated according to ΔCt of stimulated blood minus ΔCt of unstimulated blood.

Statistical Analysis of qPCR

A repeated-measures model with a variance-covariance structure of autoregressive order 1 within each cow was used to analyze the data, considering BCS (B4 and B5), feeding management (F75, F100 and F125), and time (Pre, wk +1, wk +2 and wk +4) as well as the interaction of BCS and feeding level as fixed effects. A transformation (natural log) was applied to match the normality assumption of the modeling. Normalized, log-2 transformed data were subjected to “nlme” and “predictmeans” in R 3.1.1 (R Core Team, 2015); *P*-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Cellular Composition of Blood from Periparturient Cows

Banos et al. (2013) reported several significant correlations between immune traits and other recorded traits, including incidence of lameness and percentage CD335-positive NK cells, subclinical mastitis and CD4:CD8 ratio, as well as fertility and percentage of CD8-positive CTL. To detect changes in these cell populations during the peripartum period, whole-blood samples from study animals were analyzed for expression of cell type-specific markers using flow cytometric analysis. The numbers of T helper lymphocytes (CD4+), cytotoxic T lymphocytes (CD8+), NK cells (CD335+), and $\gamma\delta$ T lymphocytes (WC1+), as well as their activation status [IL-2 receptor (CD25)+], were highly variable between animals, which was expected because dairy cows are an outbred population. No effects of BCS, feeding level, or time (Figure 1) were evident. Across all time points and treatment groups, $3.9 \pm 1.9\%$ of the cells were CD335+ NK cells, of which $4.8 \pm 2.8\%$ were activated; WC1+ $\gamma\delta$ -T cells comprised $8.1 \pm 2.4\%$ of the cells and $6.9 \pm 3.2\%$ of them were activated. In total, $12 \pm 3.9\%$ of the cells were CD4+ T helper cells, of which $25 \pm 14\%$ were activated; CD8+ CTL contributed $8.2 \pm$

3.6% of the cells and $19 \pm 10\%$ of them were activated. The CD4:CD8 ratio was 2.1 ± 1.7 , suggesting a normal distribution of these cell types.

The size of the cellular subpopulations was similar to that in previous reports (Boysen and Storset, 2009; Hoek et al., 2009; Mašlanka et al., 2012; Banos et al., 2013). An increase in NK cell number from pre- to postcalving, as described in a previous study (Heiser et al., 2015), was not evident in this study. These results suggest that BCS and feeding level do not influence the size of lymphocyte subpopulations during the peripartum period. These cell types also do not generally change during this time. However, individual animals can experience changes in lymphocyte subpopulations that subsequently may influence susceptibility to infectious diseases. Investigation of such potential correlations between cell populations, cytokine responses, metabolic parameters, and animal health, productivity, and reproduction is subject to current and ongoing analysis.

Cytokine Gene Expression and Secretion in Immunocompetence Assay

Posttranscriptional mechanisms contribute to the control of protein expression in immune effector cells (Ivanov and Anderson, 2013). To ensure the relevance of measuring mRNA levels, expression of *IFNG* and *IL17* in the immunocompetence assay was compared at the mRNA and protein levels (Figure 2). Across all data pairs, combining treatments and time points, the in vitro-stimulated samples secreted $1,200 \pm 2,300$ pg/mL of IFNG and $1,400 \pm 3,100$ pg/mL of IL17, as measured by ELISA ($n = 245$; mean $\pm$ SD). These results correlated well (Figure 2) with the Ct values obtained from qPCR from the same samples, which were 5.6 ± 2.3 for *IFNG* ($R^2 = 0.87$) and 5.6 ± 2.7 for *IL17* ($R^2 = 0.87$). These results confirm the relevance of qPCR for the measurement of cytokine release from bovine cells.

