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Reducing prepartum urine pH by supplementing anionic feed ingredients: Effects on physiological and productive responses of Holstein × Gir cows

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ABSTRACT

This study compared physiological and productive parameters in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate containing ammonium chloride to reduce urine pH near 7.0 (CON; n = 17), or a commercial anionic supplement to reduce urine pH near 6.0 (SUPP; n = 17). Nonlactating, multiparous, pregnant cows were assigned to receive SUPP or CON beginning 21 d before expected date of calving. Cows were maintained in a single drylot pen with ad libitum access to corn silage, and individually received their prepartum concentrate once daily (0800 h) before calving. Cows from both treatments completely consumed their concentrate allocation within 30 min after feeding. Cow body weight and body condition score were recorded once weekly, urine pH measured every 3 d, and blood samples collected on d −21, −14, −9, −6, and −3 relative to expected calving date. After calving (d 0), cows were moved to an adjacent drylot pen with ad libitum access to water and a total mixed ration, and were milked twice daily (0600 and 1700 h). Cow body weight and body condition score were recorded once weekly and individual milk production was recorded daily until 30 d in milk (DIM). Blood samples were collected before each milking during the first 5 DIM, as well as at 6, 9, 16, 23, and 30 DIM before the morning milking. Based on actual calving dates, cows received SUPP or CON for (mean ± standard error) 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. Urine pH was less in SUPP versus CON cows during the last 15 d of gestation (6.12 vs. 7.15, respectively). Milk yield during the first 5 DIM and throughout the experimental period was greater in SUPP versus CON cows (by 20 and 14%, respectively), whereas serum Ca concentrations did not differ between treatments during the first 5 DIM. Serum concentrations of fatty acids were greater in SUPP versus CON cows 3 d before and at calving (by 52 and 22%, respectively), whereas SUPP cows had lower serum glucose and cortisol concentration at calving (by 23 and 27%, respectively). Hence, the SUPP treatment decreased prepartum urine pH near 6.0 in Holstein × Gir dairy cows without depressing concentrate intake compared with CON, although total dry matter intake was not evaluated to fully investigate feed intake responses. Moreover, the SUPP treatment transiently affected serum glucose, fatty acids, and cortisol concentrations near the time of calving, and resulted in greater milk yield during the initial 30 DIM compared with CON.

Key words: anionic supplement, dairy cow, prepartum, urine pH

INTRODUCTION

Hypocalcemia is a disorder resulting from decreased blood Ca concentrations at the time of parturition known to depress immunocompetence and productivity of dairy cows (Daniel, 1983; Goff, 2008; Martinez et al., 2012). Feeding prepartum diets with low or negative DCAD is a traditional strategy to mitigate hypocalcemia in dairy herds (Block, 1994). These diets result in metabolic acidosis leading to reduced blood and urinary pH, increased Ca mobilization from bone to blood and urine, and enhanced intestinal absorption of Ca (Takagi and Block, 1991; Moore et al., 2000; Grunberg et al., 2011). Accordingly, blood Ca concentrations and milk yield postpartum were increased in dairy cows receiving diets with negative DCAD during the prepartum period (Moore et al., 2000; DeGroot et al., 2010; Weich et al., 2013).

Reducing DCAD is typically achieved with the addition of anionic salts such as ammonium chloride to concentrate or TMR, which may decrease their palatability and voluntary intake (Oetzel et al., 1991; Goff,

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2008). Indeed, a meta-analysis by Charbonneau et al. (2006) reported that reducing DCAD linearly decreased prepartum DMI, which can impair welfare and productive responses of dairy cows during lactation (Drackley, 1999). Charbonneau et al. (2006) also suggested that diets with DCAD near 0 mEq/kg result in urinary pH of 7.0 and mitigate hypocalcemia without major detriments to prepartum DMI, whereas lowering DCAD to acidify urine beyond pH of 7.0 substantially depresses DMI. Grunberg et al. (2011) reported that prepartum dairy cows fed to achieve urine pH near 7.0 experienced greater urinary calcium excretion before calving and increased plasma Ca concentration on the day after calving compared with cohorts with urine pH around 8.0. Nevertheless, urinary pH of 6.0 has been associated with enhanced prepartum Ca metabolism and postpartum performance in dairy cattle (DeGroot et al., 2010; Weich et al., 2013; Leno et al., 2017).

Recently, Leno et al. (2017) evaluated a commercial anionic supplement (**ANM**) used to formulate acidogenic prepartum diets, which was expected to preserve voluntary DMI by dairy cows. More specifically, these authors reported that ANM inclusion to yield negative DCAD and urine pH near 6.0 did not reduce prepartum DMI compared with control diets with positive DCAD. However, no other experimental studies examined the use of ANM to reduce urine pH below 7.0, particularly in Holstein × Gir cows in semi-confined systems typical of tropical regions of the planet. Cattle within these systems often receive concentrate separate from forage during the prepartum period (Leiva et al., 2015; Brandão et al., 2016), which may accentuate the detrimental effects of anionic supplements on concentrate intake. Holstein × Gir cows also have different productive and metabolic aspects compared with Holstein cattle (Madalena et al., 1979), the principal animal model used in DCAD and hypocalcemia research conducted to date. Based in this rationale, we hypothesized that including ANM in the prepartum concentrate fed to Holstein × Gir cows to reduce urine pH near 6.0 would not impair concentrate intake and would increase serum Ca concentrations and milk yield during early lactation compared with feeding ammonium chloride. Hence, the primary objective of this experiment was to compare serum Ca concentrations and milk production in Holstein × Gir cows receiving a concentrate containing ammonium chloride to reduce urine pH near 7.0, with cows receiving ANM to reduce urine pH near 6.0 during the prepartum period. A secondary objective was to determine whether treatment differences on serum Ca and milk production were associated with BW, BCS, and metabolic responses during the transition period, such as serum glucose, fatty acids, haptoglobin, cortisol, insulin, and IGF-I.

MATERIALS AND METHODS

This experiment was conducted at the São Paulo State University–Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. The animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the São Paulo State University Animal Ethics Committee (#108/2018).

Animals and Diets

Thirty-four nonlactating, multiparous, pregnant 3/4 Holstein × 1/4 Gir cows (BW = 611 ± 14 kg, BCS = 3.25 ± 0.06, 2.5 ± 0.1 parities) were ranked in a decreasing order by parity, then by BCS (Wildman et al., 1982) and by BW, and alternatingly assigned to receive a concentrate containing ANM (Animate; Phibro Animal Health, Teaneck, NJ; **SUPP**; n = 17) or ammonium chloride (**CON**; n = 17) beginning 21 d before expected date of calving. This allocation procedure was adopted to ensure that both treatment groups had similar parity, BW, and BCS at the beginning of the experiment.

