Effects of early postpartum sodium salicylate treatment on long-term milk, intake, and blood parameters of dairy cows

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ABSTRACT

Previous research has shown that cows who receive treatment with nonsteroidal anti-inflammatory drugs after calving may have increased milk yield beginning near peak lactation, resulting in greater 305-d milk production. It has not been demonstrated whether this response is associated with greater feed intake following the first 3 wk of lactation. Dry matter intake (DMI) and milk yield were measured daily for 56 cows over the first 120 d in milk. Cows in their second parity and greater were blocked by parity and alternately enrolled 12 to 36 h after calving into 1 of 2 treatments: either 3 daily drenches of water or 3 daily drenches of a similar volume of water containing 125 g of sodium salicylate (SAL) beginning 12 to 36 h after calving. Cows were housed in individual stalls to monitor DMI. Blood samples were collected before calving and on the last day of treatment, as well as at 7, 11, 14, 18, 21, 35, 49, 63, 77, 91, 105, and 120 d in milk. The SAL treatment did not affect estimated 305-d milk, fat, or protein yields (from monthly test days), daily milk yield or components, energy-corrected milk, fat-corrected milk, or DMI; however, an interaction between parity and treatment was observed for DMI, where second-parity SAL cows had decreased intake with no differences observed in older cows. This resulted in a parity by treatment interaction for the ratio of energy-corrected milk to DMI. Similarly, no main effects of treatment were observed for plasma glucose, β -hydroxybutyrate (BHB), or fatty acid concentrations, but we noted interactions between treatment and parity for glucose, BHB, and insulin. Older cows had greater plasma glucose and insulin concentrations and decreased plasma BHB following SAL but no differences were observed in second parity animals. Alterations in glucose and insulin resulted in a tendency for a treatment by time interaction for

the revised quantitative insulin sensitivity check index. Feeding behavior was also altered following SAL administration, resulting in fewer but longer meals, as well as a tendency for greater meal weight. A tendency for a treatment by week interaction for inter-meal interval was observed, as well as a parity by treatment interaction for meal weight. Despite the lack of a milk yield response, SAL had a prolonged programming effect on feeding behavior and blood variables over the first 120 DIM, with responses largely dependent on parity.

Key words: inflammation, feeding behavior, nonsteroidal anti-inflammatory drug, metabolism, dairy cow

INTRODUCTION

The transition period—defined as 3 wk before and 3 wk following parturition in dairy cattle—is notorious for its potential pitfalls and challenges to dairy farm management. In addition to the event of parturition itself, milk production requires an enormous amount of energy, resulting in a vast shift in metabolism (Bell, 1995). As a result of numerous metabolic, dietary, and social pressures, early-lactation dairy cattle are prone to a plethora of metabolic disorders. Metritis, retained placenta, and excessive negative energy balance (Staples et al., 1990) can lead to reproductive failures in the ensuing lactation; therefore, a successful lactation is dependent on a successful transition period.

A growing body of research indicates that systemic metabolic inflammation is elevated in dairy cows at parturition, and that this inflammation may play a role in the development of metabolic disorders during the transition period (Bradford et al., 2015). Furthermore, inflammation has been linked to negative production outcomes (Bionaz et al., 2007; Bertoni et al., 2008; Yuan et al., 2013); however, it is not fully understood what role inflammation plays in the early-lactation dairy cow.

Farney et al. (2013b) demonstrated that postpartum administration of the nonsteroidal anti-inflammatory drug (NSAID) sodium salicylate (SS) administered in the drinking water for the first 7 d after calving

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increased 305-d milk production. Similarly, Carpenter et al. (2016) showed that short-term NSAID treatment (3 daily drenches of SS or 1 meloxicam bolus) increased weekly milk yields beginning at 7 wk in lactation, ultimately resulting in increased 305-d milk yield. Interestingly, Farney et al. (2013b) did not detect a difference in milk production during the first 21 d of milk production, and Carpenter et al. (2016) demonstrated that elevated milk production due to NSAID treatment did not occur until 7 wk in milk, with a tendency for treatment effects by 4 wk. Thus, the fact that Farney et al. (2013b) did not detect a difference in feed intake in the 2 wk following SS administration is not surprising, as the milk production response was delayed. Carpenter et al. (2016) performed their experiment on a commercial dairy without measurement of DMI, so it is unknown whether feed intake was also affected by NSAID treatment and to what extent.

