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Diet starch concentration and starch fermentability affect markers of inflammatory response and oxidant status in dairy cows during the early postpartum period

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ABSTRACT

Our objective was to evaluate the effects of diet starch concentration and starch fermentability on inflammatory response markers and oxidant status during the early postpartum (PP) period and its carryover effects. Fifty-two multiparous Holstein cows were used in a completely randomized block design experiment with a 2 × 2 factorial arrangement of treatments. Treatments were starch concentration and starch fermentability of diets; diets were formulated to 22% (low starch, LS) or 28% (high starch, HS) starch with dry-ground corn (DGC) or high-moisture corn (HMC) as the primary starch source. Treatments were fed from 1 to 23 d PP and then switched to a common diet until 72 d PP to measure carryover (CO) effects. Treatment period (TP) diets were formulated to 22% forage neutral detergent fiber and 17% crude protein. The diet for the CO period was formulated to 20% forage neutral detergent fiber, 17% crude protein, and 29% starch. Coccygeal blood was collected once a week during the TP and every second week during the CO period. Liver and adipose tissue biopsies were performed within 2 d PP and at 20 ± 3 d PP. Blood plasma was analyzed for concentrations of albumin, haptoglobin, reactive oxygen and nitrogen species (RONS), and antioxidant potential (AOP), with lipopolysaccharide-binding protein (LBP) and TNFα evaluated during the TP only. Oxidative stress index (OSi) was calculated as RONS/AOP. Abundance of mRNA from genes involved in inflammation and glucose metabolism in liver and genes involved in lipogenesis in adipose tissue were determined. Data were analyzed separately for the TP and CO periods. During the TP, treatments interacted to affect concentrations of TNFα, haptoglobin, and LBP, with HMC increasing their concentrations for HS (9.38 vs. 7.45 pg/mL, 0.45 vs. 0.37 mg/mL, and 5.94 vs. 4.48 μg/mL, respectively) and decreasing their concentrations for LS (4.76 vs. 12.9 pg/mL, 0.27 vs. 0.41 mg/mL, and 4.30 vs. 5.87 μg/mL, respectively) compared with DGC. Effects of treatments diminished over time for LBP and haptoglobin with no differences by the end of the TP and no main CO effects of treatment for haptoglobin. The opposite treatment interaction was observed for albumin, with HMC tending to decrease its concentration for HS (3.24 vs. 3.34 g/dL) and increase its concentration for LS (3.35 vs. 3.29 g/dL) compared with DGC, with no carryover effect. Feeding DGC increased the OSi during the first week of the TP compared with HMC, with this effect diminishing over time; during the CO period HMC increased OSi for HS and decreased it for LS compared with DGC, with this effect diminishing toward the end of CO. Feeding HMC increased the abundance of genes associated with inflammation and gluconeogenesis in liver for HS and decreased it for LS compared with DGC. Feeding HS increased the mRNA abundance of genes associated with adipose tissue lipogenesis compared with LS. Results during the TP suggest that feeding LS-DGC and HS-HMC elicited a more pronounced inflammatory response and induced an upregulation of genes associated with inflammation and gluconeogenesis in liver, without effects on OSi, but effects on plasma markers of inflammation diminished during the CO period.
Key words: postpartum period, starch concentration, starch fermentability, inflammation

INTRODUCTION

Failure of cows to adapt physiologically to challenging conditions during the peripartum period (e.g., in-

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creased nutrient demand and milk production) triggers metabolic stress (Sordillo and Mavangira, 2014), which is associated with excessive oxidative stress and inflammation (Abuelo et al., 2015). The postpartum (**PP**) period involves a series of nutritional changes (e.g., new diet ingredients, diet fermentability) and environmental stressors (e.g., commingling, movements to the parlor) that can further challenge metabolic adaptation. Exacerbated metabolic stress can have negative short- and long-term implications on cow well-being, production, and reproduction (Sordillo and Aitken, 2009; Huzzey et al., 2015; Nightingale et al., 2015) and both oxidative stress and inflammation can be modulated with nutritional interventions (Miller et al., 1993; Bertoni et al., 2015).

Research in ruminants has shown that nutrition can affect the extent and severity of the inflammatory response (Bertoni et al., 2015). Some studies have reported that ruminal acidosis can induce a severe inflammatory response in dairy cattle as well as oxidative stress (Khafipour et al., 2009; Guo et al., 2013). Whereas the methodologies implemented in these studies to cause an acidosis challenge do not represent common feeding conditions, high levels of starch in the diet (28% or more) have yielded an inflammatory response in dairy cows (Emmanuel et al., 2008; Gott et al., 2015) and increased oxidative stress in lactating ewes (Sgorlon et al., 2008). In addition, a review of several studies concluded that feeding cows more than 44% of dietary concentrate containing highly fermentable starch sources (e.g., barley or wheat grain) linearly increased circulating markers of systemic inflammation (Zebeli et al., 2012). The inflammatory response from highly fermentable diets might be from absorption of bacterial LPS derived from lysis of gram-negative bacteria in the gastrointestinal tract (Khafipour et al., 2009). Even though there is a lack of research on the effects of diet starch fermentability on oxidative stress, a more acute inflammatory response when cows receive high quantities of rapidly fermentable starch could increase the production of reactive oxygen species and cow's susceptibility to oxidative stress (Mavangira and Sordillo, 2018). Cows that elicit a pronounced inflammatory response compared with those that elicit a low-grade inflammatory response have different metabolic adaptations (e.g., hepatic glucose metabolism) that may affect the rate at which the inflammation is resolved, likely influencing the short- and long-term effects on health and productive and reproductive performance mentioned previously (Bradford et al., 2015). Further, the degree of inflammation and metabolic changes induced by the type and amount of starch in rations fed during the early PP period have not been investigated. Our objective was to evaluate effects of diet starch concentration and starch fermentability on the inflammatory response and oxidant status of cows during the early PP period and its carryover effects. We hypothesized that cows fed a high-starch ration with highly fermentable starch will elicit a more pronounced inflammatory response and shifts in oxidant status during the early PP period.

MATERIALS AND METHODS

Animal Care

This study was conducted from February 1 to November 15, 2015, at the Dairy Cattle Research and Teaching Center at Michigan State University with all experimental procedures approved by the Michigan State University Institutional Animal Care and Use Committee (East Lansing, MI; AUF 11/13-254-00). Cows were housed individually in tie stalls, allowing for daily recording of feed offered and refused and collection of feed refused for analysis, and fed once a day (0800 h) at 115% of expected intake and milked at the parlor twice a day (0400 and 1430 h). All cows were in apparent good health at the beginning of the experiment, and standard farm health and reproductive protocols were maintained during this study.

Experimental Design and Treatments

Fifty-two multiparous Holstein cows were used in a completely randomized block design experiment with 2 × 2 factorial arrangement of treatments with 13 cows per treatment. Blocking criteria consisted of BCS observed within 1 wk before expected calving date (up to 1-unit difference using a 5-point scale, where 1 = thin and 5 = fat; Wildman et al., 1982), previous lactation 305-d mature-equivalent milk production (within 5,000 kg) and date of parturition (within 60 d). A common close-up diet was fed from 21 d before expected parturition date until calving. This diet contained corn silage, mature grass hay, dry ground corn, soybean meal, SoyChlor (West Central Soy, Ralston, IA) and a mineral and vitamin mix, and was formulated to contain 42.5% NDF, 38.3% forage NDF, 21.5% starch, and 13.5% CP.

Treatments differed in diet starch concentration (**SC**; low starch = 22%, **LS**, or high starch = 28%, **HS**) and diet starch fermentability (**SF**; dry-ground corn, **DGC**, or high-moisture corn, **HMC**). At calving, cows were randomly assigned to 1 of the 4 diet treatment combinations (LS-DGC, LS-HMC, HS-DGC, HS-HMC). Dry-ground corn grain was stored in a covered gravity wagon and HMC was ground and ensiled in a bag (Ag-Bag Plastic, Cottage Grove, MN) for at

least 4 mo after harvest before utilization. Differences in SF were confirmed by 7-h in vitro starch digestibility analysis (44.1% for DGC and 61.9% for HMC) before the experiment, and with additional sampling throughout the experiment, as reported by Albornoz and Allen (2018). Starch concentration of diets was adjusted by altering the percentages of the corn grain treatments and soyhulls to maintain the same forage NDF concentration across treatment diets. Diets contained alfalfa silage, grass hay, corn grain treatments, soyhulls, soybean meal, minerals, and vitamins and were formulated to 17% CP, and 22% forage NDF (Table 1). Cows received their respective diets during the treatment period (**TP**), which began on the day of calving if they calved before feeding time (0800 h) or at the following morning's feeding and lasted until 23 d PP. During the carryover period (**CO**) from 24 to 72 d PP, all cows received a common diet to evaluate possible residual effects of treatment diets (Table 1). Dry matter concentration of fermented feeds was determined twice per week throughout the experiment and diets were adjusted accordingly. All rations were formulated to meet or exceed cows' predicted requirements for protein, minerals, and vitamins according to NRC (2001), and ingredient and nutrient composition of treatment and CO diets are described (Table 1). Incidences of infectious and metabolic disorders during the TP and CO periods are reported in Table 2.

Data and Sample Collection

Blood samples were collected via coccygeal venipuncture at 0730 h on the same day of the week ± 3 d relative to day of parturition within a week before parturition and at 6, 13, 20, 27, 41, 55, and 69 d PP. Samples collected prepartum were used as a covariate for statistical analysis. Blood was collected in tubes containing K_2-EDTA and immediately centrifuged (3,000 × *g* for 15 min) to harvest plasma, which was then flash frozen in liquid nitrogen and stored at −80°C. Liver and adipose tissue biopsies were performed at 0800 h within 2 d PP and at 20 ± 3 d PP. Liver samples were collected by needle biopsy as described by Bradford and Allen (2005) and adipose tissue samples were collected from the dorsal subcutaneous depot in the tail head region as described by Harvatine et al. (2009). Both liver and adipose tissue samples were flash frozen in liquid nitrogen immediately after collection and stored on dry ice for transportation to be stored at −80°C until further analysis.