Prior to calving, cows were maintained in a single drylot pen with ad libitum access to corn silage (1.5 m of linear bunk space/cow) and water. Cows individually received a limit-fed prepartum concentrate through self-locking head gates once daily (0800 h; Table 1). The CON concentrate was designed to result in a DCAD of 0 mEq/kg and urinary pH of 7.0 (Charbonneau et al., 2006) based on a predicted corn silage intake of 7.8 kg of DM/cow daily, estimated by the Spartan Dairy Ration Evaluator/Balancer (version 3.0, Michigan State University, East Lansing). In turn, ANM inclusion into the concentrate was designed to yield urinary pH of 6.0 as in Leno et al. (2017). More specifically, urine samples were scheduled to be collected every 3 d from d −21 to expected calving day, immediately before concentrate feeding (0800 h). A midstream urine sample from each cow was collected into a paper cup following manual stimulation below the vulva, and immediately analyzed with a portable glass electrode pH meter (DM22, Digimed Analítica Ltda, São Paulo, Brazil) that was calibrated daily (0600 h) with a 3-point calibration curve (pH 4, 7, and 10). Urine pH of each SUPP cow was used to regulate individual ANM inclusion rate into the concentrate, which was sustained when urinary pH approached the 6.0 target. The same

adjustment was not performed nor required for CON cows. It should be noted that basal concentrate intake of SUPP cows was constant; only ANM inclusion rate into the concentrate varied according to urinary pH. General composition and daily intake of concentrates are described in Table 1, and were formulated to be iso-caloric and iso-nitrogenous. Cows from both treatments completely consumed their concentrate allocation within 30 min after feeding.

After calving, cows from both treatments were moved to an adjacent single drylot pen with ad libitum access to water and a TMR (1.5 m of linear bunk space/cow). The TMR consisted (DM basis) of 47.8% corn silage and 52.2% concentrate (Table 1) and was formulated with the Spartan Dairy Ration Evaluator/Balancer (version 3.0) to yield 35 kg of milk/d. Cows were milked twice daily in a side-by-side milking system (0600 and 1700 h).

Sampling

During the entire experimental period, cows were monitored daily by 2 trained research technicians for incidence of health disorders, including retained placenta, ketosis, mastitis, and metritis by trained personnel as in Lima et al. (2012). Cows were also observed for clinical signs of hypocalcemia (Allen, 2018) including hypersensitivity and excitability (stage 1), inability to stand but maintenance of sternal recumbency (stage 2), and loss of consciousness (stage 3). No clinical health disorders were detected during the experiment. Cows were weighed on a platform scale (Precision Balanças, Tupã, SP, Brazil), and BCS was assessed according to Wildman et al. (1982) by the same 2 evaluators blinded to distribution of cows across treatments.

Samples of the offered corn silage, ingredients of prepartum concentrate, and lactation TMR were collected every 2 wk, pooled into 1 sample, and analyzed for nutrient content via wet chemistry procedures by a bromatology laboratory (3rlab, Belo Horizonte, Brazil). Calculations of ME used the equation proposed by NRC (2001). Concentration of DM was 47.3% in corn silage and its nutritive value (DM basis) was 47% NDF, 2.21 Mcal/kg of ME, 9.1% CP, 1.11% K, 0.01% Na, 0.11% S, and 0.16% Cl. Nutritive values of prepartum concentrates and lactation TMR are described in Table 1. The SUPP treatment provided a greater amount of Mg and S compared with CON, due to mineral composition of ANM (Table 1). Nevertheless, daily Mg and S intake by cows from both treatments were above requirements when predicted corn silage intake of 7.8 kg of DM/cow daily was accounted for (332 and 191% above requirements for absorbed Mg, 178 and 115% above requirements for absorbed S in SUPP and CON cows, respectively; NRC, 2001).

Precalving

Cow BW and BCS were scheduled to be recorded once weekly (d −21, −14, and −7) relative to expected calving date (d 0) before concentrate feeding (0800 h). Blood samples were also scheduled to be collected on d −21, −14, −9, −6, and −3 relative to expected calving date, immediately before concentrate feeding. Based on actual calving dates, SUPP and CON cows were assigned to treatments and experimental procedures on d 19.2 ± 1.2 and 19.0 ± 0.9 before calving, respectively. Hence, day of BW and BCS assessment, blood collection, as well as urine pH relative to actual calving date, was rounded into the nearest pre-scheduled sampling date.

Postcalving

Cows were evaluated for BW and BCS immediately after calving (d 0) and then once weekly, whereas individual milk production was recorded daily until 30 DIM. Milk samples were collected once a week from each cow following each milking of the day. More specifically, 50 mL were retrieved from a composite milk sampler (#AMS/200; Ambic Equipment Limited; Oxfordshire, UK) attached to each individual milk collector (GEA Farm Technologies, Bönen, Germany), immediately mixed with a bronopol preservative, and stored at 4°C. Samples from both milkings of the day were combined into 1 daily sample (100 mL) and shipped to a commercial laboratory (Clínica do Leite, Universidade de São Paulo, Piracicaba, Brazil) within 48 h of sampling. Milk samples were analyzed for SCC via flow cytometry (AOAC, 1990) with a Somacount 300 instrument (Bentley Instruments Inc., Chaska, MN), and concentrations of fat, lactose, protein, casein, and TS via infrared spectrometry (method 972.16; AOAC International, 1999). Daily milk yield was adjusted to FCM, ECM, or TS-corrected milk (NRC, 2001) based on milk concentrations of fat, protein, and TS of the concurrent week. Milk nutrient output was estimated based on milk composition and daily milk yield, and energy output calculated according to the NRC (2001). Blood samples were collected before each milking during the first 5 DIM (0600 and 1700 h), as well as at 6, 9, 16, 23, and 30 DIM before the morning milking (0600 h).

Table 1. Composition and nutritional profile of prepartum concentrates or lactation TMR offered to 3/4 Holstein × 1/4 Gir dairy cows[1]

Item	Prepartum		Lactation
	SUPP	CON	
Composition, % (DM basis)			
Corn silage	—	—	47.82
Ground corn	33.3	32.8	26.09
Soybean meal	33.7	43.6	23.22
Limestone	11.3	10.1	—
Sodium chloride	0.65	0.65	—
Dicalcium phosphate	3.95	3.72	—
Prepartum mineral mix A[2]	3.10	9.13	—
Prepartum mineral mix B[3]	—	—	—
Animate[4]	14.0	—	—
Lactation mineral mix[5]	—	—	2.61
Urea	—	—	0.260
Total intake, kg/d (DM basis)	2.91	2.96	Ad libitum
Nutritional value[6] (DM basis)			
DM, %	90.0	91.1	44.3
NDF, %	10.4	9.6	28.7
ME, Mcal/kg	2.74	2.68	2.58
CP, %	23.5	25.3	19.0
Ca, %	5.94	5.68	0.80
P, %	1.20	1.17	0.56
Mg, %	1.07	0.41	0.27
Cl, %	2.41	1.56	0.25
K, %	0.99	1.12	1.18
Na, %	0.44	0.39	0.22
S, %	1.04	0.57	0.23
Co, mg/kg	2.74	2.65	0.13
Cu, mg/kg	46.7	45.2	19.0
I, mg/kg	1.55	1.52	0.65
Fe, mg/kg	1,060	988	101
Mn, mg/kg	168	163	38.7
Se, mg/kg	0.82	0.77	0.46
Zn, mg/kg	177	170	70.6
Vitamin A, IU/kg	38,700	38,000	5,200
Vitamin D, IU/kg	9,700	9,500	1,300
Vitamin E, IU/kg	387	380	26.1
DCAD,[7] mEQ/100 g	−88.8	−33.8	20.1
Predicted DCAD,[8] mEQ/100 g	−11.3	0.0	—

[1]Prepartum concentrates were designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving. Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively.