The overall aim of our experiment was to determine whether long-term milk production response to early-lactation treatment with SS is achieved through effects on metabolic efficiency, DMI, or both. To address these questions, our objectives were to monitor milk production, feed intake, feeding behavior, production efficiency, and blood parameters for 120 d in dairy cows who received SS or placebo treatment following calving.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee. Holstein cows ($n = 28/\text{treatment}$) entering at least their second lactation were enrolled on this study between January 24, 2014, and December 24, 2014. Farney et al. (2013b) demonstrated that SS tended to decrease 305-d milk production in primiparous cows, so they were not enrolled in the current study. A total of 40 cows were in their second parity, whereas 16 cows were in their third parity and greater. Cows were blocked according to parity and sequentially assigned to either SS drench (**SAL**) or water drench (**CON**) at calving. Treatment drenches were given according to the procedure of Carpenter et al. (2016). In short, oral drenches were given once daily for 3 consecutive days between 1400 and 1600 h, beginning 12 to 36 h after parturition. Cows assigned to SAL received 125 g/d of SS dissolved in approximately 375 mL of water, whereas CON animals received an equal volume of water without SS.

All animals were housed at the tiestall facility at the Kansas State University Dairy Teaching and Research Center from calving to 120 DIM. Cows were fed twice

daily (0630 and 1730 h) at 110% of expected intake and milked 3 times daily (0000, 1000, and 1700 h); milk weights were recorded at each milking. A total of 4 cows, all in their second parity, were excluded from the study due to injury or health events. Of these, 2 were suspected to have ruptured colonic ulcers ($n = 1/\text{treatment}$) and 2 experienced injury ($n = 1/\text{treatment}$). In total, 52 cows remained on study for the full 120 d.

Sampling and Analysis

Feed delivered and refused were measured daily to determine feed intake for all cows. Samples of TMR were collected weekly and stored at -20°C , then composited in 2-mo intervals and analyzed for chemical composition with near infrared reflectance spectroscopy by Dairy One (Ithaca, NY). Ingredient and chemical composition of the ration is presented in Table 1. For all cows of parity 3+ and 26 second-parity cows, feeding behavior was measured via feed bunks suspended from load cells for continuous monitoring of bunk weight. Feeding behavior variables (meal weight, meal length, number of meals/day, and intermeal interval) were determined as described by Yuan et al. (2015).

Blood samples were collected via the coccygeal vein on the first and last day of treatment (3 DIM), as well as d 7, 11, 14, 18, 21, 35, 49, 63, 77, 91, 105, and 120 following calving, before the morning feeding. Two tubes (approximately 7 mL each) were used for each blood sample time point; one tube contained potassium EDTA and the other contained potassium oxalate with sodium fluoride as a glycolytic inhibitor (Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ). Plas-

Table 1. Average composition of diet fed to cows up to 120 DIM¹

Item	% of diet DM	Mean	SD
Alfalfa hay	19.6		
Corn silage	20.9		
Wet corn gluten feed ²	29.3		
Cottonseed	3.8		
Fine rolled corn	15.9		
Expeller soybean meal ³	4.9		
Straw	1.5		
Vitamin and mineral mix	4.3		
DM (% as fed)		51.2	1.59
CP (% of DM)		17.8	0.55
ADF (% of DM)		21.2	1.30
NDF (% of DM)		33.2	1.57
Ether extract (% of DM)		4.8	0.36
NE _L (Mcal/kg of DM)		1.67	0.027

¹Samples of TMR were collected once per week throughout the experiment and pooled into 2-mo intervals for analysis. Means and SD of all values across time are reported. NE_L estimated according to NRC (2001).

²Sweet Bran; Cargill Inc., Blair, NE.

