

Effects of feeding intensity during the dry period on leukocyte and lymphocyte sub-populations, neutrophil function and health in periparturient dairy cows

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Abstract

The objective of this investigation was to study (1) the numbers of leukocytes, (2) the proportions of lymphocytes expressing CD4, CD8, WC1, B or IL2R and (3) neutrophil phagocytosis and oxidative burst activity in blood around parturition in three groups of dairy cows fed different levels of a total mixed ration during the last eight weeks before calving. All cows were fed *ad libitum* during the first eight weeks of lactation. Serum concentration of the acute phase protein serum amyloid A (SAA), the milk somatic cell count (SCC) and disease incidence were also recorded. Special emphasis was given to the weeks just before and just after calving as dairy cows are known to be immune suppressed during this period.

Dry period diet had only minor effects on leukocyte numbers, and did not influence neutrophil phagocytosis and oxidative burst. In addition, no effect was observed on disease incidence or SAA concentrations. However, an increase in the proportion of B-lymphocytes and a decrease in the proportion of WC1+ T lymphocytes were observed after calving in cows fed high or low energy rations during the dry period, but not in cows fed a medium energy ration. The weeks just before and after parturition were characterised by neutrophilia, eosinopenia, lymphopenia and monocytosis, but time had no effect on neutrophil phagocytosis and oxidative burst. The proportions of CD4+, CD8+, B+ and IL-2R+ lymphocytes increased in early lactation relative to the mid dry period. In addition, the concentration of SAA increased dramatically at calving. The results emphasise the need for more studies to clarify the complex interactions between nutrition and immunity during the peripartum period in dairy cattle.

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1. Introduction

A well-functioning immune defence is essential for a host's resistance to infection. Dairy cows are more vulnerable to infectious diseases around calving due to immune suppression during this period (see, for example, reviews by Kehrl et al., 1998; Mallard et al., 1998). The reasons for the suppression are not fully known, but several factors such as management, feeding and changes in hormonal levels are involved.

Proper nutrition of the dairy cows during the dry period is a key factor for performance during lactation, and mismanagement of the late gestation diet predisposes the animals to a negative energy balance, and to both metabolic and infectious health disorders during early lactation (reviewed by Østergaard and Sørensen, 1998; Rukkwamsuk et al., 1999). Indeed, a negative energy balance in early lactation can lead to hepatic lipidosis and ketosis, suppression of the immune system by a negative influence on leukocyte function (Targowski and Klucinski, 1983; Ropstad et al., 1989; Hoeben et al., 1997; Wentink et al., 1997; Suriyasathaporn et al., 1999) and a greater susceptibility to infectious diseases, such as mastitis, during this period.

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Blood leukocyte numbers and their functions change, sometimes dramatically, around parturition, resulting in suppression of the immune response from a few weeks before to a few weeks after calving (see Kehrli et al., 1998; Mallard et al., 1998). For example, neutrophil trafficking is impaired at calving, as is neutrophil phagocytosis and killing (see, for example, Kehrli et al., 1989a; Lee and Kehrli, 1998). In addition, lymphocyte numbers decrease around parturition mainly due to reduced lymphocyte proliferation (see, for example Kehrli et al., 1989b; Saad et al., 1989). Although suppression of leukocyte functions in dairy cows has been associated with negative energy balance around calving and in early lactation, few, if any, studies have investigated the numbers and proportions of different leukocyte subpopulations, and how their functions are influenced by over- and/or underfeeding during the dry period. Variations in cell populations, especially lymphocytes, and their release of important cytokines, can influence the immune response and the susceptibility to disease.

The aim of the present study was to examine the following parameters in blood collected from dairy cows during the late dry period and in early lactation: (1) the numbers of neutrophils, eosinophils, lymphocytes and monocytes, (2) the proportions of CD4+, CD8+ and WC1+ T-cells, B-cells and IL2R+ lymphocytes (indicating the degree of cell activation) and (3) neutrophil phagocytosis and oxidative burst activity.

Three groups of cows were fed different amounts of energy during the last eight weeks before calving and ad libitum during the first eight weeks of lactation. Special emphasis was given to the weeks just before and just after calving as dairy cows are known to be immune suppressed during this period. Moreover, serum concentrations of the acute phase protein serum amyloid A (SAA), as an indicator of inflammatory reactions and as a help to identify subclinical disease, was also measured, and the disease incidence during the study period recorded. The composite milk somatic cell count (SCC) was also measured after calving.

The overall experiment had been designed to investigate the effects of feeding intensity during the period around calving on feed intake, body weight, milk production, metabolic and hormonal responses during the dry period and early lactation. These results as well as a detailed description of the study design are given in Agenäs et al. (2003) and Holtenius et al. (2003).

2. Materials and methods

2.1. Animals, management and experimental design

Twenty-three high-producing, clinically healthy Swedish Red and White dairy cows were used. They were multiparous and belonged to selection lines of

high, or low milk fat percentage at the same amount of energy produced in milk.

Before the experiment, the cows were grouped according to lactation number and selection line for milk fat. Within each group, cows were randomly allocated to one of three dietary treatments: low (L), medium (M) and high (H) energy rations during the dry period. The groups contained eight, eight and seven animals, respectively. All animals were dried off approximately 10 weeks before the predicted date of parturition and introduced to the experimental diets eight weeks before parturition was expected.

The groups received 6 (L), 9 (M) and 14.5 (H) kg dry matter (DM) of a dry period total mixed ration (TMR) mix providing 71, 106 and 177 MJ metabolisable energy (ME) per day, respectively. The diets provided on average 75%, 110% and 178% of the energy requirements for maintenance and pregnancy according to the Swedish feeding recommendations (Spörndly, 1999). At calving, all cows received a bolus containing 110 g calcium chloride and 44 g calcium sulphate per os to avoid hypocalcaemia in overconditioned cows. From parturition until the end of the experimental period, all cows were fed another TMR mix ad libitum. The animals were housed in individual tie stalls at the university research farm and feed refusals were collected daily. A detailed description of the composition of the TMR feeds has been reported elsewhere (Agenäs et al., 2003).

The experimental design and all handling of the animals were approved by the Uppsala Local Ethics Committee.

2.2. Blood and milk sampling and analysis

2.2.1. Blood

From each cow, EDTA blood (Terumo Europe) for total and differential leukocyte counts was taken in the morning from the coccygeal vein, once weekly from eight weeks prior to the predicted parturition date until eight weeks postpartum. Total (WBC) and differential (neutrophils (NEU), lymphocytes (LYM), eosinophils (EOS) and monocytes (MON)) leukocyte counts were determined using a Cell-Dyn 3500 (Abbott Diagnostics, Abbott Laboratories). Additional jugular blood samples were collected in the morning at five time points, i.e. 4–5 weeks and 7–10 days before estimated calving, and 0–3 days, 7–10 days and 4–5 weeks after calving. Blood samples with EDTA were taken for immunostaining of lymphocyte subpopulations, and neutrophil phagocytosis and oxidative burst assay. Blood without additives (Terumo Europe) was taken for analyses of SAA. The tubes were centrifuged at 1500g for 35 min and serum was frozen at –20 °C until analysed using the Tridelta Phase SAA Assay (Tridelta Development Limited).

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