Table 1. Ingredient and nutrient composition of treatment and carryover diets

Item	LS[1]		HS[1]		Common diet
	DGC	HMC	DGC	HMC	
Ingredient, % of DM					
Corn silage	—	—	—	—	25.6
Alfalfa silage	37.0	37.1	37.7	37.0	17.3
Grass hay	8.25	8.35	8.35	8.21	—
DGC	27.5	—	35.4	—	17.8
HMC	—	28.1	—	36.2	9.30
Soyhulls	11.0	11.0	1.87	2.18	—
Soybean meal	11.7	11.1	12.2	12.4	15.3
Cottonseed	—	—	—	—	7.2
Wheat straw	—	—	—	—	4.47
Mineral-vitamin mix[2]	2.02	2.02	2.02	2.02	2.15
Limestone	0.55	0.55	0.55	0.55	0.72
Sodium bicarbonate	0.95	0.95	0.95	0.95	0.75
Dicalcium phosphate	0.95	0.95	0.95	0.95	—
Nutrient composition, % of DM					
DM, %	58.4 ± 3.2	55.2 ± 7.8	59.2 ± 3.6	53.1 ± 7.3	56.1 ± 2.5
OM	89.5 ± 0.9	89.4 ± 0.9	89.8 ± 0.9	89.6 ± 0.9	91.8 ± 0.4
NDF	33.0 ± 1.4	33.0 ± 1.4	28.3 ± 1.4	27.6 ± 1.4	28.1 ± 1.0
Forage NDF	22.4 ± 2.4	22.8 ± 2.4	22.6 ± 2.4	22.2 ± 2.4	20.4 ± 7.9
CP	17.2 ± 0.6	16.7 ± 0.6	17.3 ± 0.6	16.9 ± 0.6	16.9 ± 0.4
Starch	21.4 ± 0.4	21.9 ± 0.4	27.1 ± 0.6	27.8 ± 0.5	28.9 ± 1.1
Ash	10.5 ± 0.9	10.5 ± 0.9	10.2 ± 0.9	10.3 ± 0.9	8.18 ± 0.4
Gross energy, Mcal/kg	4.21 ± 0.04	4.21 ± 0.05	4.25 ± 0.04	4.25 ± 0.04	ND[3]

[1]LS, low starch = 22% starch, HS, high starch = 28% starch, DGC = dry ground corn, HMC = high moisture corn.

[2]Mineral-vitamin mix contained in a DM basis: 25.6% NaCl, 10.0% Ca, 2.0% Mg, 2.0% P, 30 mg/kg Co, 506 mg/kg Cu, 20 mg/kg I, 2,220 mg/kg Fe, 2,080 mg/kg Mn, 15 mg/kg Se, 2,030 mg/kg Zn, 300 kIU/kg vitamin A, 50 kIU/kg vitamin D, and 1,500 kIU/kg vitamin E.

[3]Not determined.

Table 2. Health disorder events during treatment period (1 to 23 d postpartum) and carryover period (24 to 72 d postpartum)

	LS[1]		HS[1]	
Item	DGC	HMC	DGC	HMC
During the treatment period				
Ketosis	2	4	3	2
Displaced abomasum	1	2	3	0
Milk fever	0	2	0	2
Retained placenta	1	0	0	1
Metritis	1	0	0	0
Mastitis	0	1	0	1
During the carryover period				
Ketosis	1	0	0	0
Mastitis	1	0	0	2
Pneumonia	0	0	1	0
Udder eczema	0	0	1	0

[1]LS, low starch = 22% starch, HS, high starch = 28% starch, DGC = dry ground corn, HMC = high moisture corn.

Markers of Inflammation and Oxidant Status

Blood plasma samples were analyzed with commercial kits for the negative acute phase protein albumin (Albumin, Beckman Coulter Inc., Brea, CA) by colorimetric measurement with an autoanalyzer (Beckman Coulter AU680, Beckman Coulter Inc.) at the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI). Haptoglobin was determined as positive acute phase protein with a commercial photometric colorimetric kit [Phase Haptoglobin Assay; Tridelta Development Ltd., Maynooth, Ireland; intraassay coefficient of variation (**CV**): 3.7%, and interassay CV: 8.6%]. Additional analyses were performed in blood plasma samples collected prepartum and during the TP to determine concentration of LPS binding protein (**LBP**) and tumor necrosis factor-α (**TNFα**). The concentration of LBP was determined with a commercially available kit (LBP ELISA for various species, Hycult Biotech, Uden, the Netherlands; intraassay CV: 5.1%, and interassay CV: 8.1%). The concentration of TNFα was determined by an ELISA method described by Farney et al. (2011; intraassay CV: 5.1%, and interassay CV: 3.8%). Reactive oxygen and nitrogen species (**RONS**) were determined with a commercially available assay (ROS and RNS assay, Cell Biolabs, San Diego, CA; intraassay CV: 6.3%, and interassay CV: 9.2%) as described by Abuelo et al. (2016) and antioxidant potential (**AOP**) using the Trolox equivalents antioxidant capacity as described by Re et al. (1999; intraassay CV: 1.6%, and interassay CV: 3.2%). Changes in oxidant status were assessed with the oxidative stress index (**OSi**) calculated from the ratio between pro-oxidant and antioxidant (RONS/AOP; Abuelo et al., 2013).

Hepatic Triglyceride Content

Liver samples were analyzed for triglyceride content as described by Zhou et al. (2016) and results expressed on a wet tissue basis.

RNA Extraction from Liver and Adipose Tissue

For RNA extraction, ~30 mg of liver and ~50 mg of adipose tissue were homogenized in TRizol reagent (ThermoFisher Scientific, Waltham, MA) using 2.3-mm zirconia/silica beads (Biospec, Bartlesville, OK) and a bead mill homogenizer (Precellys, Bertin Instruments, Montigny-le-Bretonneu, France). Following homogenization, the TRizol homogenate was phase-separated using chloroform. Total RNA was extracted from the clear phase using the EZ gene Tissue RNA Miniprep Kit and genomic DNA eliminated using DNase I Digestion Kit according to manufacturer's instructions (Biomiga, San Diego, CA). Purity, concentration, and integrity of total RNA were evaluated using a NanoDrop 1000 spectrophotometer (ThermoFisher Scientific) and an Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA). All samples had a 260:280 nm ratio between 1.96 and 2.05 and a RNA integrity number for adipose tissue between 5 and 6, and >7 for liver tissue. Reverse transcription was performed using the qScript cDNA SuperMix (Quantabio, Beverly, MA) with 1 μg of total RNA.

Quantitative Real-Time PCR Analysis

Transcriptional studies were performed on the cDNA samples using high-throughput quantitative (**q**)PCR reactions on Wafergen SmartChip Real-time PCR system

(Takara Bio Inc., Mountain View, CA). In liver tissue, SYBR-Green qPCR assays were performed based on custom-designed primers with most of them previously reported by Gualdrón-Duarte and Allen (2018). Additional primers were designed either using web-based primer design software (Integrated DNA Technologies, Coralville, IA) or by Primer Express Software v3.0.1 (Supplemental Table S1; https://doi.org/10.3168/jds.2019-16398). These assays included SYBR-Green select master mix (ThermoFisher Scientific), 900 nmol of primers (Supplemental Table S1; Millipore-Sigma), and 1.5 ng/μL cDNA generated from 1 μg of RNA. Cycling conditions used were 50°C for 2 min and 95°C for 10 min holding; 45 cycles, 95°C for 15 s denaturation and 60°C for 1 min amplification; final melting curve analysis was performed at 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. The quantitative PCR assays for adipose tissue were performed using TaqMan gene expression assays (Supplemental Table S2, https://doi.org/10.3168/jds.2019-16398; Applied Biosystems/ThermoFisher Scientific). A standard protocol provided by the manufacturer was used as previously described by Contreras et al. (2017a), and each 100-nL real-time PCR reaction contained 1× Applied Biosystems TaqMan Universal PCR Master mix (ThermoFisher Scientific), 1× TaqMan gene expression assays, and 1.5 ng/μL of cDNA generated from 1 μg of RNA. The following real-time PCR cycling conditions were used for the chip: initial enzyme activation at 95°C for 10 min, 45 cycles of denaturation at 95°C for 10 s, and annealing at 60°C for 53 s. All qPCR reactions were performed in duplicate and no-template controls were included in each chip for each TaqMan gene expression assay/custom-designed primer. Finally, qPCR results were analyzed using SmartChip qPCR software (v 2.8.6.1), with amplification efficiency beyond the range (1.5–2.2), a threshold cycle (**Ct**) >40, and samples with multiple melting peaks were discarded. The Ct values from qPCR reactions were subsequently analyzed with qBase+ (Biogazelle, Ghent, Belgium). The software qBase+ calculates the stability of endogenous control genes and provides a value called the M value. The geometric mean of 2 endogenous control genes with optimal pairwise variation value (V value, <0.15) and acceptable low M value in both adipose (<0.35) and liver (<0.36) samples was used to normalize the Ct values of target genes (Contreras et al., 2017a). Endogenous control genes (*PGK1* and *RPS9* for liver; *EIF3K* and *RPS9* for adipose) were identified according to Hellemans et al. (2007). Relative quantitation analysis was performed using the ΔΔCt method as previously described by Livak and Schmittgen (2001).

