J. Dairy Sci. 101:11342–11353
https://doi.org/10.3168/jds.2018-15058

Effects of oral administration of acidogenic boluses at dry-off on performance and behavior of dairy cattle

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ABSTRACT

The aim of this study was to evaluate the potential of oral acidogenic mineral boluses (196 g) containing anionic salts to facilitate the transition from lactation to the dry stage by inducing a mild and temporary metabolic acidosis at dry-off. In experiment 1, 84 lactating cows were randomly allocated to 1 of 3 treatment groups consisting of an oral administration of 0, 1, or 2 boluses 5 d before dry-off to evaluate the effects on milk production. In experiment 2, 16 lactating cows were involved in a crossover study to evaluate the effects of the administration of 2 boluses on milk production, feed intake, and urine pH. In experiment 3, 152 lactating cows were allocated to 1 of 2 treatments (control: no treatment; bolus: 2 oral boluses the day before last milking) to evaluate udder pressure, incidence of milk leakage, and lying behavior during the first days following dry-off. Also, milk yield in the subsequent lactation for all enrolled cows was recorded during the first 60 DIM. In experiment 1, cows receiving 2 boluses had the greatest reduction in milk production (−2.56 kg/d of milk) compared with those receiving 1 bolus or no treatment (−1.15 and −0.23 kg/d, respectively) the second day after bolus application. In experiment 2, the application of oral boluses decreased feed intake of cows during the first 3 d following treatment, and milk production was reduced on d 2 and 3 after bolus application. Reduced urine pH at 8 and 24 h after treatment was observed in bolus cows compared with control cows. In experiment 3, bolus cows had lower udder pressure after drying off, but incidence of milk leakage did not differ between treatments. Bolus cows had an additional 85 min of lying time in the 24 h following dry-off. Serum P and β-OH-butyrate concentrations were lower in bolus cows than in control cows after dry-off, but no other differences in blood parameters between treatments were observed. Also, no differences in milk yield in the subsequent lactation were observed between treatments. It is concluded that oral bolus application diminishes feed intake and milk production, and, if applied at dry-off, it decreases udder pressure and increases lying time during the first 24 h after dry-off.
Key words: dry-off, lying behavior, milk leakage, oral bolus

INTRODUCTION

Drying-off has been widely recognized as a critical period of the production cycle of dairy cows (Capuco and Akers, 1999; Bachman and Schairer, 2003; Choudhary, 2014). It has been suggested that with increasing milk yields over the last decades, the transition from lactating to dry has progressively become more challenging for cows in terms of animal health and welfare (Thornton, 2010; Zobel et al., 2015). From an animal health perspective, cessation of milking has been associated with increased risk of new IMI that may persist into the following lactation with detrimental consequences on milk production (Pantoja et al., 2009; Oliver and Murinda, 2012; Leelahapongsathon et al., 2016). One of the factors contributing to this increased risk is the continued milk production in the mammary gland in the immediate period following drying-off: the milk is accumulated in the udder, and the increased udder pressure may cause milk leakage (**ML**) from the teats (Schukken et al., 1993; Bradley and Green, 2004; Rajala-Schultz et al., 2005). Milk leakage may allow microorganisms to colonize the udder, coinciding with a moment of impaired natural protective activity in the mammary gland due to the involution process (Burvenich et al., 2007). In addition to ML, the increased intramammary pressure after cessation of milking has been suggested to potentially cause discomfort in cows, which in turn may alter lying behavior (O'Driscoll et al., 2011). In fact, several studies evaluating lying behavior of high-producing cows have indicated reduc-

Received May 14, 2018.
Accepted August 6, 2018.
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tions in lying time on the day of dry-off (Chapinal et al., 2014; Rajala-Schultz et al., 2018).

Because of the potential risks on cow health and welfare resulting from milk accumulation in the udder, several studies have examined the effects of different management strategies aimed at reducing milk production the days before dry-off as well as accelerating the involution process early after drying-off. These include dietary changes (Odensten et al., 2005; Valizaheh et al., 2008), gradual cessation of milking (Tucker et al., 2009; Zobel et al., 2013; Rajala-Schultz et al., 2018), and the use of pharmacological options such as intramammary infusions of casein hydrolysate (Leitner et al., 2007; Ponchon et al., 2014) or a single intramusular administration of cabergoline (Bach et al., 2015; Bertulat et al., 2017; Boutinaud et al., 2017). Another unexplored strategy to reduce milk production and facilitate drying-off could be the use of acidifying agents as a feed additive (e.g., ammonium chloride, calcium chloride) several hours before the last milking. Ammonium chloride (NH_4Cl) is a strong systemic and urinary acidifying agent. Ammonium chloride supplementation in ruminants has been shown to induce metabolic acidosis (Augustinsson and Johansson, 1986; Mavangira et al., 2010) and reduce feed intake (Mckinnon et al., 1990). Therefore, it is foreseen that NH_4Cl supplementation could help reduce milk production in dairy cows, either directly by modifying the acid–base balance of dairy cows at dry-off or indirectly by reducing DMI. To our knowledge, the effects of acidifying agents in dairy cows at dry-off have not been previously studied. We hypothesized (1) that an anionic or acidogenic supplementation to cows before drying would reduce milk production at dry-off as a consequence of an alteration in the acid–base status or a depression of feed intake or both and (2) that this reduction would minimize discomfort, udder pressure, and incidence of ML following dry-off. Thus, the first objective of this study was to determine the effects of the application of 1 or 2 fat-coated boluses containing anionic salts on milk production. The second objective was to investigate the effects of the oral application of 2 acidogenic boluses (196 g each) on daily milk production and feed intake. The third objective was to evaluate the effects of the application of 2 oral acidogenic boluses on the metabolic response, udder pressure, ML, and lying behavior after dry-off as an indication of discomfort after dry-off.