Cytokine Expression in Bovine Blood Leukocytes from Periparturient Cows

In immunocompetence studies, cytokine levels are usually measured from stimulated lymphocytes and results are normalized against unstimulated cells. Here, cytokine expression in unstimulated cells was analyzed to investigate potential effects of BCS, feeding level, and time on the baseline expression of cytokines. Expression of the cytokines *IFNG*, *TNF*, *IL17*, and *IL10* was variable between individual animals, with an average coefficient of variation of 26% across all time points and treatments. Almost all groups presented with an increase in expression of all 4 cytokines in the week

Figure 1. Cellular composition of bovine blood during the peripartum period. Blood was sampled from 60 dairy cows precalving (Pre) and 1, 2 and 4 wk after calving (wk +1, +2, +4) and analyzed using flow cytometry for the relative number of cells expressing CD335 [natural killer (NK) cells], WC1 ($\gamma\delta$ T cells), CD4 [T helper (Th) cells], and CD8 (cytotoxic T cells, CTL); top]. CD25 (IL-2 receptor) was detected on subpopulations of these cell types (bottom) and can be interpreted as being an activation marker (mean $\pm$ SD).

after calving (wk +1; Figure 3), although this was significant only for *IFNG* in the BCS4 groups ($P < 0.05$). A clear exception was group B5F125, which had stable levels of *IFNG* from Pre to wk +2 and a decrease at wk +4 ($P < 0.05$). Expression of *IL10* was lower ($P < 0.05$)

in BCS5 groups compared with BCS4 groups; *IL10* is an antiinflammatory cytokine (Couper et al., 2008) that prevents immune system-mediated damage to the host by limiting the immune response (Banchereau et al., 2012). An earlier study in transition cows that

Figure 2. Correlation of cytokine gene expression and cytokine secretion. The graphs represent gene expression, as measured by quantitative real-time PCR, for *IFNG* (top) and *IL17* (bottom) in correlation with cytokine secretion for IFN- γ and IL-17 measured by ELISA in the same cultures ($n = 245$). Ct = threshold cycle.

were managed differently identified no significant time effects (Heiser et al., 2015) in the expression of these cytokines. The higher baseline expression of these cytokines in the week after calving supports the observation that cows experience a degree of inflammation around parturition (Bertoni et al., 2008; Trevisi et al., 2012) even at a systemic level. Dairy cows going into transition with a BCS5 were less prone to such a systemic inflammatory state.

Cytokine Expression in In Vitro-Stimulated Bovine Blood Leukocytes from Periparturient Cows

To gain a better understanding of actual leukocyte function, blood cells from dairy cows from all BCS and feeding level groups and from 4 time points were stimulated in vitro with SEB/LPS (as per Heiser et al., 2015),

and mRNA expression for *IFNG*, *TNF*, *IL17*, and *IL10* was analyzed by qPCR and compared with expression in unstimulated cells. Some samples presented with very low or absent cytokine expression in unstimulated samples, which resulted in high fold-changes when relative expression was calculated. Because the biological relevance of these values is questionable, cytokine RNA expression is presented as $\Delta\Delta Ct$ (Figure 4).

During the weeks immediately precalving, *IFNG* was affected by feeding management and time (see Figure 4), but we found no effect of precalving BCS. Feeding management affected *IFNG*, with levels higher in B4F125 compared with B4F75 ($P < 0.05$) and B4F100 ($P < 0.01$), as well as B5F125 compared with B5F75 and B5F100 ($P < 0.05$). Expression of *IFNG* was also affected by time. Compared with Pre, all groups except for B5F125 presented with a significant increase at wk +1 ($P < 0.01$ for B4F75 and B4F100; $P < 0.05$ for B4F125, B4F75, and B5F100). Levels of *TNF* had less pronounced effects (see Figure 4). An effect of precalving BCS was not detected. Feeding management affected *TNF* only in the BCS4 group, with levels being higher in B4F125 compared with B4F75 ($P < 0.05$). Time affected *TNF*, with all groups except for B4F75 and B4F125 showing a significant increase at wk +1 ($P < 0.05$). The *IL17* expression pattern over time was similar to that of *IFNG* (see Figure 4), with effects of feeding management and time but not BCS. Levels were higher in B4F125 compared with B4F75 and B4F100 ($P < 0.01$) as well as in B5F125 compared with B5F75 and B5F100 ($P < 0.01$). Expression of *IL17* was also affected by time, with all groups increasing at wk +1 ($P < 0.01$) relative to the Pre sample and returning to precalving levels in wk +2 and wk +4. We also noted that *IL17* expression was lower in B4F75 compared with B4F100 after calving and compared with B4F125 at all times ($P < 0.01$). Levels of *IL10* showed similar expression patterns to *IFNG* but were less prominent. An effect for BCS was detected, with *IL10* expression lower in B5F125 than in BCS4F125 ($P < 0.01$). Feeding management also affected *IL10*, with levels higher in B4F125 ($P < 0.01$) compared with B4F75 and B4F100. Moreover, *IL10* was affected by time. All groups, except for B4F125, were higher at wk +1 ($P < 0.01$ for B4F100, $P < 0.05$ for B4F75, all BCS5 groups).