³Soybest; Grain States Soya, West Point, NE.

ma from the tube containing potassium oxalate was used for glucose measurement, and plasma from the tube containing potassium EDTA was used for all other analyses. Plasma was collected and stored at -20°C until analyzed for glucose by a colorimetric kit (kit #439-90901; Wako Chemicals USA Inc., Richmond, VA), fatty acids using an enzymatic colorimetric procedure (NEFA-HR; Wako Chemicals USA Inc.), insulin using a bovine-specific sandwich ELISA (no. 10-1201-01; Mercodia AB, Uppsala, Sweden) with a detection limit of $0.025\text{ pg}/\mu\text{L}$, and BHB using a commercial kit (kit #H7587-58; Pointe Scientific Inc., Canton, MI). Haptoglobin was measured by the method of Cooke and Arthington (2013), a colorimetric technique that uses differences in peroxidase activity to measure haptoglobin-hemoglobin complexing. Absorbance was measured with a spectrophotometer (PowerWave XS; BioTek Instruments Inc., Winooski, VT), and calculations were performed using Gen5 software (BioTek Instruments Inc.). Insulin sensitivity was estimated using the revised quantitative insulin sensitivity check index (RQUICKI), as described by Holtenius and Holtenius (2007) using the equation $\text{RQUICKI} = 1/[\log(G_b) + \log(I_b) + \log(\text{FA}_b)]$, where G_b is the blood plasma concentration of glucose in milligrams per deciliter, I_b is the blood plasma concentration of insulin in microunits per milliliter, and FA_b is the blood plasma concentration of fatty acids in millimoles per liter.

Milk samples were collected twice weekly and were analyzed for concentrations of fat, true protein, lactose (B-2000 Infrared Analyzer; Bentley Instruments Inc., Chaska, MN), urea nitrogen (MUN spectrophotometer; Bentley Instruments Inc.), and somatic cells (SCC 500, Bentley Instruments Inc.; Heart of America DHIA, Manhattan, KS). In addition to milk sampling by research personnel, milk composition and yield were tested for individual cows at approximately 4-wk intervals by DHIA technicians, and 305-d mature-equivalent lactation yields were calculated by DHIA for animals that remained in the herd for at least 90 d (VanRaden, 1997). Whole-lactation (305-d), mature-equivalent yield values were collected and analyzed after all cows were beyond 305 DIM. A total of 16 cows had daily lactation records over 120 DIM, but less than 305 DIM; 6 of these cows had records up to 120 to 199 DIM ($n = 3/\text{treatment}$), 4 cows had records up to 200 to 249 DIM ($n = 2/\text{treatment}$), and 6 cows had records up to 250 to 305 DIM ($n = 2\text{ CON and }4\text{ SAL}$).

Data Analysis

Daily milk yield, DMI, and feeding behavior variables were averaged by week for analysis. Somatic cell linear

score was determined through the following equation: $\log_2(\text{SCC}/100) + 3$ (Shook, 1993). Energy-corrected milk yield was calculated as $(0.327 \times \text{milk yield}) + (12.95 \times \text{fat yield}) + (7.65 \times \text{protein yield})$, and FCM yield was calculated as $(0.432 \times \text{milk yield}) + (16.216 \times \text{fat yield})$ (NRC, 2001). Insulin and BHB data were log-transformed for analysis, and reported means are back-transformed. The BOXPLOT procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) was used to identify extreme outliers for milk, DMI, and feeding behavior variables. Outliers were identified by the procedure as being greater than 1.5 times the interquartile range above the 75th percentile, or less than 1.5 times the interquartile range below the 25th percentile.

Data were analyzed using PROC MIXED in SAS to model the main effects of treatment, parity, time (week or day), the interaction between treatment and time, and the interaction between treatment and parity. Three-way interactions were tested for plasma variables but were found to be insignificant. Cow was included as a random effect, and repeated measures were used with an autoregressive covariance structure, selected based on the Bayesian information criterion value. Plasma variables were analyzed using the pretreatment value as a covariate. Daily milk yield and DMI were analyzed with pretreatment haptoglobin as a covariate, but this was removed from the final model due to $P > 0.20$. The interaction between treatment and haptoglobin in the model for daily milk yield and DMI was tested and removed for both variables ($P \geq 0.53$). Significance was declared at $P < 0.05$, and tendencies were identified at $0.05 \leq P < 0.10$.

