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Research paper

Elevated plasma haptoglobin concentrations following parturition are associated with elevated leukocyte responses and decreased subsequent reproductive efficiency in multiparous Holstein dairy cows

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ABSTRACT

The objectives were to describe the relationship between the intensity of the acute phase response and the metabolic status and leukocyte responses of early postpartum, multiparous cows and determine if subsequent reproductive performance was impaired in cows with a greater acute phase response. Peripheral blood was collected from 240 Holstein cows, 2–8 days in milk and 2nd–8th parity from 8 dairies in Western TX and Eastern NM across 5 days ($n = 6$ cows/dairy/day). Plasma concentrations of haptoglobin were measured and cows were classified as Low (1st quartile), Moderate (2nd and 3rd quartiles), or High (4th quartile) responders. Metabolic measurements included: plasma glucose, urea nitrogen, non-esterified fatty acids and β-hydroxybutyrate concentrations. Leukocyte response measurements included: total leukocyte counts and differentials, neutrophil surface expression of L-selectin, neutrophil oxidative burst capacity when co-cultured with an environmental *Escherichia coli*, as well as the secretion of tumor necrosis factor-α and interferon-γ when diluted whole blood were co-cultured with lipopolysaccharide and phytohemagglutinin-P, respectively. All data are reported as Low, Moderate, and High haptoglobin responders. Plasma haptoglobin concentrations ranged from below the limit of detection to 8.4 μg/mL, 8.5 to 458 μg/mL, and 459 to 1757 μg/mL. The High cows had more severe neutropenia (3.45, 3.31, and $2.23 \pm 0.31 \times 10^6$ cells/mL; $P = 0.013$) Additionally, the innate leukocyte responses of the High cows were stimulated as evident by increased secretion of tumor necrosis factor-α (568, 565, and 730 ± 73.4 pg/mL; $P = 0.003$), surface expression of L-selectin on neutrophils (70.8, 71.9, and 119.8 ± 7.9 geometric mean fluorescence intensity; $P = 0.001$), and greater neutrophil oxidative burst capacity (37.9, 40.4, and 47.9 ± 0.31 geometric mean fluorescence intensity; $P = 0.002$). In contrast, the secretion of the T-lymphocyte derived cytokine, interferon-γ, was suppressed in both the Moderate and High cows when compared with Low cows (718, 408, and 322 ± 92.2 pg/mL; $P = 0.01$). Haptoglobin class had an overall effect on days to conception ($P = 0.039$). The number of days in milk for 75% of the cows in each haptoglobin class to conceive increased from 123 d in the Low group, 139 d in the Moderate group, and 183 d in the High group. These data indicate that a stronger acute phase response during the early postpartum period that is characterized by an activated innate immune system and a suppressed mitogen-induced interferon-γ secretion resulted in impaired reproductive efficiency, and this response was consistent across the large commercial dairy herds sampled.

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1. Introduction

The transition from gestation to lactation in high producing dairy cows continues to plague the dairy industry, with high incidences of both metabolic and infectious diseases. This period is stressful to a cow and many management practices and therapies to improve the success of this transition were investigated. While it is unlikely that a commercial dairy herd will eliminate all transition disorders, early detection could be beneficial in dampening the severity of disease outcomes. A moderate body of evidence suggests that blood metabolites can be useful in the identification of at-risk animals prior to them exhibiting clinical symptoms and corresponding intervention strategies will provide a more favorable outcome (Ospina et al., 2010, Huzzey et al., 2012). A sensitive and specific biomarker provided in real-time would enable dairy producers to focus at the individual cow level, rather than the herd level, and allow for timely culling or treatment decisions, favorably impacting the economic bottom line of the dairy enterprise.