Statistical Analysis

Data were analyzed separately for the TP (from 1 to 23 d postpartum) and for the CO period (from 24 to 72 d postpartum) as required to evaluate treatment effects during early PP and its residual effects. Blood variables were analyzed using the MIXED procedure of JMP Pro (version 14.2, SAS Institute Inc., Cary, NC) with repeated measures and first-order autoregressive covariate structure. The repeated variable was day PP and cow was the subject, with the following model:

$$Y_{ijsf} = \mu + B_i + C(B_iS_sF_f)_j + J + S_s + F_f + S_sF_f + T + S_sT + F_fT + S_sF_fT + T^2 + S_sT^2 + F_fT^2 + S_sF_fT^2 + COV + e_{ijsf},$$

where Y_{ijsf} = response variable, μ = overall mean, B_i = random effect of block (i = 1 to 13), $C(B_iS_sF_f)_j$ = random effect of cow (j = 1 to 4) within block and treatment diet, J = random effect of Julian date, S_s = fixed effect of SC (s = 1 to 2), F_f = fixed effect of SF (f = 1 to 2), S_sF_f = interaction between SC and SF, T = linear fixed effect of sampling day PP, S_sT = interaction between SC and day PP (linear), F_fT = interaction between SF and day PP (linear), S_sF_fT = interaction between SC, SF, and day PP (linear), T^2 = quadratic fixed effect of sampling day PP, S_sT^2 = interaction between SC and day PP (quadratic), F_fT^2 = interaction between SF and day PP (quadratic), $S_sF_fT^2$ = interaction between SC, SF, and day PP (quadratic), COV = TP covariate variable corresponding to the response variable, and e_{ijsf} = residual error. Day postpartum was included in the model as a continuous measure for the analysis of linear and quadratic interactions between main effects and sampling day PP during TP and CO periods. Blood variables analyzed in samples collected prepartum were included in the model as covariates for analysis of variables for the TP.

Liver and adipose tissue variables were analyzed using the Fit model procedure of JMP Pro (version 14.2, SAS Institute Inc.) according to the following model:

$$Y_{ijosf} = \mu + B_i + C(B_i)_j + J + O_o + S_s + F_f + S_sF_f + COV + e_{ijosf},$$

where Y_{ijosf} = response variable, μ = overall mean, B_i = random effect of block (i = 1 to 13), $C(B_i)_j$ = random effect of cow (j = 1 to 4) within block, J = random effect of Julian date, O_o = day offset from fixed weekly sampling day (o = −3 to +3), S_s = fixed effect of SC

(s = 1 to 2), F_f = fixed effect of SF (f = 1 to 2), S_sF_f = interaction between SC and SF, COV = TP covariate variable corresponding to the response variable, and e_{ijosf} = residual error.

Normality of the residuals was checked with normal probability and box plots and homogeneity of variances with plots of residuals versus predicted values. Goodness of fit to the normal distribution was also tested with the Shapiro-Wilk test, and variables were transformed when necessary to fit a normal distribution. Data from the last 4 wk of the CO period from a cow receiving the HS-DGC treatment were removed because of abnormal recovery from an udder infection from eczema. Treatment effects were declared significant at $P < 0.05$ and tendencies at $P < 0.10$. Interactions were declared significant at $P < 0.10$ and tendencies at $P < 0.15$, with mean separation test performed using Tukey's honestly significant difference test for SC × SF interactions with $P < 0.15$.

RESULTS

Production and Metabolism

Companion publications from this study report production (e.g., DMI, milk yield, and components) and blood metabolites and hormone results (Albornoz and Allen, 2018) and energy intake, energy balance, and milk fatty acids concentrations and yields (Albornoz et al., 2019). In these companion publications, we reported that during the TP, HMC decreased intakes of DM and NE_L more when included in the HS (3.9 kg/d and 3.2 Mcal/d) than in the LS (0.9 kg/d and 0.6 Mcal/d) diets, and HMC decreased yields of milk, fat, protein, 3.5% FCM, and milk NE_L by 4.3 kg/d, 0.19 kg/d, 0.18 kg/d, 4.8 kg/d, and 2.8 Mcal/d, compared with DCG, respectively. Energy balance was improved by HS compared with LS (−14.7 vs. −16.8 Mcal/d). Of interest was the interaction observed for BHB, with DGC increasing concentrations of plasma BHB compared with HMC in HS diets (13.4 vs. 7.90 mg/dL) but decreasing its concentration compared with HMC in LS diets (10.4 vs. 14.2 mg/dL; interaction, $P = 0.02$; Table 3). During the CO period, treatment effects on DMI diminished over time, with no main effects of treatment for the entire period. However, treatments interacted to affect yields of milk, milk fat, and FCM during the CO period, which were greater for HS-DGC and LS-HMC (54.8 and 52.8, 1.76 and 1.81, and 51.3 and 52.2 kg/d, respectively) than for LS-DGC and HS-HMC (51.2 and 51.0, 1.68 and 1.64, and 48.4 and 48.6 kg/d, respectively).

Markers of Inflammation

During the TP, haptoglobin and LBP concentrations decreased over time, with all treatments reaching similar concentrations by wk 3 PP, but during wk 1 and wk 2 PP, haptoglobin concentration was greatest for HMC for HS and lowest for LS compared with the DGC treatments, which were intermediate and similar to each other ($P = 0.07$, quadratic), whereas LBP was greater for HS-HMC and LS-DGC compared with HS-DGC and LS-HMC ($P = 0.02$, quadratic; Table 3; Figure 1A, B). Treatments interacted to affect concentrations of haptoglobin, LBP, and TNFα ($P < 0.04$), with greater concentrations for HMC compared with DGC with HS (0.45 vs. 0.37 mg/mL, 5.94 vs. 4.48 μg/mL and 9.38 vs. 7.45 pg/mL, respectively) and lower concentrations for HMC compared with DGC with LS (0.27 vs. 0.41 mg/mL, 4.30 vs. 5.87 μg/mL, and 4.76 vs. 12.9 pg/mL, respectively). Over the TP, HMC tended to decrease the concentration of the negative acute phase protein albumin for HS (3.24 vs. 3.34 g/dL) and increase its concentration for LS (3.35 vs. 3.29 g/dL) compared with DGC (interaction $P = 0.14$). Albumin tended to increase quadratically over time for SC treatments ($P = 0.13$, quadratic) and SF treatments ($P = 0.10$, quadratic). During the CO period, treatments did not affect concentrations of albumin, but the concentration of haptoglobin was similar between SC treatments early in the CO period but decreased more for LS compared with HS by the end of the period ($P = 0.10$; Table 3).

Markers of Oxidant Status

Over the TP, HMC increased RONS compared with DGC (8.8 relative fluorescence units/μL; $P = 0.02$; Table 3), increasing RONS quadratically over time compared with DGC, with greater concentrations during wk 2 PP ($P = 0.03$, quadratic) but similar concentrations by wk 3 PP. No overall effects of treatment were observed for AOP but both SC and SF affected AOP quadratically over time, with HS increasing AOP during wk 2 PP compared with LS ($P = 0.03$, quadratic) and HMC increasing AOP during wk 1 PP compared with DGC, with the difference diminishing over time ($P = 0.07$, quadratic). Whereas no overall effects of treatment were observed for OSi, OSi was greater for HMC than for DGC during wk 1 PP but the difference decreased over time ($P = 0.01$, quadratic).

Despite a lack of interaction among treatments during the CO period, AOP increased linearly over time for both SC treatments ($P = 0.02$, linear) and both SF treatments ($P = 0.02$, linear), but more for LS

Table 3. Effects of diet starch concentration (SC) and starch fermentability (SF) on markers of inflammation and oxidant status during the treatment period (1 to 23 d postpartum) and carryover period (24 to 72 d postpartum)

	LS[2]		HS[2]			*P*-value[3]						
Variable[1]	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF	Time	SC × Time	SF × Time	SC × SF × Time
During treatment period												
Albumin, g/dL	3.29	3.35	3.34	3.24	0.06	0.55	0.74	0.14	0.15^{LQ}	0.13^{Q}	0.10^{Q}	0.16
Haptoglobin,[4] mg/mL	0.41^{ab}	0.27^{b}	0.37^{ab}	0.45^{a}	0.15	0.20	0.48	0.04	$<0.01^{LQ}$	0.98	0.18	0.07^{LQ}
LBP,[4] μg/mL	5.87	4.30	4.48	5.94	0.14	0.86	0.93	0.03	$<0.01^{LQ}$	0.43	0.44	0.02^{LQ}
TNFα,[4] pg/mL	12.9^{a}	4.76^{b}	7.45^{ab}	9.38^{ab}	0.26	0.80	0.14	0.02	0.56	0.28	0.28	0.15
RONS, RFU/μL	51.2	61.2	50.6	58.2	3.67	0.62	0.02	0.75	0.07^{LQ}	0.87	0.03^{Q}	0.34
AOP, TE/μL	4.64	5.42	5.67	5.55	0.47	0.22	0.48	0.34	0.01^{LQ}	0.03^{Q}	0.07^{Q}	0.76
OSi[4]	11.9	11.4	7.69	11.2	0.17	0.19	0.33	0.23	$<0.01^{L}$	0.24	0.01^{Q}	0.18
BHB,[4] mg/dL	10.4^{ab}	14.2^{a}	13.4^{ab}	7.90^{b}	0.17	0.33	0.54	0.02	0.01^{Q}	0.31	0.16	0.01^{LQ}
During carryover period												
Albumin, g/dL	3.46	3.50	3.52	3.49	0.05	0.61	0.93	0.50	0.26	0.53	0.68	0.53
Haptoglobin,[4] mg/mL	0.38	0.37	0.41	0.35	0.13	0.87	0.47	0.57	0.01^{L}	0.10^{L}	0.21	0.45
RONS, RFU/μL	45.0	41.9	46.5	45.4	5.85	0.67	0.72	0.86	0.32	0.48	0.67	0.28
AOP, TE/μL	7.21	7.40	7.04	6.93	1.10	0.77	0.97	0.89	0.04^{LQ}	0.02^{L}	0.02^{L}	0.20
OSi[4]	6.34	5.69	6.30	6.64	0.18	0.74	0.90	0.72	$<0.01^{L}$	0.84	0.15^{L}	0.09^{L}

[a,b]Means in the same row with different superscript letters differ.