RESULTS

Milk Production and Feed Intake

Data for whole-lactation production are presented in Table 2, and daily milk and feed intake are presented in Table 3. Whole-lactation milk, fat, and protein yields were not affected by treatment ($P \geq 0.39$) or the interaction between treatment and parity ($P \geq 0.20$; Table 2). The main effect of week was significant for daily milk yield, ECM, FCM, DMI, ratios of milk yield to DMI and ECM to DMI, SCC, and all milk components ($P < 0.01$) with the exception of MUN concentration, which only tended to differ by week ($P = 0.07$; Table 3). Daily milk production was not affected by treatment ($P = 0.79$), and no treatment by parity or treatment by week interactions were detected ($P \geq 0.82$). Similarly, milk fat, protein, lactose, and MUN concentrations were not affected by treatment ($P \geq 0.18$) or by treatment interactions with parity or week ($P \geq 0.16$). As a result,

Table 2. Whole-lactation production responses following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d)

Item	Treatment					<i>P</i> -value		
	CON		SAL		SEM			
	Parity 2	Parity 3+	Parity 2	Parity 3+		Treatment	Parity	Treatment × parity
305-d MEq ¹ milk yield (kg)	14,809	14,124	14,589	13,635	426	0.39	0.28	0.76
305-d MEq fat yield (kg)	518	488	497	522	20.5	0.78	0.91	0.20
305-d MEq protein yield (kg)	429	400	416	399	10.3	0.60	0.09	0.65

¹MEq = mature equivalent yields calculated and reported by DHIA (n = 52 total). A total of 16 cows had daily lactation records over 120 DIM, but less than 305 DIM; 6 of these cows had records up to 120–199 DIM (n = 3/treatment), 4 cows had records up to 200–249 DIM (n = 2/treatment), and 6 cows had records up to 250–305 DIM (n = 2 CON and 4 SAL).

we observed no treatment effects or interactions ($P \geq 0.35$) for fat, protein, or lactose yield; ECM and FCM were also unaffected (all $P \geq 0.76$). No effects of treatment were observed for somatic cell linear score (all $P \geq 0.63$). Dry matter intake was not affected by SAL overall ($P = 0.70$), but we noted a significant interaction of parity and treatment ($P = 0.03$) with no DMI response observed in older cows but a decrease in DMI among second-parity cows (Table 3). The interaction between treatment and week was not significant ($P = 0.75$). Due to the lack of any observable effect on milk yield, this change in DMI contributed to a tendency for an interaction between treatment and parity for the ratio of milk yield to DMI ($P = 0.08$) and a significant interaction between treatment and parity for the ratio of ECM to DMI ($P = 0.02$), although treatment was not significant for either of these variables ($P \geq 0.33$; treatment by week: $P \geq 0.46$). Cows in parity 3+ re-

ceiving SAL tended to have decreased ECM-to-DMI ratio compared with CON ($P = 0.06$).

Blood Parameters

Results of plasma analyses are shown in Table 4. Predictably, the effect of time was significant for plasma glucose, fatty acids, BHB, insulin, and haptoglobin concentrations ($P < 0.01$); however, it is interesting to note that RQUICKI only showed a tendency to change due to time ($P = 0.098$). Pretreatment plasma concentrations were tested as covariates for all plasma variables and found to be significant predictors of plasma insulin and haptoglobin ($P < 0.01$), whereas pretreatment concentrations of glucose, fatty acids, and BHB were not significant predictors ($P \geq 0.11$). Glucose was not affected by treatment overall ($P = 0.11$) or by the interaction between treatment and day ($P = 0.90$), but we did note

Table 3. Milk and feed responses in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d)

Item ¹	Treatment					<i>P</i> -value		
	CON		SAL		SEM			
	Parity 2	Parity 3+	Parity 2	Parity 3+		Treatment	Parity	Treatment × parity
Milk yield (kg/d)	52.6	55.3	52.6	54.6	1.37	0.79	0.12	0.12
Fat (%)	3.69	3.69	3.69	3.60	0.118	0.66	0.80	0.77
Protein (%)	2.82	2.68	2.81	2.77	0.048	0.42	0.09	0.37
Lactose (%)	4.96	4.86	4.88	4.89	0.038	0.60	0.26	0.16
MUN (mg/dL)	15.2	14.6	14.9	13.6	0.44	0.18	0.03	0.50
Fat yield (kg/d)	1.92	1.99	1.91	1.97	0.059	0.76	0.37	0.84
Protein yield (kg/d)	1.49	1.47	1.46	1.52	0.038	0.89	0.55	0.35
Lactose yield (kg/d)	2.61	2.72	2.53	2.80	0.082	0.99	0.03	0.35
ECM yield (kg/d)	53.6	55.5	52.7	55.6	1.53	0.79	0.16	0.76
FCM yield (kg/d)	54.2	56.6	53.3	56.0	1.62	0.65	0.14	0.93
DMI (kg/d)	26.2	25.7	25.1‡	27.3	0.57	0.70	0.16	0.03
Milk yield:DMI	2.01	2.25	2.10	2.13	0.058	0.85	0.03	0.08
ECM:DMI	2.07	2.31	2.15	2.14‡	0.049	0.33	0.03	0.02
Somatic cell linear score	0.68	1.18	0.78	1.28	0.097	0.64	0.03	0.91

¹No significant interactions between treatment and time were detected ($P > 0.10$).