[2]Containing 14% Ca, 1.5% S, 3.7% Na, 12,500 mg/kg of P, 4,375 mg/kg of Mn, 4,375 mg/kg of Zn, 2,400 mg/kg of methionine, 1,875 mg/kg of monensin, 1,125 mg/kg of Cu, 462 mg/kg of ethoxyquin, 125 mg/kg of biotin, 125 mg/kg of Mg, 75 mg/kg of Co, 50 mg/kg of I, 43.7 mg/kg of Cr, 22 mg/kg of Se, 63×10^9 cfu of *Saccharomyces*, 1,250,000 IU/kg of vitamin A, 312,500 IU/kg of vitamin D_3, and 12,500 IU/kg of vitamin E (NC Máxima K2 Pré-parto; Nutron Alimentos Ltda., Campinas, Brazil).

[3]Containing 18% NH_4Cl, 9% Ca, 3.2% S, 1.2% Na, 4,200 mg/kg of P, 1,460 mg/kg of Mn, 1,460 mg/kg of Zn, 800 mg/kg of methionine, 625 mg/kg of monensin, 375 mg/kg of Cu, 154 mg/kg of ethoxyquin, 41.7 mg/kg of biotin, 40 mg/kg of Mg, 25 mg/kg of Co, 16.7 mg/kg of I, 14.6 mg/kg of Cr, 7.5 mg/kg of Se, 21×10^9 cfu of *Saccharomyces*, 416,700 IU/kg of vitamin A, 104,200 IU/kg of vitamin D_3, and 4,167 IU/kg of vitamin E (NC Máxima K2 Aniônico; Nutron Alimentos Ltda.).

[4]Containing 38.0% CP, 3.2% ether extract, 3.1% sugar, 5.8% starch, 15.9% NDF, 1.4% Ca, 0.4% P, 4.8% Mg, 5.4% S, and 13.9% Cl according to the manufacturer (Phibro Animal Health Corp., Quincy, IL). Cows receiving SUPP consumed 0.476 ± 0.036 kg/d (as-fed basis) of this supplement during the prepartum period.

[5]Containing 22% Ca, 7.5% P, 6.5% Na, 1.0% K, 3.6% Mg, 2.0% S, 0.0005% Co, 0.040% Cu, 0.002% I, 0.040% Mn, 0.001% Se, 0.150% Zn, 200,000 IU/kg of vitamin A, 50,000 IU/kg of vitamin D_3, and 0.070% of vitamin E (Milk MAC, M. Cassab Tecnologia Animal, São Paulo, Brazil).

[6]Based on nutritional composition of concentrate ingredients analyzed via wet chemistry procedures by a commercial laboratory (3rlab, Belo Horizonte, Brazil).

[7]DCAD = [(Na % of DM/0.023) + (K % of DM/0.039)] − [(S % of DM/0.016) + (Cl % of DM/0.0355)].

[8]Cows were maintained in a single drylot pen with ad libitum access to corn silage (1.5 m of linear bunk space/cow) and water during the prepartum period. Expected DCAD was based on concentrate intake and predicted corn silage intake (7.8 kg/d of DM per cow) estimated by the Spartan Dairy Ration Evaluator/Balancer (version 3.0; Michigan State University, East Lansing).

Blood Analysis

Blood samples were obtained from either the coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL, Becton Dickinson and Company, Franklin Lakes, NJ), placed immediately on ice, centrifuged at 3,000 × *g* at 4°C for 30 min for serum collection, and stored at −20°C on the same day of collection. All samples were centrifuged within 5 min after collection to prevent degradation of metabolites and hormones. Samples collected during the first 5 DIM were analyzed for total serum Ca concentration (colorimetric kit #K051, Bioclin Diagnostics, Belo Horizonte, Brazil). Cows were classified with hypocalcemia when total serum Ca was ≤2.125 mmol/L (Martinez et al., 2016). Precalving (−21, −14, −9, −6, and −3 relative to expected calving date) and postcalving (0, 3, 6, 9, 16, 23, and 30 DIM) samples were also analyzed for serum concentrations of glucose (colorimetric kit #G7521; Pointe Scientific Inc., Canton, MI), fatty acids (colorimetric kit HR Series NEFA–2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA), haptoglobin (colorimetric assay; Cooke and Arthington, 2013), IGF-I, cortisol, and insulin (chemiluminescent enzyme immunoassay, Immulite 1000, Siemens Medical Solutions Diagnostics, Los Angeles, CA). The intra- and interassay CV were, respectively, 2.9 and 3.9% for glucose, 3.8 and 5.3% for fatty acids, 3.0 and 8.7% for haptoglobin, 4.8 and 6.3% for cortisol, and 4.0 and 5.0% for insulin. Serum IGF-I concentration was analyzed within a single assay, and the intraassay CV was 2.7%.

Statistical Analysis

Sample size was determined by the capacity of the research facility, as in previous studies from our group testing similar research designs and hypotheses (Leiva et al., 2015; Brandão et al., 2016). Our sample size of n = 17 per treatment would allow for detection of a 3.5 kg/d difference in milk yield between treatments (primary production response) based on 95% confidence, 80% power, and 3.0 kg/d standard deviation (G*power 3 software; Faul et al., 2007). Two cows from the SUPP treatment had twins and were removed from the experiment.

All statistical analyses were performed with SAS (version 9.4, SAS Institute Inc., Cary, NC), with cow as the experimental unit, cow(treatment) as the random variable, and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. All data were analyzed using values obtained from the sampling before the beginning of treatment administration when appropriate (d −21 relative to expected calving), and days receiving treatment before calving as independent covariates. All data were initially tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure (SAS Institute Inc.), and milk SCC data were not normally distributed (W = 0.41). Therefore, SCC data were log-transformed (base-10 log) to achieve normality (W = 0.96) for statistical analysis, and results back transformed to facilitate interpretation.

