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Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows

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ABSTRACT

Increased disease rates are commonly reported among high-yielding dairy cows in the transition period, extending from 3 weeks before to 3 weeks after calving, and characterized by the occurrence of an inflammatory response in terms of both positive and negative acute phase proteins (APP+ and APP−). To determine the above inflammatory response, the authors had developed the Liver Functionality Index (LFI), which defines the above condition on the basis of some APP− responses (albumin, cholesterol *sensu stricto* + bilirubin) during the first month of lactation. In this respect, low LFI values are associated to a high inflammatory response and vice versa. The relationship between LFI and inflammatory cytokine response was investigated from day −28 to day +28 with respect to calving in 12 periparturient dairy cows showing the six highest and six lowest LFI values within a cohort of 54 high-yielding dairy cows. The hypothesis being tested was that LFI and APP− on the whole could be used as readout of successful vs. non-successful adaptation to the transition period, with a strong association to disease occurrence. In fact, low LFI cows experienced many more disease cases (13 vs. 3 in high LFI Group) and related drug treatments till day +28. Interleukin-6 (IL-6) serum concentrations were always higher in low LFI cows ($P < 0.05$ on day +28). The greater IL-6 levels were correlated with higher ceruloplasmin (APP+) and lower lysozyme serum concentrations ($P < 0.05$ and <0.1, respectively). This latter finding was correlated with a clear role *in vitro* of lysozyme in a dose-dependent modulation of the inflammatory response of swine intestinal epithelial cells and bovine peripheral blood mononuclear cells. Hematological examinations showed no significant differences between the two groups under study. On the whole, our results indicate that LFI and LFI-related parameters could be used to identify cows at risk in the transition period toward an improved farm management. Also, our study indicates that disease cases in periparturient, high-yielding dairy cows are correlated with signs of accentuated IL-6 response and other markers of inflammatory phenomena. These likely start in the late lactation period or around dry-off, as suggested by our prepartal data, and proceed at much greater levels after calving.

1. Introduction

The health condition of high-yielding dairy cows is particularly at risk in the transition period, which includes the 3 weeks before and after parturition (Drackley, 1999). As a result, culling is highest because of diverse disease cases such as mastitis, metritis, clinical ketosis, placental retention, and left/right abomasal displacement (Goff and Horst, 1997; LeBlanc et al., 2006; Mulligan and Doherty, 2008). This prompted considerable research efforts in this area, aimed at investigating the serious worsening of animal health in the periparturient period. These studies disclosed critical features of the cows' adaptation physiology and identified relevant risk factors for disease occurrence. In particular, transition cows were shown to display an overt inflammatory response related to pregnancy and lactation (Sordillo et al., 2009), even without signs of microbial infections and/or otherwise determined pathology (Bionaz et al., 2007; Bertoni et al., 2008); this can increase metabolic stress, thus compromising the host's immune defences. The extent, the seriousness and the consequences of the above inflammatory response can be characterized by the Liver Functionality Index (LFI) (Bertoni et al., 2006) which defines the above condition on the basis of the time-course of plasma albumin, cholesterol and bilirubin, i.e. negative acute phase response proteins during the first month of lactation. Interestingly, clear signs of immunosuppression (Kehrli et al., 1989) and reduced immune functions in late pregnancy (Lacetera et al., 2005) may be observed in correlation with an inflammatory response and a metabolic stress-related

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condition in early lactation, even though contradictory data have been obtained about the functions of polymorphonuclear neutrophilic leukocytes in the same period (Sander et al., 2011). Owing to the above, a clear correlation does exist among immunosuppression, disease occurrence, inflammation and metabolic stress, which demands a proper investigation approach to unravel critical cause-effect relationship, as well as useful prognostic parameters of the cows' health status. Among these, inflammatory cytokines are involved in the response to metabolic changes in the transition period (Sordillo et al., 1995), and a central, dominant role of the cytokine network in the metabolic and inflammatory response of transition cows is in line with diverse and substantial evidence. In fact, recent data suggest that a significant worsening occurs of both inflammatory (e.g. a slower increase of negative acute phase proteins as albumin, cholesterol, paraoxonase, retinol binding protein) and metabolic (e.g. larger decrease of plasma glucose and greater values of NEFA, β-hydroxybutyrate, and reactive oxygen metabolites) indices in transition cows, after the administration of interferon-α (IFN-α) (Trevisi et al., 2009). Also, treatments with Tumor Necrosis Factor alpha (TNF-α) in late-lactating Holstein cows (Bradford et al., 2009) promote liver triglycerides accumulation, that means an increased risk of fatty liver mainly in early lactation subjects, when lipomobilization is dramatically increased. Finally, a major role is played by interleukin-6 (IL-6) gene expression, which is markedly increased in cows with induced ketosis in their post-calving period (Loor et al., 2007). Therefore, IL-6 seems to play a central role in the impairment of normal liver functions of transition cows.

Owing to the above, we hypothesized that cows showing low LFI values (high inflammatory response) could be clearly differentiated from high LFI cows (low inflammatory response) in terms of clinical conditions and mean plasma concentrations of inflammatory cytokines around calving. The hypothesis being tested was that LFI and APP− could be used as readout of successful vs. non-successful adaptation to the transition period, with a strong association to disease occurrence.