MATERIALS AND METHODS

All cows were handled according to EEC Directive 86/609 covering the protection of animals used for experimental purposes. All procedures conducted herein were conducted following the guidelines and under the supervision of the Animal Care Committee of Institut de Recerca i Tecnologia Agroalimentàries (Barcelona, Spain) with the experiment approval number 9786.

Experiment 1

The objective of this experiment was to assess the effect of the administration of different numbers of acidogenic boluses to lactating cows on milk yield. To study the effects on milk yield, this experiment was performed in pregnant and lactating dairy cows during the week prior to the scheduled date of dry-off.

Animals, Experimental Design, and Measurements. First, 84 lactating and pregnant (28.1 ± 6.17 kg/d of milk yield and 222 ± 3.2 d pregnant) Holstein cows were blocked by parity (29 primiparous and 55 multiparous) and randomly allocated (using the random generator function of Excel; Microsoft Corp., Redmond, WA) to 1 of the following 3 treatments: 1 bolus applied 5 d before dry-off (**B1**), 2 boluses applied 5 min apart 5 d before dry-off (**B2**), and a sham bolus (the bolus applicator was introduced in the esophagus of the cow but no bolus was given) applied 5 d before dry-off (**B0**). The period of 5 d before dry-off was chosen to evaluate any potential influence on milk yield in cows that were as close as possible to the situation (i.e., days pregnant, level of intake, level of milk production) found at dry-off. The mineral composition of the oral bolus, each weighing 196 g (Bovikalc Dry, Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany) was NH_4Cl = 10.4%, calcium chloride ($CaCl_2$) = 51.9%, calcium sulfate ($CaSO_4$) = 20.8%, water = 12.6%, and coating material (mono- and diglycerides of fatty acids esterified with acetic acid) = 4.3%. Each bolus provided approximately 20 g (about 10.4% of the total bolus weight) of NH_4Cl.

Cows were enrolled in a commercial dairy farm milking close to 900 cows (Murucuc, Vic, Spain) between July and August 2016 at the end of their lactation (341 ± 32.2 DIM), and daily milk production was recorded for 15 d before dry-off using electronic milk meters (Westfalia Surge Metatron Milk Meter; GEA Farm Technologies, Barcelona, Spain). Every week there were between 8 and 12 cows enrolled in the study, and they were randomly allocated to 1 of the 3 treatments. The inclusion criteria for animal enrollment were good general health based on physical inspection, daily milk yield >15 kg, no signs of clinical mastitis, and 4 functional quarters. The cutoff for milk yield was chosen to ensure that cows would be producing sufficient milk to be a challenge for drying-off, as NMC (2006) recommends not drying cows with milk yields above 15 kg/d. All enrolled cows were kept in a barn equipped with freestalls, had ad libitum access to water, were fed twice

Table 1. Ingredient and nutrient composition of the different rations fed in this study

Item	Experiment 1	Experiment 2	Experiment 3	
			Lactation ration	Dry ration
Ingredient, % of DM				
Alfalfa hay	15.65	1.57	5.96	—
Alfalfa silage	7.14	0.00	—	—
Fescue hay	—	22.26	—	—
Ryegrass hay	—	1.53	—	—
Grass silage	—	8.14	17.52	30.59
Corn silage	6.69	0.00	14.52	—
Barley straw	9.48	3.24	—	38.53
Brewers grains	11.15	0.00	—	—
Corn	36.99	20.37	24.7	22.14
Soybean meal	7.40	10.88	8.43	8.74
Canola meal	—	—	13.52	—
Sunflower meal	—	4.41	—	—
Barley	1.75	21.67	6.83	—
Molasses	—	1.48	—	—
Soybean hulls	1.75	2.88	—	—
Beet pulp	—	0.45	6.43	—
Palm oil	0.97	0.00	—	—
Urea	0.11	0.00	—	—
Calcium carbonate	0.37	0.63	0.74	—
Magnesium oxide	0.11	0.13	0.22	—
Sodium chloride	0.30	0.27	0.3	—
Sodium bicarbonate	—	—	0.65	—
Vitamin-mineral premix	0.15	0.09	0.18	—
Nutrient, DM basis				
CP, %	15.6	15.3	16.5	12.4
NE_L, Mcal/kg	1.65	1.64	1.72	1.29
NDF, %	32.8	34.9	30.4	50.2
DCAD,[1] mEq/100 g	18.28	25.1	28.4	17.9

[1]Calculated as {[Na (g/kg)/0.0023] + [K (g/kg)/0.00391]} − {[Cl (g/kg)/0.00355] + [S (g/kg)/0.00321] × 2}.

daily a lactation TMR (Table 1) following NRC (2001) recommendations, and were milked 3 times daily.