Primary Objectives. The effects of treatment on urine pH, serum Ca concentration, and milk production were modeled using linear mixed models (PROC MIXED). The model statement contained the fixed effects of treatment, time (hour, day, or week according to response and sampling schedule), and the resultant interaction. Previous 305-d mature-equivalent milk yield from each cow was also included as independent covariate for all analysis of daily milk production and composition. The specified term for the repeated statements varied according to response (hour, day, or week), and cow(treatment) was included as subject. The covariance structure used for all analyses was autoregressive, which yielded the lowest Akaike information criterion value. Prevalence of hypocalcemia (total serum Ca ≤2.125 mmol/L; Martinez et al., 2016) during the first 5 DIM was analyzed with a binomial distribution and logit link function, using a generalized linear mixed model (PROC GLIMMIX). The model statement contained the fixed effects of treatment, hour, and the resultant interaction.

Secondary Objectives. The effects of treatment on cow BW, BCS, and serum concentrations of glucose, fatty acids, cortisol, haptoglobin, insulin, and IGF-I were modeled using linear mixed models (PROC MIXED). Model statements, repeated statements, and covariance structure were the same as those used for milk production responses, without the inclusion of previous 305-d mature-equivalent milk yield as independent covariate. Analyses of the nonrepeated measures parity; BW and BCS change; and initial (no covariate included), postcalving, and final BCS and BW contained the fixed effect of treatment in the model statement.

General Criteria. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤0.10. Results reported are covariately adjusted LSM, whereas prevalence of hypocalcemia is reported as actual means. Moreover, results are presented according to main treatment effects if the treatment × time (hour, day, or week) interaction was >0.10, or within the time variable using the SLICE option when such interaction was ≤0.10.

RESULTS AND DISCUSSION

Based on allocation of cows to experimental treatments, SUPP and CON cows had similar ($P \geq 0.39$)

Table 2. Parity, BW, and BCS of 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving[1,2]

Item	SUPP	CON	SEM	*P*-value
Parity	2.6 (2.2 to 2.9)	2.4 (2.0 to 2.8)	0.2	0.50
BW, kg				
Initial BW (d −21), kg	598 (552 to 643)	625 (579 to 670)	22	0.39
Postcalving BW (d 0), kg	554 (514 to 594)	567 (530 to 605)	19	0.61
BW change (d −35 to 0), kg	−43 (−69 to −9)	−58 (−91 to −31)	14	0.30
Final BW (d 30), kg	542 (504 to 580)	549 (513 to 585)	18	0.77
BW change (d 0 to 30), kg	−12 (−29 to 5)	−18 (−34 to −2)	8	0.59
BCS[3]				
Initial BCS (d −21)	3.24 (3.04 to 3.42)	3.28 (3.10 to 3.46)	0.09	0.71
Postcalving BCS (d 0)	2.98 (2.82 to 3.15)	3.03 (2.87 to 3.18)	0.08	0.68
BCS change (d −21 to 0)	−0.25 (−0.35 to −0.14)	−0.26 (−0.37 to −0.16)	0.05	0.83
Final BCS (d 30)	2.81 (2.63 to 3.00)	2.82 (2.65 to 3.00)	0.08	0.95
BCS change (d 0 to 30)	−0.17 (−0.37 to 0.03)	−0.20 (−0.39 to −0.02)	0.09	0.77

[1]Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. Values within parentheses represent 95% CI. Values are reported as LSM within each sampling time.

[2]Prior to calving, BW and BCS were scheduled to be recorded once weekly (d −21, −14, and −7) relative to expected calving date (d 0) before concentrate feeding (0800 h). According to actual calving dates, BW and BCS were rounded into the nearest prescheduled sampling date. After calving, BW and BCS were recorded once weekly until d 30 of lactation.

[3]According to Wildman et al. (1982), and assessed by 2 evaluators that were blinded to distribution of cows across treatments.

parity, BW, and BCS (Table 2) at the beginning of the experiment.

Prepartum Urine pH and Concentrate Intake

A treatment × day interaction was detected ($P < 0.01$) for prepartum urine pH, being less ($P < 0.01$) in SUPP versus CON cows from d −15 to calving (Figure 1). Moreover, both SUPP and CON cows reached and maintained their target urine pH of approximately 6.0 and 7.0, respectively, from d −12 to calving (Figure 1). As designed, ANM intake by SUPP cows increased linearly from d −21 to −12 (day effect; $P < 0.01$), when their urine pH reached the target level, and averaged (±SE; as-fed basis) 504 ± 14 g/cow daily from d −12 until calving. It should be noted that corn silage intake was not evaluated herein to precisely determine prepartum DCAD in both treatments, which was predicted based on estimated corn silage intake as described in Table 1. However, both SUPP and CON treatments were designed to, and successfully reduced urine pH to the levels established by our hypothesis (Charbonneau et al., 2006; Leno et al., 2017). In addition, cows from both treatments completely consumed their concentrate allocation within 30 min after feeding; hence, prepartum concentrate intake was not affected by the addition of anionic salts or ANM to yield the target urinary pH. Prepartum concentrate was limit-fed as typical of semi-confined systems in tropical regions of the planet (Leiva et al., 2015; Brandão et al., 2016), whereas different outcomes could have been noted if concentrate was offered for ad libitum intake. Others have also associated reduced prepartum DMI as DCAD decreased with discomfort created by metabolic acidosis (Vagnoni and Oetzel, 1998; Charbonneau et al., 2006), whereas such outcome cannot be assessed herein due to the lack of total DMI evaluation during the prepartum period.

Serum Ca Concentrations and Milk Yield During the First 5 DIM

No treatment differences were detected ($P = 0.92$) for total serum Ca concentrations during the first 5 DIM (Figure 2), despite treatment differences in urinary pH (Figure 1). Prevalence of cows with hypocalcemia (serum Ca concentrations ≤2.125 mmol/L; Martinez et al., 2016) was also similar ($P = 0.72$) between treatments during this period (Figure 2). Although these outcomes diverge from the main hypothesis of this experiment and earlier research (Moore et al., 2000; Leno et al., 2017), others have also reported that reducing DCAD prepartum failed to increase total blood Ca concentrations in dairy cows during early lactating (DeGroot et al., 2010; Weich et al., 2013). Nevertheless, milk yield during the first 5 DIM was greater ($P = 0.05$) in SUPP versus CON cows (Figure 2), whereas colostrum and milk synthesis demand a substantial amount of Ca (Ramberg et al., 1970). Perhaps the SUPP treatment maintained serum Ca concentrations similar to that of CON cows despite increased Ca demand for milk production, although milk Ca concentration was not evaluated herein to fully support this rationale. Alternatively, lack of treatment effects on serum Ca concentrations and reduced prevalence of hypocalcemia

could be associated with moderate milk ability and subsequent Ca demand for milk synthesis of Holstein × Gir cattle, along with the parity of the cows evaluated in this experiment (Madalena et al., 1979; Rezac et al., 2014; Leno et al., 2017).