[1]LBP = LPS binding protein; TNFα = tumor necrosis factor α; RONS = reactive oxygen and nitrogen species; AOP = antioxidant potential; OSi = oxidative stress index (RONS/AOP); RFU = relative fluorescence units; TE = Trolox equivalents.

[2]LS, low starch = 22% starch, HS, high starch = 28% starch, DGC = dry ground corn, HMC = high moisture corn.

[3]Time = day postpartum. Polynomial interactions are identified with superscripts L = linear and Q = quadratic.

[4]Data were log-transformed to fit normal distribution and least squares means were back-transformed.

compared with HS, and more for DGC compared with HMC after the second week of the CO period (Table 3). Treatments interacted over time to linearly decrease OSi, with all treatments reaching similar values by the end of the CO period, but LS-HMC and HS-DGC decreased OSi compared with LS-DGC and HS-HMC over most of the CO period ($P = 0.09$, linear).

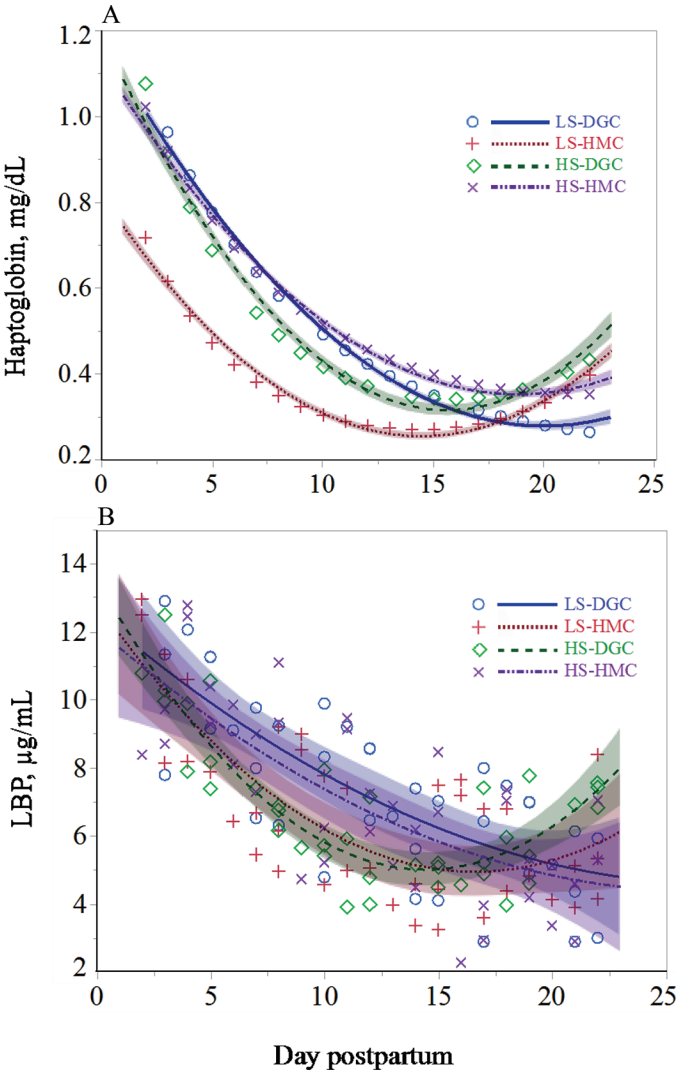

Figure 1. Effects of diet starch concentration and fermentability on blood plasma concentration of (A) haptoglobin ($P = 0.07$, quadratic) and (B) LPS binding protein (LBP; $P = 0.02$, quadratic) during treatment period (1 to 23 d postpartum). Treatments are represented as 22% starch with dry ground corn (LS-DGC), 22% starch with high moisture corn (LS-HMC), 28% starch with dry ground corn (HS-DGC), and 28% starch with high moisture corn (HS-HMC). Haptoglobin concentration decreased quadratically ($P < 0.0001$) over days postpartum for all treatments. Concentration of LBP decreased linearly over days postpartum for HS-HMC ($P < 0.0001$) and quadratically ($P < 0.0001$) for LS-DGC, LS-HMC, and HS-DGC. Shaded areas represent the 95% confidence interval for each treatment.

Hepatic Triglyceride Concentration and Gene Expression

Treatments did not affect triglyceride concentration in liver but we observed effects on hepatic abundance of mRNA (Table 4). For genes associated with glucose metabolism in liver, HMC increased expression of *NR2C2*, *FBP1*, *G6PC*, *PC*, and *PCK1* compared with DGC when included in an HS diet, and decreased their expression compared with DGC when included in an LS diet ($P = 0.01$, $P = 0.12$, $P = 0.07$, $P = 0.08$, and $P = 0.08$, respectively). The HS treatment decreased expression of *GCGR* and *LIPIN2* ($P = 0.04$ and $P = 0.09$, respectively; Table 4) compared with LS. Treatments interacted to affect abundance of *AHSG*, *CD204*, *CD206*, and *SOCS1* ($P < 0.08$), with HMC increasing their expression for HS and decreasing their expression for LS compared with DGC. Treatment effects on additional genes are presented in Supplemental Table S3 (https://doi.org/10.3168/jds.2019-16398).

Adipose Tissue Gene Expression

Relative abundance of key regulators of lipogenesis (*AGPAT2*, *GLUT4*, *GPAT1*, *LPL*, *PGK1*, and *SREBF1*) increased or tended to increase ($P = 0.02$, $P = 0.10$, $P = 0.09$, $P = 0.06$, $P = 0.09$, and $P < 0.01$, respectively) for HS compared with LS, and DGC increased expression of *ADIPOR2* and *GLUT4* compared with HMC ($P = 0.03$ and $P = 0.04$, respectively; Table 4). Abundance of mRNA for *LIPE*, encoding the lipolysis enzyme hormone sensitive lipase, decreased for HMC when included in HS and increased when included in LS compared with DGC ($P = 0.11$). Related to fatty acid transport genes, expression of *FATP3* tended to decrease for HMC with HS and increase with LS compared with DGC ($P = 0.13$). Finally, expression of the antigen *CD44* increased for HMC compared with DGC ($P = 0.03$). Treatment effects on additional genes are presented in Supplemental Table S4 (https://doi.org/10.3168/jds.2019-16398).

DISCUSSION

Treatment Period

The objective of our study was to evaluate the interaction between metabolic stress occurring during the early PP period and the concentration and fermentability of starch in dairy cattle diets. During the TP, LS-DGC and HS-HMC elicited a more pronounced inflammatory response, identified by the increase in plasma concentration of the positive acute phase pro-

teins haptoglobin and LBP and the proinflammatory cytokine TNFα, and the decrease in plasma concentration of the negative acute phase protein albumin compared with LS-HMC and HS-DGC. Evidence suggests that increasing the concentration of grain in the diet (from 15 to 30% of diet DM) with a highly fermentable starch source (e.g., rolled barley) increases blood concentration of markers of inflammation (Emmanuel et al., 2008), but this response did not occur with a lower concentration of grain (from 9.9 to 19.9% of diet DM) using a starch source with moderate ruminal fermentability (e.g., dry ground corn; Gott et al., 2015). The increase in inflammatory response in the Emmanuel et al. (2008) study was attributed to an increased absorption of LPS derived from lysis of gram-negative bacteria in the gastrointestinal tract as indicated by an increased concentration of serum LBP. In accordance, we observed a more pronounced inflammatory response and increase in LBP in cows fed a more fermentable starch source in HS diets; however, the opposite occurred in LS diets and reasons for this are not clear.

Inflammation has implications on energy requirements and partitioning, and it has been estimated that a challenged immune system (with jugular infusion of LPS) increases glucose demand by up to 1 kg within 12 h post-challenge in mid-lactation cows (Kvidera et al., 2017). Treatments that increased inflammation also decreased plasma BHB and increased lactate concen-

Table 4. Effects of diet starch concentration (SC) and starch fermentability (SF) on hepatic triglyceride (TG) content and hepatic and adipose mRNA abundance at 20 d postpartum

	LS[1]		HS[1]			*P*-value		
Variable	DGC	HMC	DGC	HMC	SEM	SC	SF	SC × SF
Hepatic TG content, mg/g of wet weight	10.7	8.52	9.90	9.51	1.59	0.95	0.44	0.40
Hepatic mRNA abundance[2]								
Carbohydrate metabolism								
FBP1	1.30[a]	1.20[a]	1.12[a]	1.42[a]	0.157	0.84	0.45	0.12
G6PC	1.59[a]	1.32[a]	1.25[a]	1.57[a]	0.203	0.78	0.88	0.07
GCGR	1.13	1.10	0.97	0.98	0.072	0.04	0.91	0.76
NR2C2	1.23[a]	1.01[a]	0.98[a]	1.21[a]	0.087	0.74	0.97	0.01
PC	1.00[a]	0.81[a]	0.73[a]	0.95[a]	0.121	0.61	0.92	0.08
PCK1	1.92[a]	1.53[a]	1.47[a]	1.80[a]	0.224	0.67	0.88	0.08
Fatty acid metabolism								
LIPIN2	1.42	1.40	1.15	1.18	0.142	0.09	0.97	0.83
Inflammation								
AHSG	1.99[a]	1.80[a]	1.49[a]	2.05[a]	0.210	0.53	0.37	0.07
CD204	1.27[a]	0.88[ab]	0.82[b]	1.11[ab]	0.101	0.24	0.59	<0.01
CD206	1.14[a]	1.05[a]	0.92[a]	1.09[a]	0.072	0.24	0.57	0.08
SOCS1	1.44[a]	0.90[a]	1.23[a]	2.32[a]	0.420	0.19	0.58	0.07
Adipose mRNA abundance[3]								
Lipogenesis								
AGPAT2	0.23	0.51	1.10	0.82	0.283	0.02	1.00	0.24
ADIPOR2	1.07	0.84	1.40	0.87	0.175	0.29	0.03	0.36
GLUT4	1.14	0.76	1.43	1.07	0.190	0.10	0.04	0.94
GPAT1	0.83	1.08	1.56	1.17	0.225	0.09	0.80	0.18
LPL	0.62	0.88	1.05	1.29	0.209	0.06	0.25	0.97
PGK1	0.82	0.86	0.95	0.92	0.061	0.09	0.90	0.56
SREBF1	0.51	0.75	1.35	1.11	0.180	<0.01	0.98	0.22
Lipolysis								
LIPE	1.07[a]	1.12[a]	1.33[a]	0.93[a]	0.143	0.80	0.23	0.11
Fatty acid transport								
FATP3	0.96[a]	1.16[a]	1.45[a]	1.10[a]	0.169	0.23	0.70	0.13
Inflammation								
CD44	0.71	1.11	0.85	1.25	0.177	0.40	0.03	0.97

[a,b]Means in the same row with different superscript letters differ.