[‡]0.05 ≤ P < 0.10.

Figure 1. Revised quantitative insulin sensitivity check index (RQUICKI) response over time in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d). Error bars indicate SEM. An asterisk (*) represents differing means due to treatment ($P < 0.05$); a double cross (‡) represents means tending to differ due to treatment ($0.05 \leq P < 0.10$).

a tendency for an interaction between treatment and parity ($P = 0.06$), with glucose concentrations being elevated by SAL only in older cows. A similar pattern was observed for BHB, with lower plasma concentrations observed due to SAL, but again only in parity 3+ (treatment by parity: $P = 0.02$; treatment by day: $P = 0.94$). Insulin tended to differ between treatments ($P = 0.08$), with a significant interaction between treatment and parity ($P = 0.03$) but no interaction observed between treatment and time ($P = 0.73$). Once again,

this treatment and parity interaction resulted from the fact that second-parity animals were not affected by SAL, whereas older cows had elevated insulin concentrations when treated with SAL. We found no effect of treatment or treatment interactions on fatty acids. Interestingly, although RQUICKI did not differ due to treatment ($P = 0.14$) or its interaction with parity ($P = 0.21$), a significant treatment by day interaction was observed ($P = 0.05$), reflecting treatment effects in wk 1 and 11 to 13 (Figure 1). Haptoglobin was measured

Table 4. Blood parameter responses in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d)¹

Item ²	Treatment					<i>P</i> -value		
	CON		SAL		SEM	Treatment	Parity	Treatment × parity
	Parity 2	Parity 3+	Parity 2	Parity 3+				
Glucose (mg/dL)	49.2	42.5	48.4	47.4‡	1.47	0.12	0.01	0.06
Fatty acids (μM)	400.3	417.6	418.4	380.7	25.17	0.72	0.70	0.29
BHB (μM)	590.5	755.4	635.5	628.3‡	1.05	0.32	0.04	0.02
Insulin (ng/mL)	0.25	0.19	0.24	0.26*	0.034	0.08	0.16	0.03
Haptoglobin ³ ($\mu g/mL$)	200.5	184.3	192.8	148.9	38.03	0.59	0.54	0.72

¹Blood samples were collected before calving and on the last day of treatment, as well as at 7, 11, 14, 18, 21, 35, 49, 63, 77, 91, 105, and 120 DIM.

²No significant interactions between treatment and time were detected ($P > 0.10$).

³Haptoglobin was only monitored up to 21 d.

* $P < 0.05$; ‡ $0.05 \leq P < 0.10$.

Table 5. Feeding behavior responses in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d)

Item ¹	Treatment				SEM	<i>P</i> -value		
	CON		SAL					
	Parity 2	Parity 3+	Parity 2	Parity 3+		Treatment	Parity	Treatment × parity
Meal count (d ⁻¹)	12.3	14.3	11.8	12.5	0.504	0.03	0.01	0.18
Meal weight (kg)	4.45	3.78	4.36	4.59*	0.467	0.10	0.30	0.04
Meal length (min)	19.9	17.9	20.9	20.5	0.807	0.03	0.15	0.34
Intermeal interval (h)	1.70	1.45	1.72	1.64	0.078	0.18	0.04	0.29

¹For effects of time and interactions between treatment and time, refer to Figure 2.

*P < 0.05.

from samples collected up to 21 d on study, and no treatment effects or interactions were observed.

Feeding Behavior

Overall effects of treatment on feeding behavior measurements are reported in Table 5. Feeding behavior data are available for 16 cows in parity 3+ and 26 cows in parity 2. Cows receiving SAL ate fewer but longer meals, although intermeal interval did not differ between groups (*P* = 0.18). Treatment with SAL increased meal weight for the parity 3+ cows (*P* < 0.05; treatment by parity interaction *P* = 0.04). The main effect of parity was significant for meal frequency and intermeal interval (*P* ≤ 0.04), but not meal weight and length (*P* ≥ 0.15; Table 5). The main effect of week was significant for all measurements (*P* < 0.01), and we observed a tendency for a treatment by week interaction for intermeal interval (*P* = 0.06; Figure 2).