Overall Milk Yield and Components

Milk yield was also greater ($P = 0.04$) in SUPP versus CON cows throughout the experimental period (Figure 3; Table 3), whereas no treatment differences were detected ($P \geq 0.13$) for milk fat, total, casein, lactose, TS, and SCC (Table 3). Milk output of total protein, casein, and lactose were greater ($P \leq 0.02$) in SUPP versus CON cows, whereas milk output of energy, fat, and TS did not differ ($P \geq 0.12$) between treatments (Table 3). Moreover, milk casein output was greater in SUPP versus CON cows during the first week of lactation (0.56 vs. 0.43 kg/d of casein, respectively, during first 7 DIM; SEM = 0.03). Given that 65% of Ca in bovine milk is associated with casein (Griffin et al., 1988), it seems plausible that SUPP prevented the decrease in serum Ca concentrations during the first 5 DIM despite increased Ca excretion in milk. It should be noted that previous 305-d mature-equivalent milk yield was not a significant covariate for daily milk yield ($P = 0.72$) and constituents ($P \geq 0.33$) in the present experiment. Others have also reported increased milk yield in early-lactation dairy cows receiving prepartum diets with low or negative DCAD (Siciliano-Jones et al., 2008; Rezac et al., 2014; Leno et al., 2017), including research that failed to detect alterations in blood Ca concentrations (DeGroot et al., 2010; Weich et al., 2013). Such increase in milk yield has been attributed to improved metabolic health and increased postpartum DMI in cows fed reduced DCAD diets (DeGroot et al., 2010; Leno et al., 2017), although total feed intake was not evaluated herein. In contrast, the effects of prepartum DCAD on milk components have been variable, with research studies reporting increased milk lactose and reduced SCC (Weich et al., 2013), reduced milk fat (DeGroot et al., 2010), or no substantial changes in milk composition as DCAD decreased (Rezac et al., 2014; Leno et al., 2017). Likewise, milk composition was not affected by treatments in the present experiment, although numerical differences noted on milk fat and TS resulted in similar ($P \geq 0.12$) FCM, ECM, and TS-corrected milk between SUPP and CON cows (Table 3). Perhaps the sample size used herein prevented the numerical differences reported for milk fat, lactose, ECM, and TS-corrected milk from reaching statistical significance, despite being sufficient to detect treatment differences in milk yield and milk casein output.

Cow BW, BCS, and Physiological Responses

Cows experienced the BW, BCS, and physiological changes associated with calving and beginning of lacta-

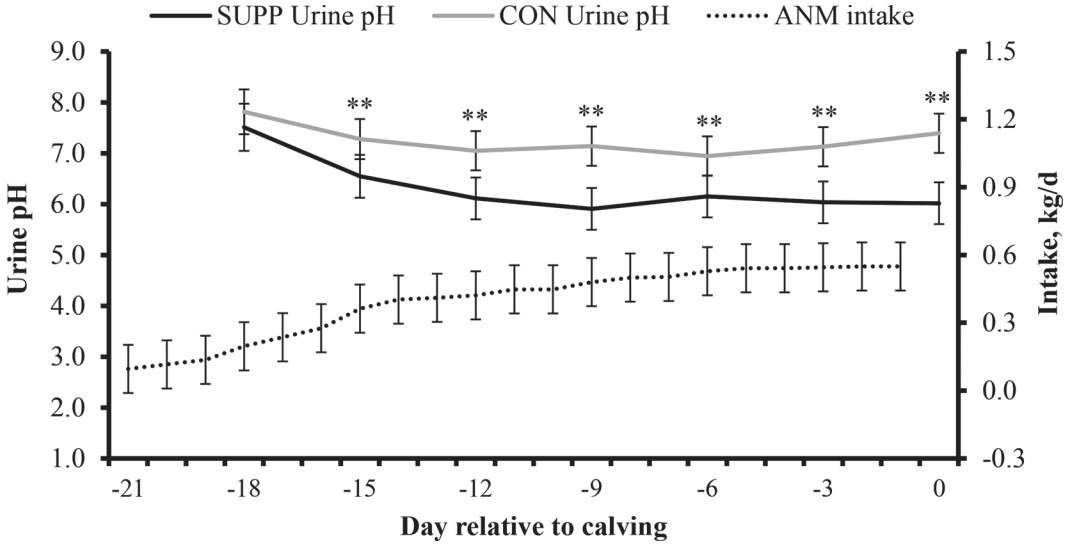

Figure 1. Urine pH (solid lines) of 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving. The dotted line represents intake of the anionic supplement (ANM) offered to SUPP cows. Error bars represent 95% CI. Based on actual calving dates, SUPP or CON cows began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively, and SUPP cows consumed 476 ± 37 g of ANM/d (as-fed basis). A treatment × day interaction was detected ($P < 0.01$) for urine pH and a day effect ($P < 0.01$) was detected for ANM intake by SUPP cows. Treatment comparison within days, $^{**}P < 0.01$.