[1]LS, low starch = 22% starch, HS, high starch = 28% starch, DGC = dry ground corn, HMC = high moisture corn.

[2]Values are least squares means of the relative expression of genes *FBP1* = fructose-1,6-bisphosphatase 1; *G6PC* = glucose-6-phosphatase; *GCGR* = glucagon receptor; *NR2C2* = nuclear receptor subfamily 2 group C member; *PC* = pyruvate carboxylase; *PCK1* = phosphoenolpyruvate carboxykinase 1; *LIPIN2* = lipin 2; *AHSG* = fetuin A; *CD204* = cluster of differentiation 204; *CD206* = cluster of differentiation 206; *SOCS1* = suppressor of cytokine signaling.

[3]Values are least squares means of the relative expression of genes *ADIPOR2* = adiponectin receptor 2; *AGPAT2* = 1-acylglycerol-3-phosphate O-acyltransferase 2; *GPAT1* = glycerol-3-phosphate acyltransferase; *GLUT4* = glucose transporter type 4; *LPL* = lipoprotein lipase; *PGK1* = phosphoglycerate kinase 1; *SERBF1* = sterol regulatory element-binding transcription factor 1; *LIPE* = lipase E; *FATP3* = fatty acid transport protein 3; *CD44* = CD44 antigen.

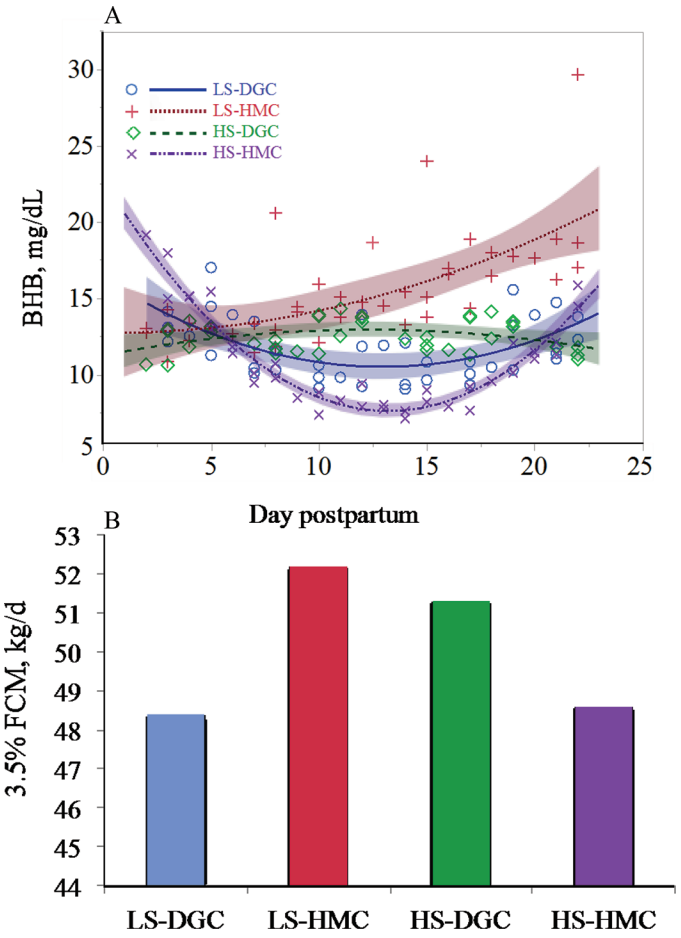

Figure 2. Effects of diet starch concentration (SC) and fermentability (SF) on blood plasma concentration of (A) BHB ($P = 0.01$, quadratic) during the treatment period (1 to 23 d postpartum) and (B) 3.5% FCM yield (interaction $P = 0.06$) during the carryover period (24 to 72 d postpartum). Treatments for BHB are represented as 22% starch with dry ground corn (LS-DGC), 22% starch with high moisture corn (LS-HMC), 28% starch with dry ground corn (HS-DGC), and 28% starch with high moisture corn (HS-HMC). Plasma BHB concentration increased over days postpartum linearly for LS-HMC ($P < 0.001$) and was affected quadratically for LS-DGC ($P < 0.0001$), HS-DGC ($P = 0.03$), and HS-HMC ($P < 0.0001$). Shaded areas represent the 95% confidence interval for each treatment.

trations during the TP and the opposite was observed for treatments that decreased inflammation (Albornoz and Allen, 2018; Table 3 and Figure 2A). Inflammatory challenges likely increase lactate concentration in blood from increased muscle protein catabolism (Doyle et al., 2011; Duan et al., 2016) and the shift in energy metabolism (from oxidative phosphorylation to glycolysis) in some activated immune cells (Palsson-McDermott and O'Neil, 2013). Further, low-grade inflammation can increase insulin resistance during the early PP period (Farney et al., 2013a) and this effect may have spared glucose for the immune system and mammary gland at the expense of increased use of BHB as an energy source by other tissues. However, increased insulin resistance normally promotes lipolysis and we observed an inconsistent relationship between the mobilization of body reserves (BCS, BW, backfat thickness) and plasma NEFA concentration among treatments that increased inflammation (Albornoz and Allen, 2018; Albornoz et al., 2019).

Elevated BHB during the early PP period has been associated with decreased health and productive performance (Ospina et al., 2010; Chapinal et al., 2012). However, ketone bodies are used as fuels by extrahepatic tissues and have beneficial effects when plasma glucose and insulin concentrations are low during the postpartum period (Allen and Piantoni, 2014). In addition, export of ketone bodies from the liver decreases the pool of acetyl CoA available for oxidation, possibly benefiting feed intake according to the hepatic oxidation theory (Allen et al., 2009). Therefore, although BHB is associated with decreased health and performance, it is not necessarily causal. The reduction in performance might be from excessive supply of acetyl CoA depressing feed intake according to the hepatic oxidation theory, and the health effects might be from other factors associated with BHB such as NEFA. The present results suggest that BHB may also be involved in modulating the inflammatory response during the early PP period. Previous studies that performed intramammary (Waldron et al., 2006; Zarrin et al., 2014) or intravenous (Werling et al., 1996) LPS challenges in bovines reported decreases in circulating BHB concentration, but mechanisms involved in reducing BHB and its role during an inflammatory response are not fully understood. Evidence suggests that BHB can act as an anti-inflammatory metabolite by inhibiting inflammation activators or signaling pathways (Grinberg et al., 2008; Fu et al., 2014; Youm et al., 2015) and therefore the increase in BHB by LS-HMC and HS-DGC might have been involved in modulating the inflammatory response. Supporting this notion, LBP was correlated positively with BHB, and hepatic abundance of markers of inflammation (*AHSG)* and macrophage infiltration (*CD206*) were negatively related to BHB (Supplemental Table S5; https://doi.org/10.3168/jds.2019-16398) during wk 3 PP. We are unable to discern whether the differences in BHB concentrations induced by treatments were from differences in energy partitioning and liver metabolism or from differences in short-chain fatty acid (e.g., butyric acid) production or absorption from the gastrointestinal tract.

Inflammation has been linked to decreased DMI, milk production (Bertoni et al., 2008; Trevisi et al., 2015), and energy balance (Esposito et al., 2014) in cows during

the early PP period. However, in our study, LS-DGC increased DMI and milk production (Albornoz and Allen, 2018) and decreased energy balance (Albornoz et al., 2019) compared with HS-HMC, with both treatments increasing inflammation. Similarly, HS-DGC increased DMI, milk production, and energy balance compared with LS-HMC (Albornoz and Allen, 2018; Albornoz et al., 2019), with both treatments decreasing inflammation. The discrepancy between results from previous studies and our results are likely associated with mechanisms involved in controlling feed intake and affecting production and energy balance. During the early PP period, different signals interact to control feed intake (e.g., metabolism of fuels, ruminal distention), and the relative contribution of each signal is dependent on several factors (e.g., degree of lipolysis, diet composition). In our study, the observed depression in feed intake by the highly fermentable HMC was likely related to hypophagic effects from propionate related to hepatic oxidation (Allen et al., 2009). In contrast, in studies associating feed intake depression with increased inflammation, a different mechanism may have been involved (e.g., signals from proinflammatory cytokines; Johnson and Finck, 2001; Dantzer and Kelley, 2007). It is possible that the degree of inflammation observed in cows in our study was not sufficient to depress feed intake and the hepatic oxidation of fuels was the dominant mechanism controlling feed intake. On the other hand, the concentrations of haptoglobin we observed in wk 1, on average, exceeded thresholds that were associated with decreased markers of liver function (Bertoni et al., 2008) and with impaired fertility in 2 large, multi-farm studies (Dubuc et al., 2012; Nightingale at al., 2015), which suggests a degree of inflammation that was not optimal for subsequent performance.