DISCUSSION

In the current experiment, no differences associated with SAL were observed for milk yield, milk composition, or milk component yields. The treatment protocol for SAL administration was based on the procedure of Carpenter et al. (2016), as milk production was significantly increased after postpartum SS treatment in that study. Farney et al. (2013b) also observed sustained increases in milk yield of cows in parity 3+ following SS administration via the drinking water for the first 7 DIM. Therefore, the current findings with regard to lactation responses to SS differ markedly from these 2 prior studies.

One potential difference between the current study and that of previous experiments is the degree of inflammation that cattle experienced at calving. On d 4 (the last day of treatment) and 7 in the current experiment, mean haptoglobin concentrations were 292

± 42.6 (±SD) and 209 ± 43.3 µg/mL, respectively, across treatment. This is much less than the mean concentrations of haptoglobin (exceeding 600 µg/mL) measured at similar time points in the previous study (Carpenter et al., 2016). The concentrations observed in the current study fall within the range of the lowest quartile reported by McCarthy et al. (2016), and the concentrations reported in Carpenter et al. (2016) fall within the second lowest quartile. McCarthy et al. (2016) reported that cows in the second lowest quartile of inflammation had different production responses following calving than those in the lowest quartile, with depressed DMI and a tendency for decreased milk yield in early lactation in the group with modestly elevated haptoglobin concentrations. Therefore, it is possible that the response to anti-inflammatory treatment that we observed previously (Carpenter et al., 2016) was greater because baseline inflammation was greater in these cows. To investigate this hypothesis within our study, milk yield and DMI were analyzed with pretreatment haptoglobin included in statistical models as a covariate, along with its interaction with treatment; however, treatment remained an insignificant predictor (*P* ≥ 0.19). Thus, we found no evidence within the current cohort of cows that those with greater pretreatment haptoglobin responded differently to SAL treatment. This does not necessarily rule out the potential utility of baseline herd measures of inflammation for predicting production responses to early-lactation NSAID administration, particularly given the limited variation in plasma haptoglobin in this cohort.

Other authors have reported that daily milk production did not differ following NSAID treatment (Priest et al., 2013; Mainau et al., 2014; Meier et al., 2014). We speculated that differences between experiments could be due at least in part to differences in the time period that milk production was measured, given that we observed effects that appeared 7 wk after treatment (Carpenter et al., 2016). However, despite the extended

Figure 2. Feeding behavior responses over time in the first 120 DIM following water (CON) or sodium salicylate (SAL) drenches in the first 3 DIM (125 g of sodium salicylate/d). Error bars indicate SEM. An asterisk (*) represents differing means due to treatment ($P < 0.05$); a double cross (‡) represents means tending to differ due to treatment ($0.05 \leq P < 0.10$).

observation period, no effects on milk production were detected in the current experiment.

Greater parity cows often have a less favorable metabolic status in early lactation, and NSAID may help to mitigate the negative effects of age on metabolism (Lee and Kim, 2006; van Dorland et al., 2009). The decrease in milk-to-DMI ratio (allowing more positive energy balance) for SAL cows in parity 3+ could have helped to prevent disorders associated with negative energy balance, although the incidence of specific

health disorders was not analyzed in the current study due to the relatively small number of animals enrolled. Administration of the NSAID meloxicam decreased the likelihood of leaving the herd in commercial dairy cattle compared with control (Carpenter et al., 2016); however, in that experiment, SS did not alter the risk of leaving the herd.