Statistical Analyses. The effect of bolus administration on daily milk production was analyzed with a mixed-effects model with repeated measures using PROC MIXED of SAS (version 9.2; SAS Institute Inc., Cary, NC). The fixed part of the model accounted for the effect of treatment, day relative to treatment application, and their 2-way interaction, and the random part accounted for the effect of batch (week of enrollment), block (parity), and cow within treatment. Day entered the model as a repeated measure using a first-order autoregressive variance-covariance matrix as it yielded the lowest Bayesian information criterion values. Average milk production between −15 and −6 d relative to dry-off was used as a covariate. Because treatment was applied at the animal level, the experimental unit was the cow. Pairwise comparisons among treatments were performed after adjusting by the method of Tukey.

The specific model was

$$Y_{ijklm} = \mu + T_k + D_i + TD_{ik} + c_{j:lk} + b_l + B_m + \beta X_{ijklm} + \varepsilon_{ijklm},$$

where Y_{ijklm} is the response due to cow j, treatment k, day i, batch l, and block m; μ denotes the overall mean; T_k denotes the fixed effect of the kth treatment; D_i denotes the fixed effect of day; TD_{ik} is the fixed effect of the interaction between the kth treatment and the ith day; $c_{j:lk}$ is the random effect associated with the jth cow nested within the kth treatment and the lth batch; b_l is the random effect of the lth batch; B_m is the random effect of the mth block; βX_{ijklm} is the covariate adjustment for each cow; and ε_{ijklm} denotes the random error.

Experiment 2

Based on the outcomes from experiment 1, the objectives of this experiment were to corroborate the effects of the administration of 2 acidogenic boluses on milk yield and to determine the potential effect on DMI and urine pH.

Animals and Experimental Design. Sixteen (8 primiparous and 8 multiparous) lactating and pregnant (154 ± 19.4 d pregnant) Holstein cows (273 ± 56.4 DIM; 31.7 ± 5.59 kg/d of milk yield) were enrolled in

January 2017 in a crossover experiment consisting of 2 periods of 9 d each and 2 treatments consisting of no supplementation (control treatment) or supplementation with NH_4Cl combined with $CaCl_2$ and $CaSO_4$ via 2 oral boluses (Bovikalc Dry, Boehringer Ingelheim Vetmedica GmbH) administered 5 min apart (bolus treatment) at d 0 of each experimental period. As in experiment 1, control cows were sham treated.

Before initiating the treatment phase, milk yield and feed intake of all cows were monitored on a daily basis for 9 d as a baseline reference period. Then, cows were randomly allocated to either the bolus or control treatments. After 9 d, treatment groups were reversed following a crossover design. The 9-d periods were chosen based on observations from experiment 1 (that showed that effects on milk yield disappeared after 4 d of bolus administration) plus 5 d as a washout interval. Cows were kept in a research farm (Blanca, Lleida, Spain) in a barn equipped with freestalls, were milked twice daily, and had ad libitum access to water and feed in the form of a TMR balanced according to NRC (2001) recommendations (Table 1).

Measurements. Daily individual feed intake was monitored throughout the study using electronic feed bins (MooFeeder, MooSystems, Cortes, Spain) that recorded time of day and amount of feed consumed at every visit (as described in Bach et al., 2018). Individual milk production at every milking was measured using electronic milk meters (AfiMilk, Afikim Ltd., Kibbutz Afikim, Israel).

Urine samples were collected at 0, 8, 24, and 48 h relative to bolus application from all cows by manual stimulation. Urine pH was immediately measured using a portable pH meter (Crison pH25, Crison Instruments SA, Barcelona, Spain) that was calibrated before sampling with pH 4.0 and 7.0 buffer solutions.

Statistical Analyses. Daily milk production, feed intake, and urine pH were analyzed using a mixed-effects model that accounted for the fixed effect of treatment, day (or hour for urine pH) of study, and their 2-way interaction plus the random effect of cow, block (parity), period, and sequence in the crossover as random effects. Day or hour entered the model as a repeated measure using a first-order autoregressive variance-covariance matrix as it yielded the lowest Bayesian information criterion values. Daily milk production and feed intake during the first 9 d of the study (baseline) were averaged and entered into the statistical model as a covariate. All analyses were performed with SAS. Because treatment was applied at the animal level, the experimental unit was the cow.

The concrete model was as follows:

$$Y_{ijklmn} = \mu + T_k + t_i + Tt_{ik} + c_{j:m} + p_l + s_m + b_n + \beta X_{ijklmn} + \varepsilon_{ijklmn},$$

where Y_{ijklmn} is the response due to cow j, treatment k, time i, period l, sequence m, and block n; μ denotes the overall mean; T_k denotes the fixed effect of the kth treatment; t_i denotes the fixed effect of time (day or hour depending on the dependent variable); Tt_{ik} is the fixed effect of the interaction between the kth treatment and the ith day or hour (depending on the dependent variable); $c_{j:m}$ is the random effect associated with the jth cow nested within the mth sequence; p_l is the random effect of the lth period; s_m is the random effect of the mth sequence; b_n is the random effect of the nth block; βX_{ijklmn} is the covariate adjustment for each cow; and ε_{ijklmn} denotes the random error.

