J. Dairy Sci. 101:1–10
https://doi.org/10.3168/jds.2018-14591

Changes in biomarkers of nutrient metabolism, inflammation, and oxidative stress in dairy cows during the transition into the early dry period

A. K. Putman, J. L. Brown, J. C. Gandy, L. Wisnieski, and L. M. Sordillo[1]
Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing 48824

ABSTRACT

Metabolic stress occurs in dairy cows when physiologic homeostasis is disrupted as a consequence of aberrant nutrient metabolism, chronic inflammation, and oxidative stress. Early-lactation cows that suffer from metabolic stress are susceptible to health disorders that cause significant production losses. However, there is little information regarding the occurrence and effect of metabolic stress during involution. Therefore, the purpose of this study was to investigate well-known biomarkers associated with metabolic stress in early-lactation cows at various time points during the early dry period when dairy cows also are subjected to dramatic changes in physiologic homeostasis. Our group conducted a descriptive study by collecting serum and whole-blood samples from the coccygeal vein of 29 healthy dairy cows at a commercial dairy herd. Sampling points included d −6, 0, +1, +2, +6, and +12 relative to dry-off date. Samples were used to quantify biomarkers related to nutrient metabolism, oxidative stress, and inflammation that included calcium, nonesterified fatty acids, β-hydroxybutyrate, albumin, haptoglobin, cortisol, reactive oxygen and nitrogen species, antioxidant potential, oxidant status index, and isoprostanes. Additionally, whole-blood leukocyte differentials for total leukocyte, neutrophils, lymphocytes, eosinophils, and monocytes were analyzed. Within altered nutrient metabolism biomarkers, calcium and nonesterified fatty acid concentrations changed most from d 0 to d +2 during the sampling period. Indicators of oxidant status, such as reactive oxygen and nitrogen species, antioxidant potential, and oxidant status index, generally increased throughout the sampling period except at d +2, suggesting altered redox status throughout early involution. In contrast, isoprostane concentrations fluctuated throughout the study, demonstrating that indicators of oxidative damage occurred more sporadically during the sampling period. Therefore, many of the biomarkers associated with early-lactation metabolic stress also changed during the transition from late lactation to the early dry period, but not to the same magnitude and duration previously reported in periparturient cows. Future studies should be directed toward assessing whether the magnitude and duration of biomarker expression can affect the health and well-being of cows during the early dry period.

Key words: dry cow, metabolic stress, involution, inflammation

INTRODUCTION

The dry period is the critical time between lactations in which a cow's mammary gland remodels and regenerates in preparation for the ensuing lactation. During this time, high-producing dairy cows are subject to stressors such as an abrupt cessation in milking, mammary gland discomfort, and physiological imbalances such as altered energy, immune, and hormonal states (Zobel et al., 2015). For instance, intramammary pressure increases when the udder becomes engorged with milk after abrupt milking cessation, leading to discomfort. Acute involution also follows abrupt milking cessation and elicits an inflammatory response, further causing cattle distress (Zobel et al., 2015). Early involution is a critical transition for dairy cows; however, most retrospective and prospective studies have focused instead on health risk factors associated with the periparturient period such as metabolic stress (Sordillo and Mavangira, 2014).

Metabolic stress is a cluster of risk factors in periparturient cows that leads to an increased susceptibility to certain health disorders, such as metritis and mastitis (Sordillo and Mavangira, 2014). Metabolic stress of periparturient cows is analogous to metabolic syndrome in humans and results from a combination of aberrant nutrient metabolism leading to dyslipidemia, chronic inflammation, and oxidative stress (Sordillo et al., 2009; Sordillo and Mavangira, 2014; Mbata et al., 2017). For instance, early-lactation cows that cannot meet energy demands through DMI experience aberrant nutrient metabolism, indicated by biomarkers