Overall, SAL appeared to have a positive effect on metabolism in older cows, with increased plasma glucose and insulin concentrations and decreased BHB

and RQUICKI in this group. This is in contrast with Farney et al. (2013a), who reported hypoglycemia in cows of parity 2+ on d 7 of lactation in response to SS. The adverse metabolic effects observed by Farney et al. (2013a) may have been, at least in part, a result of the extended treatment period (7 d) compared with Carpenter et al. (2016) and the current study (3 d). Elevated plasma glucose in older cows in the current study might indicate increased glucose entry into the blood, decreased removal from the blood into tissues, or some combination of both, but it is difficult to determine the exact cause. Decreased insulin sensitivity with SAL may have caused the observed increase in plasma glucose by slowing its uptake into tissues, by promoting gluconeogenesis in the liver, or by a combination of these (Aschenbach et al., 2010); however, inflammation is often linked to blunted insulin sensitivity (Odegard and Chawla, 2013), and it follows that anti-inflammatory treatment would be expected to result in greater RQUICKI, as observed in Farney et al. (2013a). Two of the most in-depth studies of salicylate effects on glucose kinetics were carried out in dogs (Miller et al., 1985) and rats (Pereira et al., 2013). Miller et al. (1985) revealed an intriguing dichotomy of enhanced peripheral insulin sensitivity coupled with decreased hepatic insulin sensitivity in response to salicylate, and Pereira et al. (2013) similarly found that salicylate enhanced peripheral insulin sensitivity without affecting hepatic insulin sensitivity. With the greater reliance on hepatic gluconeogenesis and insulin-independent glucose utilization in ruminants, it is conceivable that overall effects of salicylate on insulin sensitivity in ruminants may differ from responses in other animals and may vary by metabolic or inflammatory state.

A depressed responsiveness to insulin is often observed in nonmammary tissues in early lactation as a mechanism of partitioning nutrients toward milk production (De Koster and Opsomer, 2013). Bjerre-Harpøth et al. (2012) showed that early-lactation dairy cattle have increased relative change in RQUICKI in response to nutrient restriction, whereas cows in mid and late lactation do not demonstrate the same coping mechanism. However, recent direct comparison between RQUICKI and the gold-standard hyperinsulinemic-euglycemia clamp assessments of insulin sensitivity showed no relationship between the 2 in late-gestation cows, calling into question the utility of the RQUICKI metric (De Koster et al., 2016). Our findings regarding the effects of SAL on RQUICKI should be considered with this weakness in mind.

Although several authors have compared feeding behavior responses to treatment at various time points in lactation, to our knowledge, this is the longest continu-

ously monitored report of feeding behavior beginning right after parturition in cattle. Huzzey et al. (2005) reported that time spent feeding increased by approximately 3.3 min/d for the first 10 d of lactation. DeVries et al. (2003) reported significant differences in daily mealtime, meal frequency, and meal duration between ~35 and ~57 DIM, with no further changes by ~94 DIM, suggesting a stabilization in feeding behavior as cows approach peak production. Our observations are consistent with this interpretation (Figure 2). Friggens et al. (1998) did not report alterations in feeding behavior between 6 and 38 wk in lactation, with the exception of a small but significant decrease in time spent feeding during an observation window from wk 35 to 38, a time when milk production would have declined substantially from peak lactation. In the current experiment, parity was a significant predictor of meal frequency and intermeal interval (Table 5). In general, second-parity cows consumed fewer meals and had a longer IMI than cows in parity 3+. Azizi et al. (2009) reported that multiparous cattle had a decrease in meal frequency and time spent eating, and an increase in meal size compared with primiparous cows; however, a lack of prior literature exists where second-parity cows were compared with older cows.

In contrast to the lack of treatment effects on peak milk yield, we did observe clear treatment effects on feeding behavior in response to treatment (Figure 2), reflecting a shift to fewer but larger meals following SAL treatment. Intriguingly, these SAL-induced shifts in meal patterns were delayed by the same 7-wk lag that occurred before production responses in our previous work (Carpenter et al., 2016). Likewise, both responses were maintained for a substantial period of time, with feeding behavior shifts remaining through at least 16 wk into lactation. It is worth pointing out that differences in housing in the current study compared with Carpenter et al. (2016) could be meaningful. In the current study, tiestall housed cows had constant and immediate access to feed (which should also be considered when interpreting feeding behavior); in contrast, our previous work on a commercial dairy farm evaluated cows housed in freestall pens, where at least some competition for access to the feed bunk was likely. In that type of competitive environment, a treatment that enables cows to consume more feed on each visit to the bunk is likely to increase DMI, but this is not likely to be true in a noncompetitive tiestall environment. Perhaps part of the reason for the lack of a treatment effect on milk yield in the current study is the removal of that constraint on DMI. It is also possible that the treatment effect on plasma insulin among older cows may have been in part due to the larger, less frequent

meals consumed by SAL-treated cows (French and Kennelly, 1990).