Experiment 3

Animals and Experimental Design. A total of 152 Holstein dairy cows from 2 commercial dairy farms in Girona, Spain, were enrolled in this study between February and June 2017: 104 cows from SAT Sant Mer (Girona, Spain) and 48 cows from Mas Duran (Girona, Spain). On average, 8 cows were enrolled on a weekly basis. All cows enrolled were first blocked by parity and then randomly (using the random function of Excel; Microsoft Corp.) assigned to 2 treatments. Experimental treatments consisted of a control group receiving no supplementation (n = 76) and a treatment group (bolus treatment) receiving 2 oral boluses (n = 76) supplying NH_4Cl combined with $CaCl_2$ and $CaSO_4$ (Bovikalc Dry, Boehringer Ingelheim Vetmedica GmbH) administered 5 min apart about 8 to 12 h before the last milking before dry-off. The timing of treatment relative to dry-off was based on the effects on milk yield and feed intake observed in experiments 1 and 2. The 4-h range (between 8 and 12 h) provides a time window for bolus application that may be suitable in both twice- and thrice-daily milking systems. In this experiment, cows were milked 3 times daily at 0400, 1200, and 2000 h; bolus administration took place either before or after the 0400 h milking, and cows had their last milking before dry-off at 1200 h.

Cows were enrolled in the study 5 d before dry-off and were monitored for 5 d following dry-off as well as during the beginning of the subsequent lactation. The inclusion criteria for enrollment were good general health based on physical inspection, ≥220 d of pregnancy, daily milk yield >20 kg at drying-off, no signs of clinical mastitis, and 4 functional quarters. The milk yield cutoff of >20 kg/d was chosen to ensure that sufficient milk production would be present at dry-off to detect measurable increases in udder pressure. All cows from SAT Sant Mer were housed in a freestall barn and had ad libitum access to lactation TMR ration (Table 1) and water until the moment of dry-off when they were moved to pens with sawdust bedding and changed to a dry-cow TMR (Table 1) offered ad libitum along with free access to water. Cows from Mas Duran had the same feeding regimen but were housed in a compost-bedded pack barn with straw as bedding during both the lactation and dry periods. In both farms, lactating cows were milked in a 2 × 10 milking parlor 3 times per day at approximately 8-h intervals, and individual daily milk yield was automatically recorded during each milking using electronic meters. At dry-off, cows from both farms were exposed to an abrupt cessation of milk and treated with an intramammary infusion of ceftiofur (Virbactan, Virbac, Sintra, Portugal). No teat sealant was applied to any cow at dry-off. During the course of the study, all animals and housing facilities were inspected twice daily, in the morning and in the afternoon, to ensure constant availability of feed and water.

Measurements. All cows were equipped with an electronic data logger (Hobo Pendant G Acceleration Data Logger, Onset Computer Corp., Pocasset, MA) for measuring cow activity starting 5 d before to 5 d after dry-off at 1-min intervals. Each data logger was attached to one hind leg using vet wrap (Eurimex flex, Divasa-Farmavic SA, Barcelona, Spain) and oriented in a position such that the x-axis of the Hobo loggers pointed right, the y-axis was perpendicular to the ground, and the z-axis pointed away from the sagittal plane. The data collected by the Hobo loggers were downloaded using Onset HOBOware software (Onset Computer Corp.,) and processed using a script written in the Python programming language to calculate total lying time per day and cow as described by Yunta et al. (2012).

Blood samples were collected from 25 randomly chosen cows per treatment group via the coccygeal vessels using 10-mL evacuated tubes (BD Vacutainer Systems, Plymouth, UK) at 0, 8, 24, and 48 h after dry-off to determine pH, Ca, P, prolactin (**PRL**), nonesterified fatty acids (**NEFA**), and BHB. Serum was then harvested and stored at −20°C until further analysis.

Measurement of PRL concentration in serum was performed by ELISA (PRL/LTH) kit (Cusabio Biotech Co., Whuan, China). Blood Ca and P concentrations were determined by atomic absorption spectrophotometry. Blood BHB concentration in serum was measured with a colorimetric method and the kit Autokit 3-HB (Wako Chemicals USA Inc., Richmond, VA). Concentrations of NEFA in serum were measured using colorimetric methods with the kit NEFA-HR(2) (Wako Chemicals USA Inc.).

Following dry-off, presence or absence of ML was recorded thrice daily on each cow at approximately 6- to 8-h intervals (at ~0800, 1600, and 2200 h) for about 30 min each time during 3 consecutive days. Milk leakage was defined as the observation of milk dripping or flowing from 1 or more teats. During the 3 d after dry-off and on a daily basis, udder pressure was determined. For udder pressure, a digital algometer (Commander, JTech Medical Industries, Midvale, UT) that was modified by welding a 2-cm washer at 2 cm from the tip of the algometer was used as previously described and validated by Bach et al. (2015). Briefly, the measure consisted of applying force to the caudoventral side of the rear left and right half udders with the tip of the algometer at a 90° angle to the skin, stopping applying force when the skin of the udder made contact with the washer. This procedure was performed for both the right and left rare quarters with 3 repetitions on each until mean values with a coefficient of variation below 10% were obtained.