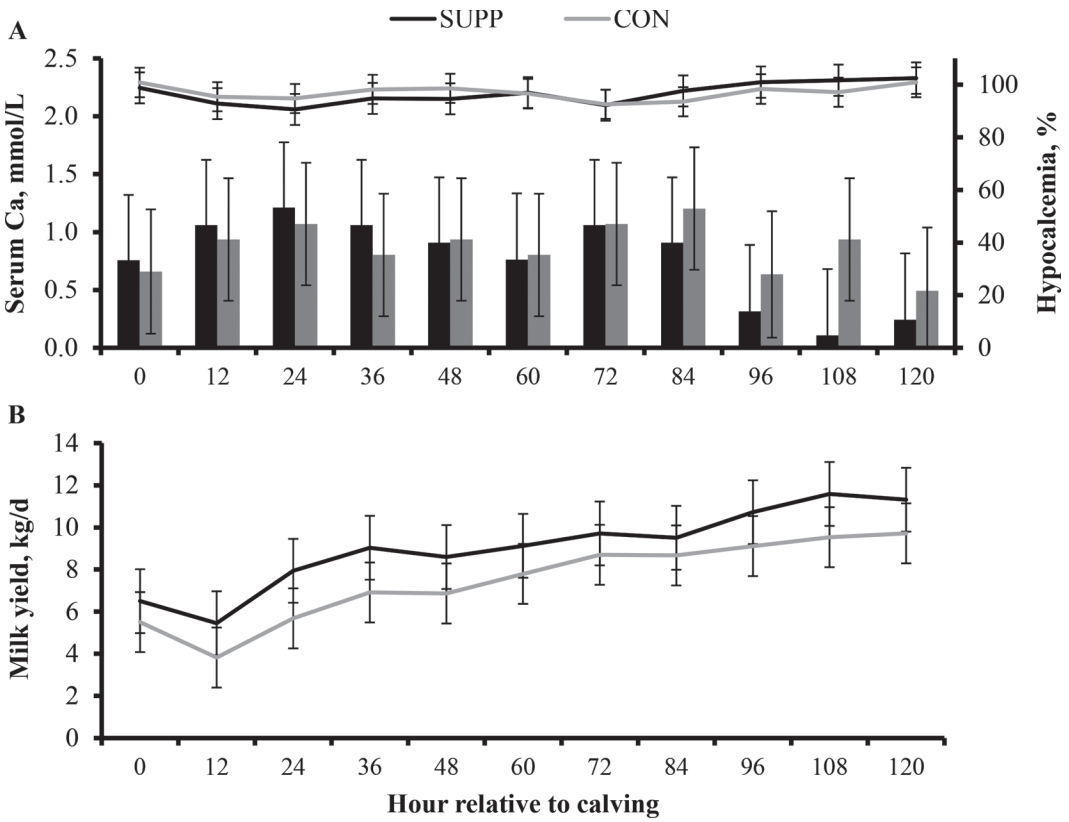

Figure 2. Serum concentrations of Ca (line graph; panel A) and prevalence of hypocalcemia (bar graph, cows with total serum Ca ≤2.125 mmol/L; panel A), as well as milk yield during the initial 120 h of lactation (panel B) in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving. Error bars represent 95% CI. Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. No treatment differences were detected for serum Ca and prevalence of hypocalcemia ($P \geq 0.72$), whereas mean milk yield was greater ($P = 0.05$) in SUPP versus CON cows [9.0 (7.8 to 10.3 CI) versus 7.5 (6.3 to 8.6 CI) kg/d, respectively; SEM = 0.5].

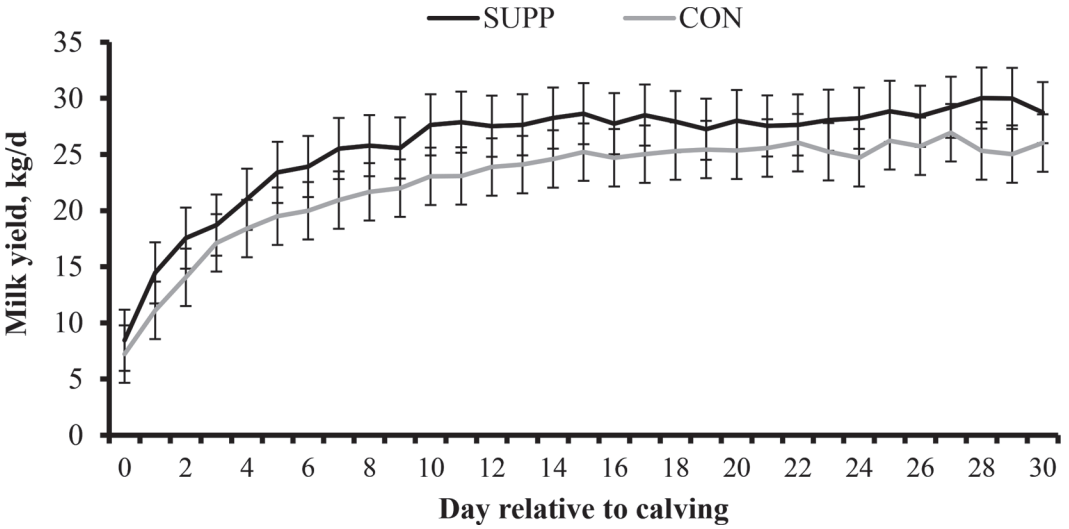

Figure 3. Milk yield of 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving. Error bars represent 95% CI. Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. Treatment and day effects were detected ($P = 0.04$ and <0.01, respectively), whereas the treatment × day interaction was not significant ($P = 0.32$). Mean milk yield was greater ($P = 0.04$) in SUPP versus CON cows [25.7 (23.3 to 28.2 CI) versus 22.5 (20.2 to 24.8 CI) kg/d, respectively; SEM = 1.1].

tion, as evidenced by temporal changes (day effects; $P < 0.01$) in BW, BCS (Table 2), and all serum metabolites and hormones (Figures 4 and 5; Vazquez-Añon et al., 1994; Jorritsma et al., 2003). No treatment differences in BW and BCS parameters were detected ($P \geq 0.30$) before and after calving (Table 2) or throughout the experimental period (data not shown), as previously reported by others (DeGroot et al., 2010; Rezac et al., 2014; Leno et al., 2017). Alternatively, lack of treatment differences in BW and BCS may also be associated with the limited sample size utilized in this experiment, despite numerical differences in BCS between SUPP and CON cows being biologically negligible (Roche et al., 2009).

No treatment differences were also detected ($P = 0.77$) for serum concentrations of IGF-I and insulin (Figure 4), which are key physiological markers of BW and BCS change, as well as nutrient status dairy cattle (Busato et al., 2002; Butler, 2003). These outcomes indicate that SUPP cows maintained BW, BCS, and nutritional status similar to CON cows despite differences noted in milk yield (Table 3), suggesting a potential increase in postpartum DMI in SUPP cows (DeGroot et al., 2010; Leno et al., 2017). In turn, a treatment × day interaction was detected ($P < 0.01$) for glucose and tended to be detected ($P = 0.08$) for serum fatty acids (Figure 5), which are also modulated by nutrient intake (Butler, 2003). Cows assigned to SUPP had less ($P < 0.01$) serum glucose concentrations at calving (d 0), and greater ($P \leq 0.01$) serum concentration of fatty acids on d −3 and at calving compared with CON cows. It seems plausible that glucose utilization and lipid mobilization near the time of calving were increased in SUPP cows to support their greater milk yield, particularly during the first 5 DIM, compared with CON cows (Grummer, 1995). Collectively, the SUPP treatment did not substantially modulate overall nutritional and metabolic status of Holstein × Gir cows during the transition period, besides transient changes in serum concentrations of glucose and fatty acids likely associated with increased milk yield in SUPP cows.