The use of NSAID can alter the human body microbiome (Rogers and Aronoff, 2016), and the same is likely true in ruminants. Ruiz-Moreno et al. (2015) and Fessenden et al. (2017) used bismuth subsalicylate in batch and continuous cultures in an attempt to mitigate production of hydrogen sulfide. Both authors reported negative effects on rumen fermentation when bismuth subsalicylate was administered to ruminal microbes in vitro. To our knowledge, no one has investigated the effects of salicylates on rumen function in vivo; however, we recently reported (Carpenter et al., 2017) that SS affects fermentation by ruminal microbes. When administered directly to batch cultures, SS decreased DM disappearance and increased final pH, indicating direct inhibitory effects on ruminal fermentation (Carpenter et al., 2017). When batch cultures were performed with rumen fluid from heifers receiving oral drenches of SS, DM disappearance was decreased relative to controls 13 and 35 d following administration (Carpenter et al., 2017), despite the lack of further treatment over those 35 d. Furthermore, the rate of in situ DM disappearance was decreased in these heifers.

We speculated that parity $\times$ treatment interactions for feeding behavior may be linked to effects of SAL on ruminal fermentation. Decreased intake observed in second-parity cows in response to SAL can be attributed primarily to a decrease in meal frequency (Table 5), which may be at least in part due to differences in gut capacity. If SAL indeed had a sustained inhibitory effect on ruminal fermentation in the current study, a corresponding delay in fiber degradation could have a negative effect on DMI because of enhanced retention time and gut fill, particularly for the younger cows with less rumen capacity. Another interesting finding was that older cows receiving SAL had significantly greater meal sizes than parity-matched controls (Table 5). A decreased fermentation rate with SAL (Carpenter et al., 2017) would likely decrease the rate of propionate production, although treatment effects on VFA were not evaluated in our studies. High rates of propionate absorption generally have hypophagic effects, but propionate infusion more dramatically decreased DMI in cows with high concentrations of liver acetyl-CoA (Stocks and Allen, 2012), indicative of a more lipolytic and ketogenic state. Younger multiparous cows had lesser BHB and fatty acid concentrations than cows in their fourth parity and greater (van Dorland et al., 2009), and similar differences in BHB were observed between parities among the control cows in the current study (Table 4). A decreased propionate supply with SAL may have attenuated satiety induced by the

interaction of propionate and a lipolytic state in cows of parity 3+, enabling longer and larger meals in that group (Allen et al., 2009).

Although it is possible that SS affects feeding behavior by altering rumen fermentation, it is also possible that SS directly alters feeding behavior, which in turn changes the rumen via differences in the rumen environment (i.e., salivary buffering, rate of passage, and so on). This may explain the sustained effect of SS on the ability of ruminal microbes to digest DM in vitro (Carpenter et al., 2017). Indeed, Weimer et al. (2010) demonstrated the importance of the animal itself in determining the microbial population of its rumen; even following near-total exchange of the contents of the rumen, animal factors eventually guided the microbial population to its original state. The microbial population of the rumen and responses of the ruminant itself have an inherent "which came first" relationship that is difficult to fully illuminate, and further research is necessary to determine the cause of alterations in feed intake and behavior in cows receiving SAL in the current experiment. It is also worth noting that the decreased RQUICKI values in SAL cows in later lactation coincided with the differences between SAL and CON in feeding behavior (Figures 1 and 2). If heightened insulin sensitivity speeds the clearance of fuels from the blood, it would be expected that hunger would occur sooner (Bradford and Allen, 2007); therefore, if responsiveness to insulin is depressed in SAL cows, perhaps it is logical that the time between meals would be lengthened (and meal frequency decreased), as we observed.

CONCLUSIONS

This experiment was conducted to determine whether the long-term milk production response to early-lactation treatment with SS was achieved through differences in DMI. In this experiment, previous results showing a response in milk production in older cows were not replicated. Despite the failure to demonstrate a change in milk production, subtle differences in feed intake, efficiency, metabolism, and feeding behavior were observed, some of which were delayed responses corresponding with time periods in which milk production responses occurred in prior studies. These may be a result of long-term effects of SAL on the rumen, or it is possible that long-term ruminal effects reported previously are a result of these differences in feeding behavior. It remains unclear why no differences in milk production were observed in this experiment, and further research is necessary to elucidate the mechanisms promoting the differences observed in metabolism in response to SAL.

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