After calving, individual daily milk yield was measured for each cow using electronic meters until 60 DIM of the subsequent lactation, and incidence of IMI was monitored for the first 200 DIM in the 104 cows from SAT Sant Mer. Intramammary infection was either diagnosed and treated by the on-farm veterinarian or detected through SCC $>300 \times 10^3$ cells/mL at the DHIA sampling.

Statistical Analyses. Because treatment was applied at the animal level, the experimental unit was the animal. Measurements of udder pressure (conducted in the 2 rear quarters) were averaged within cow and sampling time before conducting statistical analysis.

All data from this experiment, except that pertaining to ML, were analyzed with a mixed-effects model with repeated measures using PROC MIXED of SAS similar to the one used in experiment 1 but excluding the covariate. The fixed part of the model accounted for the effect of treatment, day (or hour for blood), and their 2-way interaction, and the random part accounted for the effects of cow nested within treatment and batch, batch (or week of enrolment), block (parity), and herd. All models were subjected to an autoregressive variance-covariance structure of first order as it yielded the lowest Bayesian information criterion values.

In addition, data pertaining to lying behavior collected for the 5 d preceding dry-off were averaged and used as a covariate to assess the potential effect of treatment on lying time during the 5 d following dry-off. The mixed-effects model used accounted for the fixed effect of treatment, day relative to dry-off, and their 2-way interaction using lying time before dry-off as a covariate and day as a repeated measure, plus the random effects of cow nested within treatment and batch, batch, block (parity), and herd.

Observations of ML were categorized as a binary response variable (1 = presence of ML; 0 = absence of ML). Similarly, incidence of IMI was summarized by cow for the 200-d period, and cows were then classified as healthy if they never incurred IMI or sick if they had at least 1 IMI.

Prevalence of IMI was analyzed using a logistic regression analysis with treatment as a main factor. Milk leakage data were analyzed with a mixed-effects logistic regression model using Stata (version 14.2; StataCorp LLC, College Station, TX) that included the fixed effects of treatment, day, and the 2-way interaction plus the random effect of herd and cow nested within herd.

RESULTS AND DISCUSSION

Experiment 1

One cow from the sham group (B0 treatment) was removed from the study because of a displaced abomasum.

Milk production was affected by an interaction ($P < 0.001$) between treatment and days elapsed since bolus application, with the greatest decrease in milk production attained 2 d after bolus administration in B2 (Figure 1). Overall, these results demonstrate that oral administration of 2 acidogenic boluses to pregnant and lactating dairy cows reduces milk production >2 kg/d the second day after application. To our knowledge, this is the first time that milk production was recorded in pregnant and lactating dairy cows receiving anionic salts before dry-off. However, changes in milk production of dairy cows supplemented with various concentrations of anionic salts have been previously described (Escobosa et al., 1984). These authors reported that cows receiving diets supplemented with 2.28% calcium chloride (~280 g/d) tended to produce 2.7 kg/d less milk than those receiving a diet supplemented with 1.70% sodium bicarbonate during the first trimester of lactation.

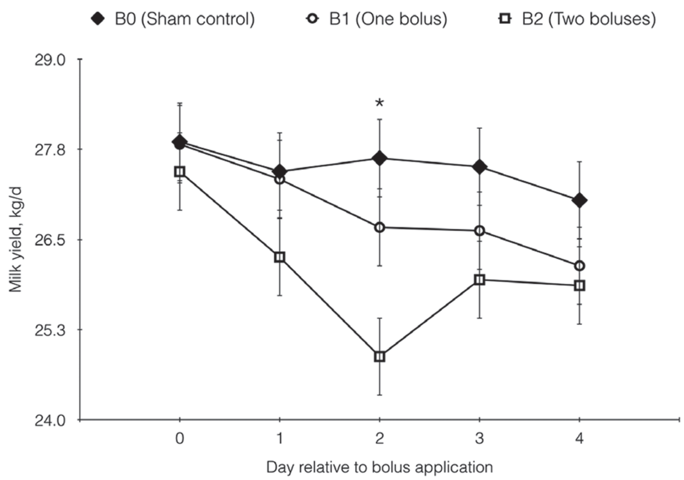

Figure 1. Experiment 1. Milk production in pregnant and lactating cows receiving no treatment (B0), cows receiving 1 acidogenic bolus after the last milking of d 0 (B1), and cows receiving 2 acidogenic boluses after the last milking of d 0 (B2). Error bars depict SEM at each time point. Asterisk depicts a difference ($P < 0.05$) between control and bolus cows.