These results allow 2 general observations: (1) cows with high energy intake precalving (125%) had higher cytokine expression precalving, and (2) all cows had higher cytokine expression in the week after calving. The latter observation confirms what was found in previous studies. A weak association between parturition and higher *IFNG* production by stimulated cells was reported and linked to a decrease in DNA methylation

Figure 3. Cytokine expression in bovine blood leukocytes from periparturient cows. The graphs represent gene expression, as measured by quantitative real-time PCR, for IFN- γ , tumor necrosis factor (TNF), IL-17, and IL-10 in unstimulated blood cells from cows of B4 (BCS = 4.0; left) and B5 (BCS = 5.0; right). Each graph represents results from groups receiving 75, 100, and 125% of estimated requirements (F75, F100, and F125). Each line represents results from 8 to 13 animals shown as mean Δ Ct $\pm$ SE, where Ct = threshold cycle.

Figure 4. Cytokine expression in stimulated bovine blood leukocytes from periparturient cows. The graphs represent gene expression as measured by quantitative real-time PCR for IFN- γ , tumor necrosis factor (TNF), IL-17, and IL-10 in *Staphylococcal* enterotoxin B/LPS-stimulated blood cells from cows of B4 (BCS = 4.0; left) and B5 (BCS = 5.0; right). Each graph represents results from groups receiving 75, 100, and 125% of estimated requirements (F75, F100, and F125). Each line represents results from 8 to 13 animals shown as mean $\Delta\Delta C_t \pm$ SE, where C_t = threshold cycle.

of the *IFNG* promoter region (Paibomesai et al., 2013). An increase in *TNF* secretion after stimulation of blood leukocytes with concanavalin A has been reported, but only when using autologous serum and not fetal bovine serum (Lessard et al., 2004). Heat stress has also been shown to affect *TNF* expression during the transition period (Tao et al., 2013). Increased expression of *IL10* can be interpreted as an attempt to control the ongoing inflammation (Banchereau et al., 2012). In a previous study using different feeding management, *IL10* also was significantly higher 1 wk after calving but *IFNG*, *TNF*, and *IL17* showed the opposite pattern (Heiser et al., 2015), underlining the importance to consider BCS and feeding level precalving. As the baseline expression, the higher stimulated expression of cytokines in the week after calving supports the understanding that transition cows develop a mild systemic inflammation (Bertoni et al., 2008; Trevisi et al., 2012).

Multiple studies have now reported an effect of nutritional state before calving on the level of cytokine expression after calving (Lessard et al., 2004; Lacetera et al., 2005; Paibomesai et al., 2013; Tao et al., 2013; Heiser et al., 2015). In this study, dairy cows going into transition with high energy intake precalving (125%) had higher stimulated cytokine expression even precalving, similar to previous findings (Zhou et al., 2015). There is growing evidence that, in other species, overfeeding causes hypertrophic expansion of adipocytes, which contributes to a heightened state of inflammation by recruiting immune cells and induces the release of proinflammatory and inflammatory cytokines, establishing an inflammatory state (Surmi and Hasty, 2008; Manteiga et al., 2013). Our results suggest that similar effects apply in cattle but others did not find a close correlation between energy overfeeding and adipose expression of inflammation-related genes (Ji et al., 2014).

For a better understanding of the underlying factors and the timing of these events, studies are needed with a tighter and more precise monitoring of the period from shortly before until 1 wk after calving.

CONCLUSIONS

The current study provides evidence that cytokine responses are, indeed, modulated during the week after calving. A recurring observation is that changes in diet and BCS might be the driving forces for immunomodulation in transition cows. Therefore, precalving feeding management becomes particularly important for the maintenance of immunocompetence after calving. Considering the current results, optimally conditioned cows might benefit from a restricted precalving diet, whereas underconditioned cows should be fed to requirements.

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