A tendency for treatment × day interaction was also detected ($P = 0.09$) for serum cortisol concentration, which was greater ($P = 0.03$) in CON versus SUPP

Table 3. Milk production during the first 30 DIM in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving[1,2]

Item	SUPP	CON	SEM	*P*-value
Milk production				
Milk yield, kg/d	25.7 (23.3 to 28.2)	22.5 (20.2 to 24.8)	1.1	0.04
FCM, kg/d	29.7 (26.5 to 32.7)	27.4 (24.5 to 30.3)	1.5	0.28
12% TS-corrected milk, kg/d	28.9 (26.1 to 31.6)	26.0 (23.4 to 28.5)	1.3	0.12
ECM, kg/d	30.0 (27.0 to 33.0)	27.3 (24.5 to 30.1)	1.4	0.18
Milk composition				
Fat, %	4.44 (4.04 to 4.84)	4.84 (4.46 to 5.22)	0.19	0.15
Protein, %	3.43 (3.31 to 3.55)	3.38 (3.27 to 3.49)	0.05	0.56
Casein, %	2.63 (2.53 to 2.74)	2.59 (2.49 to 2.69)	0.05	0.49
Lactose, %	4.56 (4.48 to 4.63)	4.48 (4.41 to 4.55)	0.03	0.13
TS, %	13.4 (13.0 to 13.9)	13.8 (13.4 to 14.2)	0.2	0.25
SCC,[3] cells/μL	130 (69 to 246)	132 (72 to 240)	1.35	0.97
Milk nutrient output				
Fat, kg/d	1.14 (1.00 to 1.28)	1.09 (0.96 to 1.22)	0.06	0.55
Protein, kg/d	0.89 (0.80 to 0.97)	0.76 (0.69 to 0.84)	0.04	0.03
Casein, kg/d	0.68 (0.62 to 0.74)	0.58 (0.53 to 0.64)	0.03	0.02
Lactose, kg/d	1.17 (1.06 to 1.28)	1.00 (0.90 to 1.11)	0.05	0.03
TS, kg/d	3.46 (3.14 to 3.79)	3.11 (2.81 to 3.42)	0.16	0.12
Energy, Mcal/d	33.8 (32.2 to 35.4)	32.4 (30.9 to 33.9)	0.76	0.19

[1]Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. Values within parentheses represent 95% CI. Values reported are LSM according to main treatment effects, given that no treatment × day interactions were detected ($P \geq 0.30$).

[2]Individual milk production was recorded daily until d 30 of lactation. Milk samples were collected once weekly from each cow following each milking of the day. More specifically, 50 mL was retrieved from the milk sampler (#AMS/200, Ambic Equipment Limited; Oxfordshire, UK) attached to each individual milk collector (GEA Farm Technologies, Bönen, Germany), immediately mixed with a bronopol preservative, and stored at 4°C. Samples from both milkings of the day were combined into 1 daily sample (100 mL), and shipped to a commercial laboratory (Clínica do Leite; Universidade de São Paulo, Piracicaba, Brazil) within 48 h of sampling. Milk samples were analyzed for SCC via flow cytometry (AOAC, 1990) with a Somacount 300 instrument (Bentley Instruments Inc., Chaska, MN), and concentrations of fat, lactose, protein, casein, and TS via infrared spectrometry (method 972.16; AOAC International, 1999). Daily milk yield was adjusted to FCM, ECM, or TS-corrected milk (NRC, 2001) based on milk concentrations of fat, protein, and TS, respectively, of the concurrent week. Milk nutrient output was estimated based on milk composition and milk yield, whereas energy output was calculated according to NRC (2001).

[3]Original SCC results were not normally distributed (Shapiro–Wilk W value = 0.41) and therefore were log-transformed (base-10 log) to achieve normality (Shapiro–Wilk W value = 0.96) for analysis. Results presented herein were back transformed to facilitate interpretation.

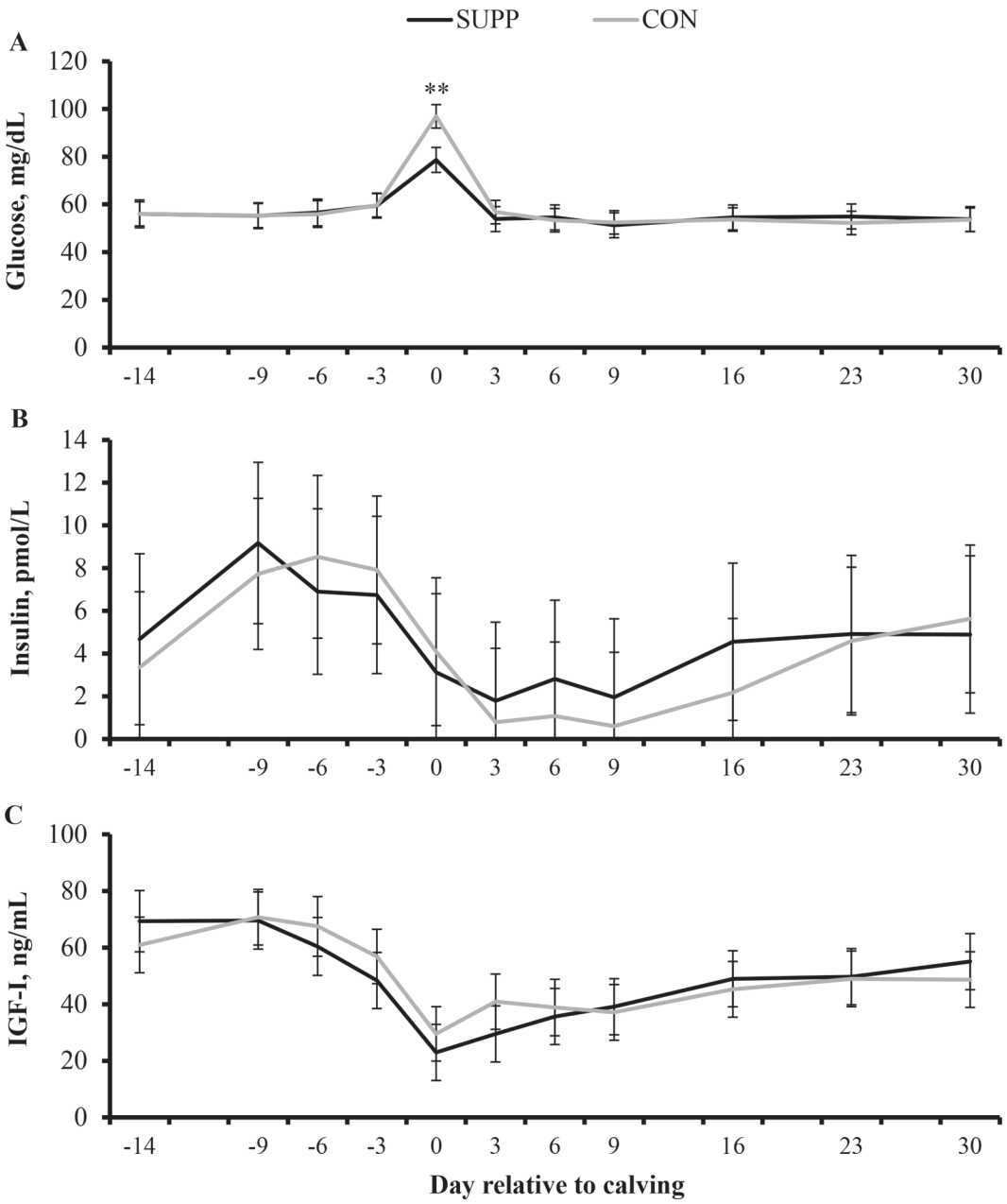

Figure 4. Serum concentrations of glucose (panel A), insulin (panel B), and IGF-I (panel C) in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving. Error bars represent 95% CI. Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. A treatment × day interaction was detected ($P < 0.01$) for glucose. No treatment differences were detected ($P \geq 0.31$) for insulin and IGF-I. Day effects were also detected for all variables ($P < 0.01$). Treatment comparison within days, $^{**}P < 0.01$.