Experiment 2

Urine pH. Urine pH of bolus cows declined ($P < 0.001$) after bolus application from 8.04 ± 0.05 at time zero to 7.37 ± 0.05 and 7.55 ± 0.05 at 8 and 24 h posttreatment, respectively, and then returned to values similar to those of time zero at 48 h (Figure 2). No differences in urine pH were observed in control cows among sampling times, and urine pH was approximately 8.07 throughout the sampling period (Figure 2). Reductions in urine pH when feeding anionic salts have been previously described in nonlactating dairy cows (Moore et al., 2000; Spanghero, 2002, 2004), and it appears to be related to renal reabsorption of HCO_3^- and enhanced urinary net acid excretion as a response mechanism to the metabolic perturbation of acid–base balance (Wang and Beede, 1992; Vagnoni and Oetzel, 1998).

Animal Performance. There was an interaction ($P < 0.001$) between treatment and day, with signifi-

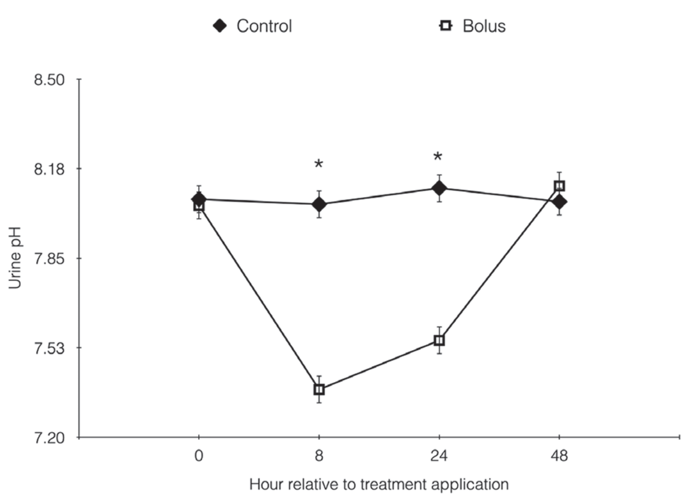

Figure 2. Experiment 2. Urine pH as affected by bolus application. Error bars depict SEM at each time point. Asterisk depicts difference ($P < 0.05$) between control and bolus cows.

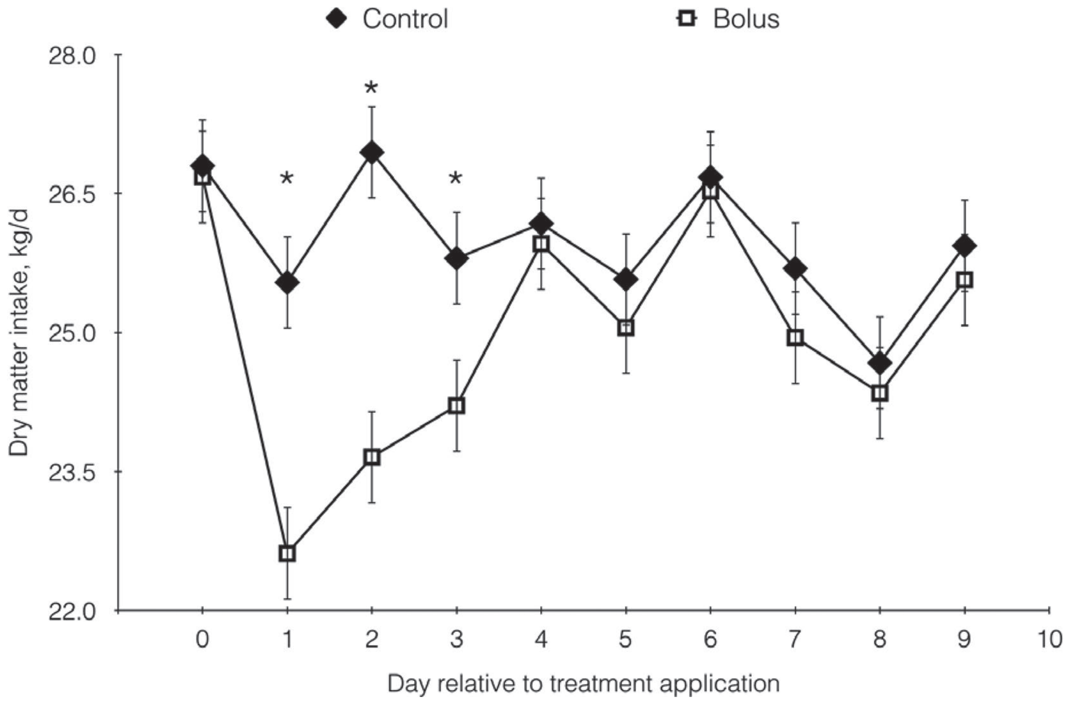

Figure 3. Experiment 2. Dry matter intake as affected by bolus application. Error bars depict SEM at each time point. Asterisk depicts difference ($P < 0.05$) between control and bolus cows.