Haptoglobin (Hp) is an acute phase protein that is near undetectable in many clinically healthy animals and whose expression is proportionally elevated in response to inflammation, such as that from tissue damage or disease. In a lipopolysaccharide challenge of non-pregnant and non-lactating cows, Jacobson et al. (2004) found that acute phase response intensity is dose dependent, but also observed a large between cow variation, indicating that acute phase protein concentrations differ among animals during similar diseased states. Humblet et al. (2006) observed significant increases in plasma Hp concentrations in both diseased and clinically healthy cows during the first week postpartum; therefore, those authors questioned the usefulness of measuring plasma haptoglobin in the first week postpartum. Ballou et al. (2011) suggested that clinical signs of disease may not be the most sensitive marker of altered health in cattle, and that many clinically healthy animals have altered homeostasis indicative of an underlying infection or pathology.

The hypotheses of this study were (1) that many clinically healthy cows would have elevated plasma Hp in the first week postpartum (2) the distribution of Low, Moderate, and High Hp responders would differ across commercial dairies, (3) cows with elevated plasma Hp would have elevated innate leukocytes and a metabolic status more indicative of negative energy balance, and (4) cows with elevated plasma Hp would have reduced reproductive performance during the subsequent lactation.

2. Materials and methods

2.1. Animals and blood collection

All procedures involving the use of cows were approved by the Animal Care and Use Committee at Texas Tech University. The study was conducted from September 19th to September 28th, 2011. Two hundred and forty clinically healthy postpartum Holstein dairy cows in their 2nd–8th lactation and 2–8 days in milk (DIM) were sampled from 8 commercial dairies in Western Texas and Eastern New Mexico. The study was completed over two weeks with 5 sampling days. On each sampling day, 6 cows were randomly chosen from each dairy from all of the eligible cows, without sampling the same cow twice. Whole blood was collected *via* jugular venipuncture from each animal (10 mL into sodium heparin and 6 mL into potassium EDTA Vacutainers; Becton Dickinson; Franklin Lakes, NJ) for measures of hematology, blood metabolites, and *ex vivo* leukocyte responses. Prior to analysis, the heparinized blood was stored at ambient temperature in an ice chest without any ice, and the EDTA blood was stored on ice based on recommendations from Sellers et al. (2013). Processing of blood samples started within two hours of collection. Blood was mixed thoroughly prior to analysis *via* laboratory rocker. The weather in the region was mild. None of the herds reported any precipitation during that time, and the mean ± standard deviation low and high temperatures recorded in the region were $13.2 \pm 1.75\,^{\circ}C$ and $32.3 \pm 2.53\,^{\circ}C$, respectively.

2.2. Herd level

Metrics describing herd size, production, and management were estimated using Dairy Comp 305 (DC 305, Valley Ag Software, Tulare, CA). Metrics included herd size, average DIM, average parity of lactating herd, average age of lactating herd, average days open, average length of dry period, percentage of herd pregnant, first service conception rate, and average herd mature equivalent lactation (305ME). In addition, the occurrences of mastitis, metritis, ketosis, displaced abomasum, retained placenta, hypocalcemia, and animals that were either sold or died were also monitored *via* DC 305. The 305ME was calculated as described by Wiggans and Dickinson (1985). Briefly, milk and fat yields were standardized by current yield multiplied by correction factors corresponding to differences in age at calving, calving month, region, breed, and yield trait.

Freshly delivered samples of the close-up and fresh cows diets were taken at each visit when blood samples were collected. Samples were composited by herd and sent in duplicate to a commercial laboratory to be analyzed by wet chemistry (Cumberland Valley Labs; Hagerstown, MD).

2.3. Cow level

All cows enrolled in the study and those included in subsequent analysis of health, leukocyte responses, and metabolic statuses where physically normal in appearance and absent of any clinical symptoms of disease. A clinically healthy cow was defined as having no retention of fetal membranes, no putrid vaginal discharge, clear milk strip in the milking parlor, and rectal temperatures less than $39.0\,^{\circ}C$.

2.4. Hematology

Total leukocyte count and differentials were measured using a commercially available hematology analyzer with bovine-specific algorithms according to manufacturer's protocols (IDEXX Procyte DX, Westbrook, ME).