In agreement with the study reported by Yuan et al. (2013) in which a low-grade inflammation was induced with daily recombinant bovine TNFα injections for 7 d during the early PP period, we did not observe effects of treatments on liver triglyceride concentration. Consistent with our findings on plasma inflammatory markers, treatments that increased inflammation (LS-DGC and HS-HMC) increased hepatic abundance of suppressor of cytokine signaling (*SOCS1*), macrophage scavenger receptors (*CD204* and *CD206*), and the acute phase protein fetuin-A (*AHSG*) compared with treatments that decreased inflammation (LS-HMC and HS-DGC). Circulating LPS bound to LBP is first recognized by toll-like receptor-4 (TLR-4), and LPS-induced inflammation in mice revealed that *SOCS1* negatively regulates LPS responses (Kinjyo et al., 2002) and enhances expression of CD204 (Hashimoto et al., 2017), a suppressor of TLR-4-mediated inflammation (Ohnishi et al., 2011). This evidence further supports the notion that cows that elicited a more pronounced inflammatory response in our study (LS-DGC and HS-HMC) were exposed to higher concentration of circulating LPS derived from enteric gram-negative bacteria. Fetuin-A is normally characterized as a negative acute phase protein with anti-inflammatory properties under acute inflammation (Wang and Sama, 2012); however, under chronic inflammation, fetuin-A can be upregulated and stimulate proinflammatory cytokines (Dasgupta et al., 2010). It is possible that a long-term (~first 3 wk PP) increase in plasma LPS concentration (as presumed from the increase in LBP concentration during wk 1 and 2 PP) in cows fed HS-HMC and LS-DGC may have induced a prolonged inflammatory response and upregulated the expression of fetuin-A.

Oxidative stress represents an imbalance between pro- and antioxidants and the OSi is a reliable indicator of the shift between pro- and antioxidants during early lactation (Abuelo et al., 2013). There is an interrelation between oxidative stress and inflammatory response (Sordillo and Aitken, 2009), and feeding a high-starch diet (25.3 vs. 32.8%) was associated with increased ruminal LPS translocation and hepatic oxidative stress in mid-lactating dairy cows (Abaker et al., 2017). However, we did not observe an increase in OSi in cows that received HS or a relationship for OSi between the 2 treatments that increased inflammation (LS-DGC and HS-HMC). The effects observed by Abaker et al. (2017) were likely not observed in our study because the HS diet used in that study caused SARA (ruminal pH <5.8 for 270 min/d) that led to milk fat depression. Subacute ruminal acidosis increases ruminal LPS production and translocation into the bloodstream (Emmanuel et al., 2008; Khafipour et al., 2009) and LPS can predispose dairy cows to oxidative stress (Shi et al., 2016; Bromfield and Iacovides, 2017). In our study, we did not measure ruminal pH, but indications of severe ruminal pH disturbances (e.g., milk fat depression or fatty acids indicative of altered ruminal biohydrogenation) by treatments were not observed (Albornoz and Allen, 2018; Albornoz et al., 2019). Induction of SARA increased plasma LBP to levels 5 times greater than what we observed in wk 1 (Khafipour et al., 2009). Further, a previous study from our group did not report indications of SARA by cows fed diets with 21 or 32% starch containing either HMC or DGC as the primary starch source during early lactation (~55 d PP; Oba and Allen, 2003). Likely, in our study, cows experienced a low-grade inflammatory response compared with cows experiencing SARA and the risk for LPS-induced oxidative stress was lower.

Treatments affected the expression of genes associated with energy metabolism in liver and adipose tissue. In liver, abundance of key regulators of gluconeogenesis

(*NR2C2*, *FBP1*, *G6PC*, *PC*, and *PCK1*) were increased by treatments that elicited a more pronounced inflammatory response (LS-DGC and HS-HMC). However, effects on plasma glucose concentration did not accompany those results (Albornoz and Allen, 2018). Previous studies have reported conflicting results in hepatic expression of genes associated with glucose metabolism during an inflammatory event. Bradford et al. (2009) simulated a low-grade inflammation in late lactation cows with daily subcutaneous injections of recombinant bovine TNFα (2 μg/kg of BW) for 7 d and reported a decrease in abundance of *PCK1* and *G6PC* compared with control, whereas Yuan et al. (2013) using the same treatment regimen at lower and higher doses (1.5 and 3 μg/kg of BW) in cows during the early PP period did not observe differences in abundance of those same genes. Further, Garcia et al. (2015) reported an increase in PC abundance when liver slices were incubated with LPS (0.2 μg of LPS/mL of total culture medium), and the abundance was greater for slices collected from cows in the early PP period than from mid-lactation cows. These studies and our data suggest that adaptation of hepatic gluconeogenesis to an inflammatory state depends on the physiological state of cows and likely on the severity of the inflammatory response. Opposite to the effects observed in liver, it is apparent that abundance of genes associated with lipogenesis in adipose tissue was primarily related to the supply of glucogenic precursors to the cow rather than inflammation. During the TP, cows fed HS increased plasma concentration of glucose and insulin over time (Albornoz and Allen, 2018) and the abundance of key regulators of lipogenesis in adipose tissue (*AGPAT2*, *GLUT4*, *GPAT1*, *PGK1*, *SREBF1*, and *LPL*). Feeding HS likely increased energy partitioned toward lipogenesis, but this effect did not accompany the observed shift in mobilization of body reserves (BW, BCS, and backfat thickness; Albornoz and Allen, 2018), likely because lipolysis prevails over lipogenesis during the early PP period (Contreras et al., 2017b) and energy intake was different among treatments (Albornoz et al., 2019). These effects suggest that a low-grade systemic inflammation might modulate hepatic metabolism of glucose but has little effect on energy partitioning in adipose tissue during the early PP period.

Trevisi et al. (2015) reported lower milk yield from cows with increased haptoglobin and decreased albumin blood concentrations. We observed a similar relationship (Figure 3A, B) and observed relationships between several inflammatory markers analyzed and the profile and yield of milk fat and individual milk fatty acids (Supplemental Table S5 and Supplemental Figure S1; https://doi.org/10.3168/jds.2019-16398) during wk 3 PP. Future work to determine mechanisms involved and the meaning of those relationships might contribute to development of indirect tools to detect the inflammatory status of cows during the early PP period.

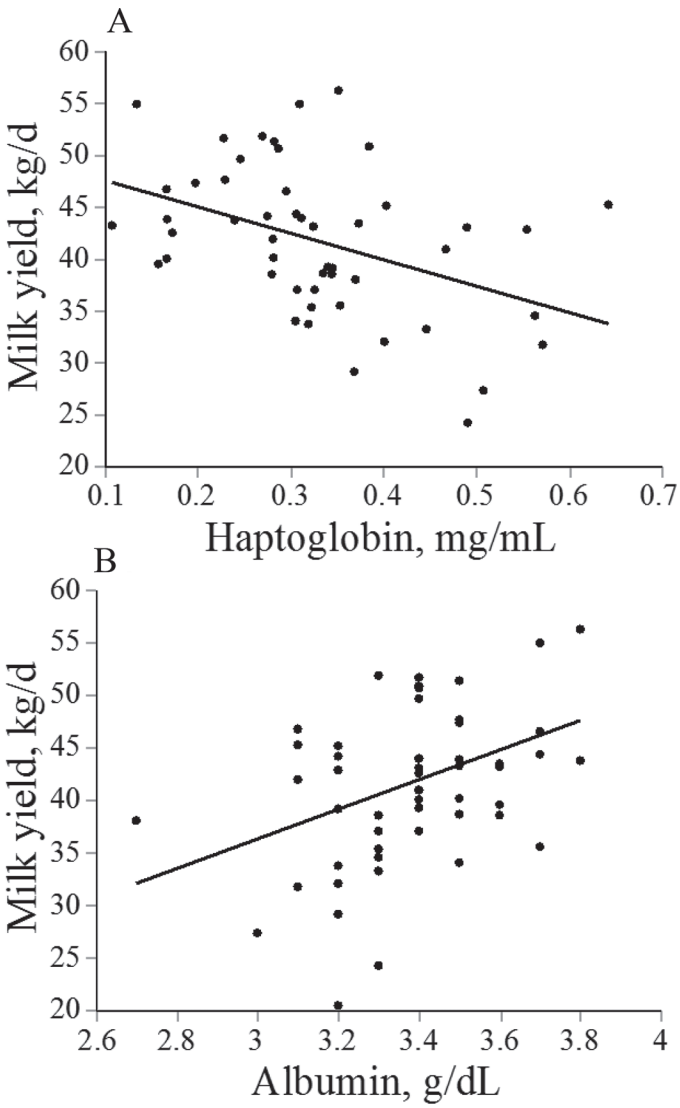

Figure 3. Relationship between milk yield and blood plasma concentration of haptoglobin and albumin during the third week postpartum. The solid lines represent the least square regressions for (A) milk yield and haptoglobin ($R^2 = 0.18$, $P < 0.01$) and (B) milk yield and albumin ($R^2 = 0.18$, $P < 0.01$).

Carryover Effects

During the CO period, cows received a common diet and effects of treatment on markers of inflammation diminished. However, during that same period, we observed improved oxidant status and production by treatments that decreased inflammation (LS-HMC and

HS-DGC) compared with those that increased inflammation (LS-DGC and HS-HMC) during the TP. Both LS-HMC and HS-DGC decreased OSi early in the CO period and increased yields of milk, fat, and 3.5% FCM (2.7, 0.125, and 3.25 kg/d, respectively) compared with LS-DGC and HS-HMC, without differences in DMI across all treatments (Albornoz and Allen, 2018). Previous studies also reported carryover effects on production, as well as health and reproductive performance, by cows with decreased or increased inflammation during the early PP period (Farney et al., 2013b; Nightingale et al., 2015; Huzzey et al., 2015; McCarthy et al., 2016). For example, Huzzey et al. (2015) reported that for each 1 mg/mL increase in plasma haptoglobin during the first 3 to 10 d PP, cows decreased 305-d mature-equivalent milk production by 464 ± 136 kg, and cows in third or greater parity that were administered the nonsteroidal anti-inflammatory drug sodium salicylate (123.3 ± 5.5 g/d) in water bowls during the first 7 d PP increased yields of milk and milk fat (2,469 ± 646 and 130 ± 29 kg, respectively) over the lactation compared with control cows of the same parity (Farney et al., 2013b). However, mechanisms to explain the relationship between inflammation and carryover effects on production have not yet been determined.