cant decreases (on average 2.6 kg/d) in DMI during the first 3 d following treatment application (Figure 3). Reductions in DMI associated with feeding anionic salts in prepartum dairy cows have been well documented in the literature (Joyce et al., 1997; Moore et al., 2000; Spears et al., 2011). Studies (Oetzel et al., 1988; Tauriainen et al., 2001; Goff, 2018) testing diets supplemented with different anionic salts (i.e., NH_4C1, $MgCl_2$, and $MgSO_4$) in lactating dairy cows reported decreases in DMI, which has been commonly associated with reduced palatability as a consequence of the presence of anionic salts in the ration. However, in this study, the salts were administrated in the form of a fat-coated bolus, which was delivered directly into the rumen, thus having no efect on the palatability of the ration. Therefore, decreases in DMI observed herein could rather be due to alterations in the acid–base status of the animal. In fact, different intake patterns have been reported in nonlactating dairy cows fed different types of anionic salts mixed in the TMR (Oetzel and Barmore, 1993), with the least severe DMI depression observed when feeding magnesium sulfate, which, in turn, was the anionic salt with the least acidifying effects (Oetzel et al., 1991). Another possible explanation for the depressed DMI could be linked to potential damage of the ruminal wall. Wentink and van den Ingh (1992) reported that administration of gels containing $CaCl_2$ caused tissue damage in the rumen wall; however, no lesions were observed when the $CaCl_2$ was administered along with oil. Whether the boluses used herein potentially could cause rumen damage is unknown, but given the relative rapid resumption of intake (4 d) the odds for tissue damage seem low.

As observed in experiment 1, milk production was reduced ($P = 0.02$) by >2 kg/d the second day after bolus application, and in this experiment, milk production was also reduced on the third day after bolus application (Figure 4). The reduction in milk yield might be at least partly explained by the decrease in DMI observed in bolus cows.

Experiment 3

Average daily milk yield 5 d before dry-off, average milk yield in the last milking before dry-off, and days pregnant were 26.3 ± 4.50 kg/d, 8.4 ± 1.48 kg, and 228.8 ± 4.31 d, respectively, in control cows and 27.4 ± 5.66 kg/d, 8.7 ± 1.53 kg, and 227.7 ± 5.32 d, respectively, in bolus cows. Average milk yield during the last 5 d of lactation, milk yield in the last milking before dry-off, and days pregnant did not differ between treatment groups.

Udder Pressure and ML. Udder pressure decreased ($P < 0.001$) with time after dry-off in both groups, and this decline was more marked in bolus than in control cows (Figure 5). Udder pressure was affected

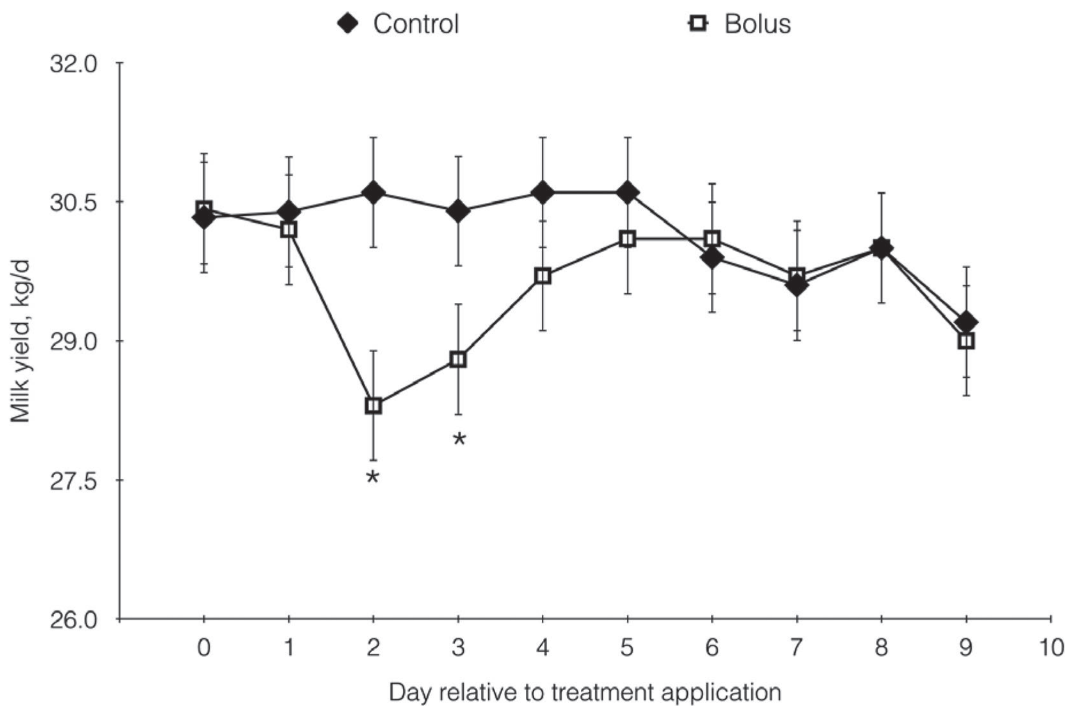

Figure 4. Experiment 2. Milk yield as affected by bolus application. Error bars depict SEM at each time point. Asterisk depicts difference ($P < 0.05$) between control and bolus cows.