2. Materials and methods

2.1. Experimental animals

This study complied with Italian laws on animal experimentation and ethics and was conducted under the direction of Bertoni and Trevisi. The trial took place in two experimental dairy farms located in Piacenza Province (Northern Italy), rearing Holstein Friesian dairy cows (parity 2–6).

On farm 1, cows were reared in loose stall barns with cubicles; they were divided into lactating and non-lactating (dry) groups and fed a group-specific total mixed ration *ad libitum*.

On farm 2, cows were housed in an artificially lit and ventilated tie-stall. Climatic conditions were maintained under constant settings: environmental temperature around 20 °C, relative humidity between 60% and 70%, photoperiod with 14 h of light (from 5:00 to 19:00 h) and 10 of dark. Cows were individually fed using the same forages of farm 1 and diet was offered *ad libitum*: forages every 12 h and concentrates by an autofeeder every 12 or 3 h in dry and lactating cows, respectively. On average, dry cows received 9–12 kg of grass hay, 8–10 kg of maize-silage and 1–2 kg of concentrate per day. After calving, the daily diet included 2 kg of grass hay and 3 kg of alfalfa, while concentrate and maize-silage were gradually increased (on day 30 of lactation, on average cows received 11–13 kg of concentrate and 18–20 kg of maize-silage).

Fifty-four multiparous cows were included in this study (thirty on farm 1 and twenty-four on farm 2), sub-divided into homogenous groups on the basis of expected time to calving, live weight, parity, genetic value and body condition score. In both herds, all cows were routinely vaccinated against bovine viral diarrhea, infectious bovine rhinotracheitis, parainfluenza type 3 and bovine respiratory syncytial viruses by a live attenuated vaccine (CattleMaster 4, Pfizer Animal Health, Exton, PA, USA) every 6 months except in the 4 weeks before and after calving. During the trial clinical inspections (rumination activity; fecal consistency; uterine discharge; presence of injuries, lameness or joint swellings; milk yield and presence of milk clots) were carried out daily on all cows, whereas rectal temperature was measured in animals twice a week (farm 1, loose stall), or every morning (farm 2, tied stall) at feeding time (8.00). Moreover, all cows were submitted to thorough gynecological examinations on days 10 ± 1 and 30 ± 3 after calving by an appointed veterinary practitioner.

2.2. Blood sampling

Cows at the feed bunk were captured with a self-locking device. Blood samples (three/sampling) were collected from each cow before feeding (7.30 h) in the morning from the jugular vein in vacuum tubes (Vacuette, Greiner Austria) containing either no anti-coagulant, or lithium-heparin, or K3EDTA (9 ml each). Samplings were performed at days −28(±2), −21(±2), −14(±1), −7(±1), +3(±1), +7(±1), +14(±1), +21(±1), +28(±1), relative to calving. Lithium–heparin and K_3EDTA tubes were immediately cooled in an ice water-bath. Lithium–heparin tubes were centrifuged at 3520*g* for 16 min at 5 °C; plasma samples were divided into five aliquots and stored at −80 °C ($n = 1$), or −20 °C ($n = 4$). Blood samples without anti-coagulant were incubated in a water-bath at 37 °C for 30 min and centrifuged at 3520*g*, 5 °C for 16 min; serum samples were stored in sterile tubes in aliquots at −80 °C. Blood samples in K3EDTA tubes were used for the preparation of blood smears and microscopic examination.

2.3. Production, nutrition, haematological and clinical chemistry examinations

Milk production was measured and recorded by the "AFIMILK" computer-controlled automated system (S.A.E. Afikim, Kibbutz Afikim, Israel) on farm 1. On farm 2, milk yield was manually weighed at every milking.

Feed intake measurement was possible only on farm 2, where cows were individually fed. Daily dry matter intake (DMI) was evaluated by weighing each feed ration and the morning refusal. Dry matter content was periodically determined on representative samples of feed and refusals.

Body Condition Score (BCS) status was individually evaluated by the same observer according to a five-point scale (ADAS, 1986), at the first blood sampling (about 30 days before the expected calving date) and then every 14 days until day 30 after calving.

Each cow was submitted to a few plasma measurements as previously described (Bionaz et al., 2007): (A) inflammatory response tests: positive (APP+, haptoglobin and ceruloplasmin) and negative (APP−, albumin, cholesterol as lipoprotein index, vitamin A as index of its carrier Retinol Binding Protein) acute phase proteins. (B) Liver tests: function (total bilirubin). (C) Energy metabolism tests: glucose, NEFA, β-hydroxybutyrate (BHB).

Plasma parameters were analyzed at 37 °C by a clinical auto-analyzer (ILAB 600, Instrumentation Laboratory, Lexington, MA, USA), using kits purchased from: Instrumentation Laboratory (IL test™; albumin, cholesterol, total bilirubin, and glucose); Wako Chemicals GmbH Neuss, Germany (NEFA); Randox Laboratories Ltd., Crumlin, Co. Antrim, United Kingdom (BHB). Plasma vitamin A was extracted with hexane and analyzed by reverse-phase HPLC