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Alterations in innate immunity reactants and carbohydrate and lipid metabolism precede occurrence of metritis in transition dairy cows

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ABSTRACT

The overall purpose of the present study was to search for early screening biomarkers of disease state. Therefore the objectives of this study were to evaluate metabolites related to carbohydrate metabolism, acute phase proteins, and proinflammatory cytokines in the blood of transition dairy cows starting at −8 weeks before calving. Blood samples were collected from 100 multiparous Holstein dairy cows during −8, −4, disease diagnosis, +4 and +8 weeks relative to parturition. Six healthy cows and 6 cows that showed clinical signs of metritis were selected for serum analysis. Overall the results showed that cows with metritis had greater concentration of lactate, interleukin-6 (IL-6), tumor necrosis factor (TNF), and serum amyloid A (SAA) versus healthy cows throughout the experiment. The disease was associated with decrease in milk production and fat: protein ratio. Cows with metritis showed alteration in metabolites related to carbohydrate metabolism, acute phase proteins, and proinflammatory cytokines starting at −8 weeks prior to parturition and appearance of clinical signs of the disease. This study suggests a possible use of cytokines as early markers of disease in dairy cows.

1. Introduction

Metritis is an important disease of transition dairy cows. Before parturition the uterine lumen is sterile and during parturition the physical barriers of the cervix, vagina, and vulva are compromised providing an opportunity for pathogenic bacteria to ascend the genital tract. Metritis is a uterine pathology that involves inflammation of all uterine layers and is associated with lower reproductive efficiency. It occurs during the first 21 days after parturition and is characterized by an enlarged uterus and watery red-brown fluid, which often have a fetid odor (Sheldon et al., 2006; Sheldon et al., 2009).

The ongoing inflammatory and immune response to uterine bacterial infection compromises animal welfare and is associated with infertility. During infection such as metritis immune cells recognize invading pathogens and become activated resulting in both local and systemic inflammation. Activated immune cells release inflammatory mediators such as cytokines tumor necrosis factor (TNF), interleukin 1 (IL-1), and interleukin 6 (IL-6) which play key roles in stimulating systemic inflammatory responses including increased body temperature and decreased feed intake (Dantzer and Kelley, 2007). Cytokines activate production of acute phase proteins including haptaglobin (Hp), serum amyloid A (SAA), and lipopolysaccharide binding protein (LBP). The importance of acute phase proteins in the response to metritis is somewhat unclear but they have gained acceptance as markers of inflammation. In fact several studies have reported alterations in blood metabolites, cytokines, and acute phase proteins in cows with metritis. For example, Hammon et al. (2006) reported that cows with metritis had significantly greater blood non esterified fatty acids (NEFA) and lower dry matter intake (DMI) beginning 2 weeks prior to parturition and greater blood β-hydroxybutyrate (BHBA) during weeks 1–4 after parturition, suggesting that cows experiencing negative energy balance prior to or around calving are predisposed to periparturient immune suppression.

Several investigators also have reported that innate immunity variables like increased concentrations of Hp and SAA are associated with metritis (Smith et al., 1998; Sheldon et al., 2001; Dubuc et al., 2010; Chan, 2010). In fact plasma Hp was elevated prior to clinical signs of metritis (Huzzey et al., 2009). In addition, serum cytokines like IL-1, IL-6, and TNF have been related to infection of the uterus in postparturient dairy cows (Sheldon and Dobson, 2004; Chapwanya et al., 2009).

Infertility is the main reason for culling cows in Canada where 15% or more than 54,000 cows are removed from dairy herds each year, leading to increasingly significant economic losses to the dairy industry (Ametaj et al., 2012). The financial losses incurred by metritis derive from infertility, increased culling for failure to conceive, and lowered milk production and the cost of treatment.

An increasing body of evidence suggests that perturbations of inflammatory and metabolic networks might begin prior to parturition

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and predispose cows to later metritis (Hammon et al., 2006; Huzzey et al., 2009; Dubuc et al., 2010; Ametaj et al., 2014). Therefore early diagnosis or detection of those alterations before appearance of the disease may help to lower the incidence rate of metritis and the economic losses to the dairy industry. The overall purpose of the present study was to search for early screening biomarkers of disease state. Therefore the objectives of this study were to evaluate metabolites related to carbohydrate metabolism (lactate, BHBA, and NEFA), acute phase proteins SAA, and Hp), and proinflammatory cytokines (IL-1, IL-6 and TNF) in the blood of transition dairy cows starting at −8 weeks before calving, during the week of disease diagnosis, and up to +4 weeks postpartum.

2. Material and methods

2.1. Animals and diets

One hundred pregnant Holstein dairy cows at the Dairy Research and Technology Centre, University of Alberta (Edmonton, AB, Canada), were used in a longitudinal study. Six cows with metritis and 6 healthy cows with no clinical disease during the entire experimental period were selected retrospectively. Cows were selected based on their parity. To avoid overlaps of diseases only cows that were free of diseases were selected in the CON group. Moreover, if the cow with metritis was diagnosed having other diseases it was excluded from the evaluation. Healthy cows had no clinical signs of any disease including metritis, lameness, milk fever, mastitis, retained placenta, or ketosis). Six pregnant multiparous Holstein dairy cows (parity: 2.7 ± 0.7, Mean ± SEM) were diagnosed with metritis (at week +1 (8 days), +2 (12 days), +2 (14 days), +3 (19 days), +3 (21 days), and +3 (20 days), respectively) and six healthy control cows (CON) that were similar in parity (3.3 ± 0.6), and body condition score (BCS), were selected for this nested case–control study.