We observed that treatments that increased BHB concentration during the TP—LS-HMC and HS-DGC—also had positive effects on production during the CO period, increasing yield of 3.5% FCM (52.2 and 51.3 kg/d, respectively) compared with treatments that decreased BHB concentration during the early PP period (LS-DGC and HS-HMC; 48.4 and 48.6 kg/d, respectively; Figure 2A, B). A similar relationship was present in previous studies with cows receiving either nonsteroidal anti-inflammatory drugs (Farney et al., 2013a,b; Carpenter et al., 2016) or dietary treatments not related to SC or SF (Piantoni et al., 2015; de Souza, 2018) within the first 4 wk PP, and these effects were not consistent with DMI, production, or blood concentrations of hormones and other metabolites during the treatment period. As suggested previously, BHB can act as an anti-inflammatory metabolite. Supporting this notion, ruminal infusion with sodium butyrate increased the concentration of BHB in plasma in the bovine (Herrick et al., 2018), and supplementation with sodium butyrate reduced inflammation of the rumen epithelium and translocation of LPS into the bloodstream, contributing to a reduction in mammary gland cell apoptosis in goats (Dai et al., 2017; Chang et al., 2018). Reduction of cell apoptosis and necrosis for several cell types such as fibroblasts, neurons, myocytes, and glial and epithelial cells when BHB is increased has been summarized by Grinberg et al. (2008), and the balance between mammary cell proliferation and apoptosis rates are deemed important factors in determining persistency of lactation in bovines (Capuco et al., 2001). Extensive reviews in human literature have demonstrated the importance of BHB and butyrate as signaling molecules and inhibitors of histone deacetylases (Newman and Verdin, 2014; Woolf et al., 2016), and the effects of some inhibitors of histone deacetylases (HDAC) as anti-inflammatory agents (Adcock, 2007) and their role on epigenetics have been extensively reviewed in the human literature (Berni Canani et al., 2012; Hull et al., 2016). Accordingly, incubation of bovine mammary epithelial cells (MAC-T) with increasing sodium butyrate concentrations inhibited class I HDAC activity, and increased histone H3 acetylation after an LPS challenge in a concentration-dependent manner in both cases (Silva et al., 2018). Further, Ylioja et al. (2018) recently demonstrated that administering sodium salicylate to dairy cows during wk 1 PP increased global DNA methylation in mammary tissue, supporting the idea that modulating the inflammatory response during the early PP period might have epigenetic effects and alter long-term production responses. Future work investigating the mechanisms by which circulating BHB concentrations affect the inflammatory response and epigenetic effects in dairy cows could help elucidate some of the carryover effects observed in our study.

CONCLUSIONS

Consistent with our hypothesis, HMC elicited a more pronounced inflammatory response than DGC when included in HS, but failed to change oxidant status during the TP. However, DGC also increased the inflammatory response compared with HMC when included in LS. Whereas treatments that increased inflammation during the TP increased abundance of genes associated with gluconeogenesis, they did not affect triglyceride accumulation in liver. The reduction in inflammation by LS-HMC and HS-DGC during the TP could be associated with positive effects observed on production during the CO period (Albornoz and Allen, 2018). Signaling effects from circulating BHB could modulate the inflammatory response during the early PP period and long-term effects on production, but further research to elucidate the mechanisms involved in mediating this response in dairy cows is required.

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REFERENCES

Abaker, J. A., T. L. Xu, D. Jin, G. J. Chang, K. Zhang, and X. Z. Shen. 2017. Lipopolysaccharide derived from the digestive tract provokes oxidative stress in the liver of dairy cows fed a high-grain diet. J. Dairy Sci. 100:666–678.

Abuelo, A., J. C. Gandy, L. Neuder, J. Brester, and L. M. Sordillo. 2016. Short communication: Markers of oxidant status and inflammation relative to the development of claw lesions associated with lameness in early lactation cows. J. Dairy Sci. 99:5640–5648.

Abuelo, A., J. Hernandez, J. L. Benedito, and C. Castillo. 2013. Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. Animal 7:1374–1378.

Abuelo, A., J. Hernandez, J. L. Benedito, and C. Castillo. 2015. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J. Anim. Physiol. Anim. Nutr. (Berl.) 99:1003–1016.

Adcock, I. M. 2007. HDAC inhibitors as anti-inflammatory agents. Br. J. Pharmacol. 150:829–831.

Albornoz, R. I., and M. S. Allen. 2018. Highly fermentable starch at different diet starch concentrations decreases feed intake and milk yield of cows in the early postpartum period. J. Dairy Sci. 101:8902–8915.

Albornoz, R. I., K. J. Harvatine, and M. S. Allen. 2019. Diet starch concentration and starch fermentability affect energy intake and energy balance of cows in the early postpartum period. J. Dairy Sci. 102:5161–5171.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334.

Allen, M. S., and P. Piantoni. 2014. Carbohydrate nutrition: Managing energy intake and partitioning through lactation. Vet. Clin. North Am. Food Anim. 30:577–597.

Berni Canani, R., M. Di Costanzo, and L. Leone. 2012. The epigenetic effects of butyrate: Potential therapeutic implications for clinical practice. Clin. Epigenetics 4:4.

Bertoni, G., A. Minuti, and E. Trevisi. 2015. Immune system, inflammation and nutrition in dairy cattle. Anim. Prod. Sci. 55:943–948.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300–3310.

Bradford, B. J., and M. S. Allen. 2005. Phlorizin administration increases hepatic gluconeogenic enzyme mRNA abundance but not feed intake in late-lactation dairy cows. J. Nutr. 135:2206–2211.

Bradford, B. J., L. K. Mamedova, J. E. Minton, J. S. Drouillard, and B. J. Johnson. 2009. Daily injection of tumor necrosis factor-a increases hepatic triglycerides and alters transcript abundance of metabolic genes in lactating dairy cattle. J. Nutr. 139:1451–1456.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650.

Bromfield, J. J., and S. M. Iacovides. 2017. Evaluating lipopolysaccharide-induced oxidative stress in bovine granulosa cells. J. Assist. Reprod. Genet. 34:1619–1626.

Capuco, A. V., D. L. Wood, R. Baldwin, K. Mcleod, and M. J. Paape. 2001. Mammary cell number, proliferation, and apoptosis during a bovine lactation: Relation to milk production and effect of bST1. J. Dairy Sci. 84:2177–2187.

Carpenter, A. J., C. M. Ylioja, C. F. Vargas, L. K. Mamedova, L. G. Mendonça, J. F. Coetzee, L. C. Hollis, R. Gehring, and B. J. Bradford. 2016. Hot topic: Early postpartum treatment of commercial dairy cows with nonsteroidal antiinflammatory drugs increases whole-lactation milk yield. J. Dairy Sci. 99:672–679.

Chang, G., J. Yan, N. Ma, X. Liu, H. Dai, M. S. Bilal, and X. Shen. 2018. Dietary sodium butyrate supplementation reduces high-concentrate diet feeding-induced apoptosis in mammary cells in dairy goats. J. Agric. Food Chem. 66:2101–2107.

Chapinal, N., S. J. LeBlanc, M. E. Carson, K. E. Leslie, S. Godden, M. Capel, J. E. Santos, M. W. Overton, and T. F. Duffield. 2012. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J. Dairy Sci. 95:5676–5682.

Contreras, G. A., C. Strieder-Barboza, J. de Souza, J. Gandy, V. Mavangira, A. L. Lock, and L. M. Sordillo. 2017a. Periparturient lipolysis and oxylipid biosynthesis in bovine adipose tissues. PLoS One 12:e0188621.

Contreras, G. A., C. Strieder-Barboza, and W. Raphael. 2017b. Adipose tissue lipolysis and remodeling during the transition period of dairy cows. J. Anim. Sci. Biotechnol. 8:41.

Dai, H., X. Liu, J. Yan, Z. U. Aabdin, M. S. Bilal, and X. Shen. 2017. Sodium butyrate ameliorates high-concentrate diet-induced inflammation in the rumen epithelium of dairy goats. J. Agric. Food Chem. 65:596–604.

Dantzer, R., and K. W. Kelley. 2007. Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun. 21:153–160.

Dasgupta, S., S. Bhattacharya, A. Biswas, S. S. Majumdar, S. Mukhopadhyay, S. Ray, and S. Bhattacharya. 2010. NF-kappaB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. Biochem. J. 429:451–462.

de Souza, J. 2018. Changing the dietary ratio of fatty acids under different physiological conditions alters energy partitioning of dairy cows. PhD Thesis. Department of Animal Science, Michigan State University, East Lansing.

Doyle, A., G. Zhang, E. A. Abdel Fattah, N. T. Eissa, and Y. P. Li. 2011. Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. FASEB J. 25:99–110.

Duan, K., Q. Chen, M. Cheng, C. Zhao, Z. Lin, S. Tan, F. Xi, T. Gao, J. Shi, J. Shen, W. Li, W. Yu, J. Li, and N. Li. 2016. Hypothalamic activation is essential for endotoxemia induced acute muscle wasting. Sci. Rep. 6:38544.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2012. Risk factors and effects of postpartum anovulation in dairy cows. J. Dairy Sci. 95:1845–1854. https://doi.org/10.3168/jds.2011-4781.