cows at calving (d 0; Figure 5). No treatment differences were detected ($P = 0.82$) for serum haptoglobin concentrations (Figure 4), although elevated cortisol has been positively associated with haptoglobin concentrations in cattle (Cooke, 2017). Research investigating the effects of prepartum DCAD on circulating cortisol and haptoglobin concentrations during the transition period is limited, although clinical hypocalcemia increases plasma concentrations of cortisol in dairy cows (Horst and Jorgensen, 1982). Yet, results from this experiment suggest that SUPP cows experienced a reduced corticosteroid reaction elicited by metabolic stress and trauma from parturition and onset of lactation, without affecting the resultant haptoglobin response (Hudson et

al., 1976; Trevisi and Bertoni, 2008; Cray et al., 2009; Cooke et al., 2012).

CONCLUSIONS

Inclusion of ANM into the SUPP treatment successfully decreased prepartum urine pH near 6.0 in Holstein × Gir dairy cows without depressing concentrate intake compared with CON cohorts, which received ammonium chloride resulting in prepartum urine pH near 7.0. Although serum Ca concentrations during the first 5 DIM were not affected by treatments, SUPP transiently affected serum glucose, fatty acids, and cortisol concentrations near the time of calving and increased

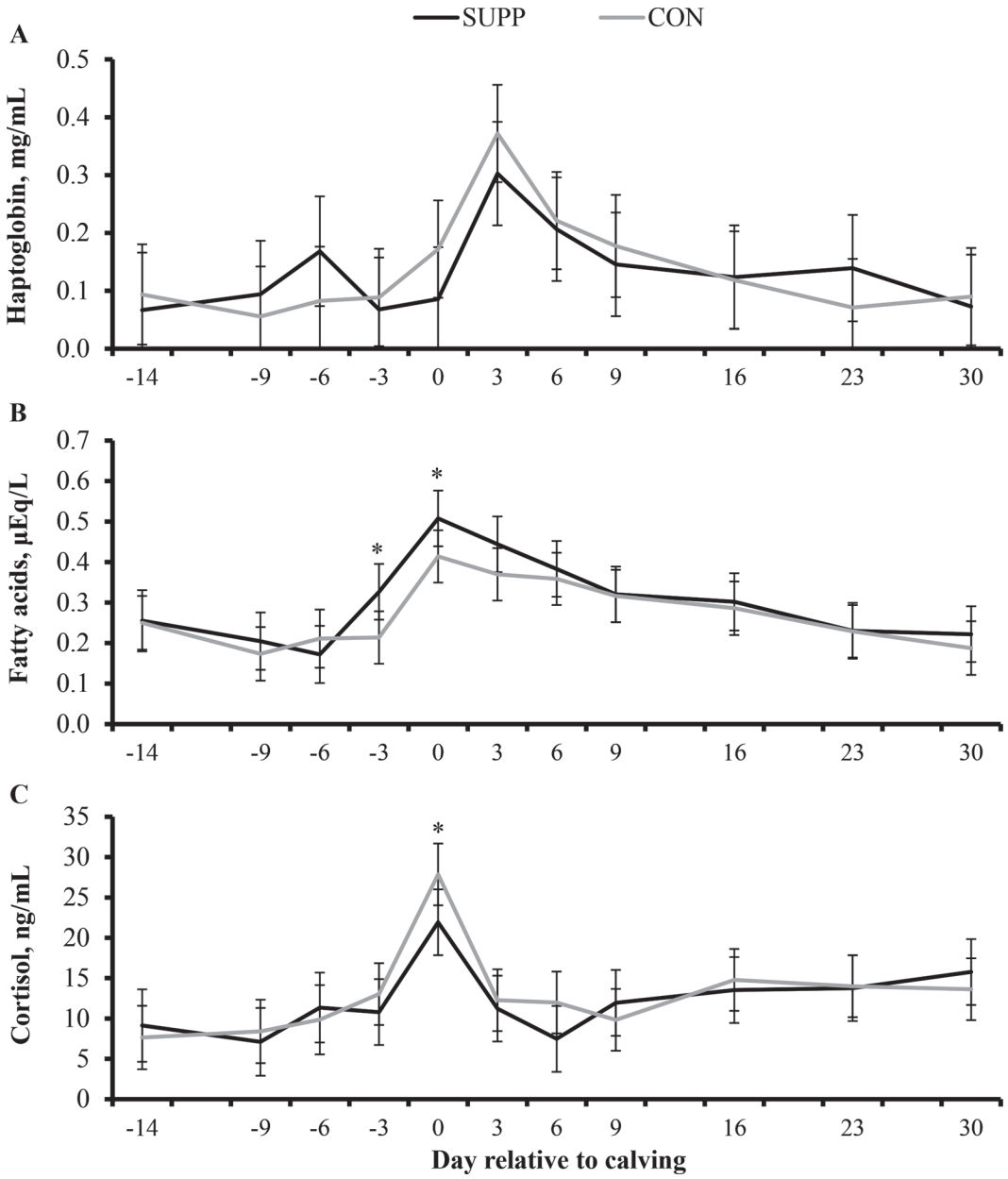

Figure 5. Serum concentrations of haptoglobin (panel A), fatty acids (panel B), and cortisol (panel C) in 3/4 Holstein × 1/4 Gir dairy cows receiving a prepartum concentrate designed to reduce urine pH to 6.0 (SUPP; n = 15) or 7.0 (CON; n = 17) before calving. Error bars represent 95% CI. Based on actual calving dates, cows receiving SUPP or CON began receiving treatments 19.2 ± 1.2 and 19.0 ± 0.9 d before calving, respectively. No treatment differences were detected ($P \geq 0.72$) for haptoglobin. Tendencies ($P \leq 0.09$) for a treatment × day interaction were detected for fatty acids and cortisol. Day effects were also detected for all variables ($P < 0.01$). Treatment comparison within days, $^*P \leq 0.05$.

milk yield during the initial 30 DIM compared with CON. Accordingly, others have reported metabolic and productive benefits of providing anionic supplements (DeGroot et al., 2010; Weich et al., 2013) such as ANM (Leno et al., 2017) to decrease prepartum urine pH near 6.0, mostly in high-producing Holstein cows receiving prepartum TMR. It could be questioned that outcomes reported herein cannot be specifically associated with urine pH or supplementation strategy, as the current experimental design did not contain a treatment yielding urine pH near 6.0 using ammonium chloride. Nevertheless, results from this experiment and Leno et al. (2017) suggest that ANM effectively decreases prepartum urine pH, resulting in enhanced milk production in dairy cattle with different milking abilities and prepartum feeding systems.

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