All experimental procedures were approved by the University of Alberta Animal Policy and Welfare Committee for Livestock and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

The experimental period lasted for 17 weeks starting from −8 weeks before parturition to +8 weeks postpartum (i.e. −8 weeks to +8 weeks, 0 week means the week of calving) for each cow. Cows were housed in individual tie stalls bedded with sawdust and with free access to water throughout the experiment. Shortly before calving cows were transferred to the maternity barn and returned to their stalls on the day following parturition. Diets were offered as TMR for ad libitum intake once daily at 0800 h to allow approximately 5% orts. All TMR were formulated to meet or exceed the nutrient requirements of dry and early lactating dairy cows with an estimated body weight of 680 kg as per National Research Council guidelines (2001). Individual feed intake (FI) was recorded daily throughout the 17 weeks period by calculating the difference between the total daily diets given to each cow with the orts on the next morning. From the onset of lactation, cows were milked in their stalls twice per day at 0500 and 1600 h, and individual milk yield (MY) was recorded electronically. Milk composition including crude protein (CP), milk fat, lactose, somatic cell count (SCC), milk urea nitrogen (MUN), and total solids (TS) were analyzed by mid-infrared spectroscopy (MilkoScan 605; A/S Foss Electric, Hillerød, Denmark) at the DHI Central Milk Testing Laboratory in Edmonton, Alberta.

2.2. Monitoring of clinical health status

Health status (HS) of cows was monitored daily based on clinical signs of disease by trained individuals and on a weekly basis by a veterinary practitioner. All periparturient diseases and veterinary treatments were recorded for each cow throughout the entire experimental period. Diagnosis of pregnancy was performed routinely by a veterinary practitioner at 60–70 days post-insemination. Based on the artificial insemination (AI) data, supported with the information of pregnancy diagnosis, the expected date of parturition was fixed by adding 280 days from the day of AI. All cows were monitored daily starting at −8 weeks prior to the expected date of calving and continuing up to +8 weeks postpartum. The various external signs observed were gait, general appearance, appetite, alertness, rectal temperature, ease of calving, body condition score (BCS), body temperature, vaginal discharges (color and consistency), udder edema, flakes in the milk, and pain in the legs.

In this study, metritis was diagnosed by trained staff and a veterinary practitioner according to the farm standard operating procedures. A metritic case was diagnosed if the cow had reddish brown vaginal discharge with fetid odor, together with fever (rectal temperature > 39.5 °C), an abnormally large uterus, and decreased feed intake and milk production within 3 weeks after parturition. Cows that had metritis and other periparturient diseases were not included in this study.

2.3. Sample collection

Blood samples were obtained from the coccygeal vein once per week at 0700 before feeding from −8 weeks before parturition to +8 weeks postpartum. Samples for analyses from 12 cows were selected at 4 time points: during −8 (53–59 days) and −4 (25–31 days) weeks before parturition, the disease week, and during +4 (25–31 days) weeks after calving. All blood samples were collected into 10-mL vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and allowed to clot and kept at 4 °C until separation of serum. Clotted blood was centrifuged at 2090 ×*g* at 4 °C for 20 min (Rotanta 460 R centrifuge, Hettich Zentrifugan, Tuttlingen, Germany). The separated serum was aspirated from the supernatant gradually by transfer pipets (Fisher Scientific, Toronto, ON, Canada) without disturbing the sediment. The separated serum was transferred to a sterile 10-mL plastic test tube (Fisher Scientific, Toronto, ON, Canada). All serum samples were stored at −80 °C until analysis to avoid loss of bioactivity and contamination and were thawed on ice for approximately 2 h before use.

Cows were milked twice per day at 0500 and 1600 h, and milk samples collected on day 0, 14, 21, 35, and 49 relative to parturition (day 0 means the day of calving), were used for the analysis of milk composition including crude protein (CP), milk fat, lactose, somatic cell count (SCC), milk urea nitrogen (MUN), and total solids (TS).

2.4. Sample analyses

2.4.1. Serum metabolites

Concentrations of serum lactate, BHBA, and NEFA were measured by an enzymatic colorimetric method using commercially available kits provided by Stanbio Laboratory (Boerne, TX, USA) and Wako Chemicals (Richmond, VA, USA), respectively. The detailed methods have been described previously by Ametaj et al. (2009). All samples were tested in duplicate and absorbance of standards and samples vs a blank for lactate, BHBA, and NEFA were read at 492, 505, and 550 nm, respectively, in a microplate reader (Spectramax 190, Molecular Devices Corporation, Sunnyvale, CA, USA). The intra-assay coefficient of variation (CV) of all the three assays was <10%.

2.4.2. Serum cytokines

Concentration of IL-1 in the serum was assayed by a commercially available bovine ELISA kit (Cusabio Biotech Co. Ltd., Wuhan, China) with mAb (monoclonal antibodies) specific for IL-1 coated on the walls of the microplate strips provided. The procedure involved the basic principle of a competitive inhibition enzyme immunoassay between biotin-conjugated IL-1 and serum IL-1 with the pre-coated antibody. The sensitivity of this assay was 250 pg/mL, and the intra-assay coefficient of variation (CV) was <10%. The detailed procedures have been described previously by Zhang et al. (2015).