Emmanuel, D. G., S. M. Dunn, and B. N. Ametaj. 2008. Feeding high proportions of barley grain stimulate an inflammatory response in dairy cows. J. Dairy Sci. 91:606–614.

Esposito, G., P. C. Irons, E. C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144:60–71.

Farney, J. K., L. K. Mamedova, J. F. Coetzee, B. KuKanich, L. M. Sordillo, S. K. Stoakes, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013a. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 305:R110–R117.

Farney, J. K., L. K. Mamedova, J. F. Coetzee, J. E. Minton, L. C. Hollis, and B. J. Bradford. 2013b. Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. J. Dairy Sci. 96:7709–7718.

Farney, J. K., L. K. Mamedova, B. H. Godsey, and B. J. Bradford. 2011. Technical note: Validation of an ELISA for measurement

of tumor necrosis factor alpha in bovine plasma. J. Dairy Sci. 94:3504–3509.
Fu, S. P., S. N. Li, J. F. Wang, Y. Li, S. S. Xie, W. J. Xue, H. M. Liu, B. X. Huang, Q. K. Lv, L. C. Lei, G. W. Liu, W. Wang, and J. X. Liu. 2014. BHBA suppresses LPS-induced inflammation in BV-2 cells by inhibiting NF-κB activation. Mediators Inflamm. 2014:983401.
Garcia, M., B. Bequette, and K. Moyes. 2015. Hepatic metabolic response of Holstein cows in early and mid lactation is altered by nutrient supply and lipopolysaccharide in vitro. J. Dairy Sci. 98:7102–7114.
Gott, P. N., J. S. Hogan, and W. P. Weiss. 2015. Effects of various starch feeding regimens on responses of dairy cows to intramammary lipopolysaccharide infusion. J. Dairy Sci. 98:1786–1796.
Grinberg, N., S. Elazar, I. Rosenshine, and N. Y. Shpigel. 2008. β-Hydroxybutyrate abrogates formation of bovine neutrophil extracellular traps and bactericidal activity against mammary pathogenic Escherichia coli. Infect. Immun. 76:2802–2807.
Gualdrón-Duarte, L. B., and M. S. Allen. 2018. Fuels derived from starch digestion have different effects on energy intake and metabolic responses of cows in the postpartum period. J. Dairy Sci. 101:5082–5091.
Guo, Y., X. Xu, Y. Zou, Z. Yang, S. Li, and Z. Cao. 2013. Changes in feed intake, nutrient digestion, plasma metabolites, and oxidative stress parameters in dairy cows with subacute ruminal acidosis and its regulation with pelleted beet pulp. J. Anim. Sci. Biotechnol. 4:31–41.
Harvatine, K. J., J. W. Perfield II, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849–854.
Hashimoto, R., R. Kakigi, K. Nakamura, S. Itoh, H. Daida, T. Okada, and Y. Katoh. 2017. LPS enhances expression of CD204 through the MAPK/ERK pathway in murine bone marrow macrophages. Atherosclerosis 266:167–175.
Hellemans, J., G. Mortier, A. De Paepe, F. Speleman, and J. Vandesompele. 2007. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 8:R19.
Herrick, K. J., A. R. Hippen, K. F. Kalscheur, D. J. Schingoethe, S. D. Ranathunga, J. L. Anderson, S. C. Moreland, and J. E. van Eys. 2018. Infusion of butyrate affects plasma glucose, butyrate, and β-hydroxybutyrate but not plasma insulin in lactating dairy cows. J. Dairy Sci. 101:3524–3536.
Hull, E. E., M. R. Montgomery, and K. J. Leyva. 2016. HDAC inhibitors as epigenetic regulators of the immune system: Impacts on cancer therapy and inflammatory diseases. BioMed Res. Int. 2016:8797206.
Huzzey, J. M., S. Mann, D. V. Nydam, R. J. Grant, and T. R. Overton. 2015. Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows. Prev. Vet. Med. 120:291–297.
Johnson, R. W., and B. N. Finck. 2001. Tumor necrosis factor alpha and leptin: Two players in an animal's metabolic and immunologic responses to infection. J. Anim. Sci. 79:118–127.
Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060–1070.
Kinjyo, I., T. Hanada, K. Inagaki-Ohara, H. Mori, D. Aki, M. Ohishi, H. Yoshida, M. Kubo, and A. Yoshimura. 2002. SOCS1/JAB is a negative regulator of LPS-induced macrophage activation. Immunity 17:583–591.
Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Fernandez, and L. H. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. J. Dairy Sci. 100:2360–2374.
Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25:402–408.
Mavangira, V., and L. Sordillo. 2018. Role of lipid mediators in the regulation of oxidative stress and inflammatory responses in dairy cattle. Res. Vet. Sci. 116:4–14.
McCarthy, M. M., T. Yasui, M. J. B. Felippe, and T. R. Overton. 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J. Dairy Sci. 99:680–700.
Miller, J. K., E. Brzezinska-Slebodzinska, and F. C. Madsen. 1993. Oxidative stress, antioxidants, and animal function. J. Dairy Sci. 76:2812–2823.
Newman, J. C., and E. Verdin. 2014. Ketone bodies as signaling metabolites. Diabetes Res. Clin. Pract. 106:173–181.
Nightingale, C. R., M. D. Sellers, and M. A. Ballou. 2015. Elevated plasma haptoglobin concentrations following parturition are associated with elevated leukocyte responses and decreased subsequent reproductive efficiency in multiparous Holstein dairy cows. Vet. Immunol. Immunopathol. 164:16–23.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.
Oba, M., and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174–183.
Ohnishi, K., Y. Komohara, Y. Fujiwara, K. Takemura, X. Lei, T. Nakagawa, N. Sakashita, and M. Takeya. 2011. Suppression of TLR4-mediated inflammatory response by macrophage class A scavenger receptor (CD204). Biochem. Biophys. Res. Commun. 411:516–522.
Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546–554.
Palsson-McDermott, E. M., and L. A. O'Neil. 2013. The Warburg effect then and now: From cancer to inflammatory diseases. BioEssays 35:965–973.
Piantoni, P., A. L. Lock, and M. S. Allen. 2015. Saturated fat supplementation interacts with dietary forage neutral detergent fiber content during the immediate postpartum and carryover periods in Holstein cows: Production responses and digestibility of nutrients. J. Dairy Sci. 98:3309–3322.
Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26:1231–1237.
Sgorlon, S., G. Stradaioli, G. Gabai, and B. Stefanon. 2008. Variation of starch and fat in the diet affects metabolic status and oxidative stress in ewes. Small Rumin. Res. 74:123–129.
Shi, H. Y., Y. M. Guo, Y. Liu, B. L. Shi, X. Y. Guo, L. Jin, and S. M. Yan. 2016. The in vitro effect of lipopolysaccharide on proliferation, inflammatory factors and antioxidant enzyme activity in bovine mammary epithelial cells. Anim. Nutr. 2:99–104.
Silva, L. G., B. S. Ferguson, A. S. Avila, and A. P. Faciola. 2018. Sodium propionate and sodium butyrate effects on histone deacetylase (HDAC) activity, histone deacetylation, and inflammatory gene expression in bovine mammary epithelial cells. J. Anim. Sci. https://doi.org/10.1093/jas/sky373.
Sordillo, L. M., and S. L. Aitken. 2009. Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol. 128:104–109.
Sordillo, L. M., and V. Mavangira. 2014. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim. Prod. Sci. 54:1204–1214.
Trevisi, E., N. Jahan, G. Bertoni, A. Ferrari, and A. Minuti. 2015. Pro-inflammatory cytokine profile in dairy cows: consequences for new lactation. Ital. J. Anim. Sci. 14:285–292.
Waldron, M. R., A. E. Kulick, A. W. Bell, and T. R. Overton. 2006. Acute experimental mastitis is not causal toward the development of energy-related metabolic disorders in early postpartum dairy cows. J. Dairy Sci. 89:596–610.

Wang, H., and A. E. Sama. 2012. Anti-inflammatory role of fetuin-A in injury and infection. Curr. Mol. Med. 12:625–633.
Werling, D., F. Sutter, M. Arnold, G. Kun, P. C. Tooten, E. Gruys, M. Kreuzer, and W. Langhans. 1996. Characterisation of the acute phase response of heifers to a prolonged low dose infusion of lipopolysaccharide. Res. Vet. Sci. 61:252–257.
Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501.
Woolf, E. C., N. Syed, and A. C. Scheck. 2016. Tumor metabolism, the ketogenic diet and β-hydroxybutyrate: Novel approaches to adjuvant brain tumor therapy. Front. Mol. Neurosci. 9:122.
Ylioja, C. M., A. J. Carpenter, M. Garcia, L. K. Mamedova, and B. J. Bradford. 2018. Anti-inflammatory treatment in early lactation alters global mammary DNA methylation. J. Dairy Sci. 101(E-Suppl.):271. (Abstr.)
Youm, Y. H., K. Y. Nguyen, R. W. Grant, E. L. Goldberg, M. Bodogai, D. Kim, D. D'Agostino, N. Planavsky, C. Lupfer, T. D. Kanneganti, S. Kang, T. L. Horvath, T. M. Fahmy, P. A. Crawford, A. Biragyn, E. Alnemri, and V. D. Dixit. 2015. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat. Med. 21:263–269.
Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8:e80316.
Zarrin, M., O. Wellnitz, H. A. van Dorland, J. J. Gross, and R. M. Bruckmaier. 2014. Hyperketonemia during lipopolysaccharide-induced mastitis affects systemic and local intramammary metabolism in dairy cows. J. Dairy Sci. 97:3531–3541.
Zebeli, Q., B. U. Metzler-Zebeli, and B. N. Ametaj. 2012. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. J. Dairy Sci. 95:2662–2672.
Zhou, Z., M. Vailati-Riboni, E. Trevisi, J. K. Drackley, D. N. Luchini, and J. J. Loor. 2016. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. J. Dairy Sci. 99:8716–8732.

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