Received February 14, 2018.
Accepted June 4, 2018.
[1] Corresponding author: sordillo@msu.edu

of fat mobilization such as nonesterified fatty acids (**NEFA**; Bell, 1995). While lipid mobilization is a normal physiological response to help the cow adapt in these situations, excessive fat mobilization can be problematic. For instance, overproduction of ketones such as BHB occurs during times of high circulating NEFA concentrations when there is not a sufficient amount of Kreb's cycle intermediates to appropriately oxidize the acetyl coenzyme A produced by NEFA (Kuhla et al., 2016; Han van der Kolk et al., 2017). Additionally, increased concentrations of certain biomarkers, such as NEFA and BHB, have been linked to early-lactation disease and altered immune competence (Erdmann et al., 2018). Furthermore, the presence of aberrant nutrient metabolism, chronic inflammation, or oxidative stress can intensify the other factors that can cause metabolic stress, causing further problems (Trevisi et al., 2012; Esposito et al., 2014; Sordillo and Mavangira, 2014). For instance, increased plasma NEFA can lead to an increase in reactive oxygen species, which can exacerbate oxidative stress when present in excess. Oxidative stress can then contribute to dysregulated inflammatory responses and dyslipidemia (Sordillo et al., 2009). Cows entering the dry period may also experience dyslipidemia, chronic inflammation, or oxidative stress; however, assessments of these biomarkers have not been made during the physiological transition from late lactation to involution.

Cows undergoing the major physiological transition from lactating to nonlactating experience many stressors that may compromise their health and well-being. For instance, a cow entering the dry period is in her last trimester of pregnancy, when fetal growth is most rapid, milk production rapidly declines, and social stressors may occur due to moving to new pens with a new group of cows (Schirmann et al., 2011). Due to the similarities of potential stressors imposed on high-producing cows entering both the early dry period and early lactation, it can be anticipated that the cows will experience some changes in biomarkers of nutrient metabolism, oxidative stress, and inflammation that collectively may contribute to metabolic stress. Understanding changes in biomarkers associated with metabolic stress will give valuable insight into how the early dry period may affect animal health and welfare. Therefore, the goal of this study was to document changes in biomarkers of nutrient metabolism, oxidative stress, and inflammation present during the early dry period of dairy cows.

Table 1. Diet of late-lactation and early dry cows for the study period

Item (% unless noted)	Late lactation	Far-off dry
NE_L[1] (Mcal/kg)	1.5	1.15
CP	19	11
Fat	5.3	2.3
NFC	38	17
Calcium	0.75	0.51
Phosphorus	0.69	0.36
Magnesium	0.78	0.31
Potassium	1.4	2.5
Sodium	0.29	0.34
Chloride	0.53	0.99
Sulfur	0.3	0.18
Selenium (mg/L)	6.2	23
Vitamin E (IU/animal)	290	1,064

[1]Estimated energy of lactation for an animal consuming 3× its requirement.

MATERIALS AND METHODS

Animals

This study was approved by the Michigan State University Institutional Animal Care and Use Committee. Animals were enrolled 56 d before the expected calving date with owner consent. Blood samples were collected from 29 Holstein dairy cows (326 ± 5 DIM; range: 299–390 DIM) from a commercial herd that was free from clinical disease with SCC $<250 \times 10^3$ cells/mL. Cows had an average milk production of 34 kg/d (range: 17.8–52.3 kg of milk/d) in the previous lactation. Parity ranged from 1 to 5, and cows had an average BCS of 3.0 at the time of dry-off (range: 2.6–4.0). Cows had ad libitum access to a TMR and water. At d −6, cows were fed a late-lactation diet and switched to a far-off dry cow diet at d 0 (Table 1). Cows remained on the far-off dry cow diet for the remainder of the study. Before drying off, cows were milked 2 times/d and were treated with a standard intramammary dry cow antimicrobial therapy to protect against new intramammary infections. All cows were housed in a freestall barn that was cooled via fans and grouped according to stage of lactation. As cows were dried off, they were moved from a late-lactation pen to a dry cow pen with a new group of animals. Animals were sampled in 4 cohorts based on dry-off date. Group 1 (n = 13), group 2 (n = 9), and group 3 (n = 5) were all sampled during the summer over a 6-wk period, whereas group 4 (n = 2) was sampled in the fall.

Sample Collection and Processing

Samples were collected aseptically in the morning between 0800 and 1000 h from the coccygeal vein in evacuated tubes containing serum separator and EDTA. Six collection time points were chosen as d −6, 0, +1, +2, +6, and +12 relative to the dry-off date. Samples were immediately stored on ice throughout the collection and processing stages.

Serum was harvested after centrifugation at 1,449 × *g* for 15 min at 4°C and aliquoted. A serum aliquot