by an interaction ($P = 0.04$) between treatment and day relative to dry-off. Udder pressure was lower at 24 h ($P < 0.001$) and 48 h ($P = 0.02$) and tended ($P = 0.06$) to be lower at 72 h after dry-off in bolus cows than in control cows (Figure 5). As a result, the average udder pressure during the first 72 h after dry-off was lower ($P < 0.001$) in bolus cows (55.0 ± 1.73 kg·m/s^2) than in control cows (61.9 ± 1.72 kg·m/s^2). Combined with the respective outcomes from experiments 1 and 2, these results support the hypothesis that application of acidogenic boluses before dry-off reduces milk production starting somewhere after the first 24 h following administration, resulting in a decrease in milk accumulation in the udder as indicated by a lower udder pressure (Figure 5). The peak udder pressure obtained herein at 24 h after dry-off for cows in both treatment groups is in contrast with other studies (Bertulat et al., 2013; Bach et al., 2015) that reported progressive increases in udder pressure following dry-off, with maximum pressures around d 2 or 3 or even at d 4 after dry-off (Leitner et al., 2007) in cows with similar milk yields as in the present study, but are similar to results by Bertulat et al. (2017), who also reported maximum udder pressure 1 d after dry-off. Nevertheless, milk production has been reported to be compromised after 18 h since last milking (Stelwagen and Lacy-Hulbert, 1996; Stelwagen et al., 1997), and tight junctions of the mammary epithelial cells become permeable after about 17 to 18 h after last milking (Stelwagen et al., 1997; Stelwagen and Ormrod, 1998).

The incidence of ML did not differ between groups. Overall, 18.8% of animals in the control group and 20.3% of animals in the bolus group showed ML at least once, with no differences between treatments. The greatest incidence of ML in both control and bolus cows was on both d 1 and 2 following dry-off, and it decreased ($P = 0.04$) on d 3 regardless of treatment. In line with our findings, Zobel et al. (2013) reported greater incidence of ML in dairy cows abruptly dried during d 1 and 2 after dry-off, whereas the incidence decreased on d 3. Although the observed decrease in udder pressure suggests a potential reduction in milk yield early after dry-off in bolus cows compared with control cows, the reduced udder pressure had no effect on the subsequent incidence of ML.

Milk Production and Udder Health. No differences in milk production between treatments were observed during the days for which milk production was recorded (first 60 DIM), with an average milk yield of 41.5 ± 1.09 kg/d for bolus cows and 41.5 ± 1.03 kg/d for control cows. To our knowledge, no information is available about the potential effects of anionic salt supplementation at dry-off on milk production in the following lactation. We had speculated that reduced udder pressure at dry-off might facilitate udder regeneration and hence have a positive effect on milk produc-

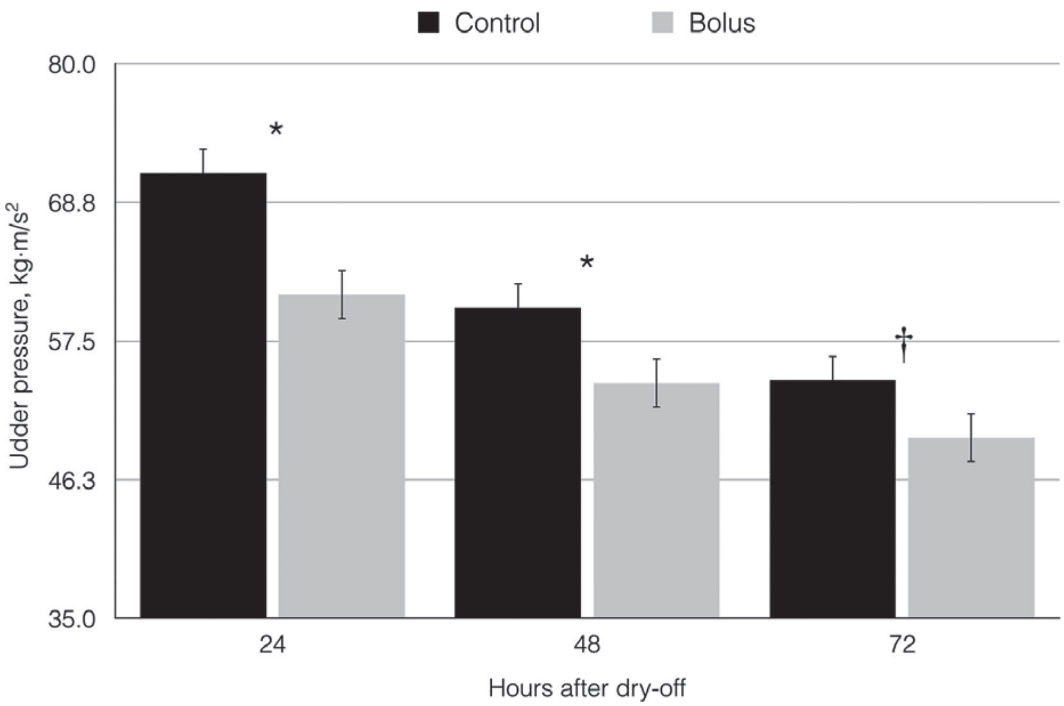

Figure 5. Experiment 3. Udder pressure (kg·m/s^2) relative to dry-off as affected by bolus application. Error bars depict SEM at each time point. *Difference ($P < 0.05$) between control and bolus cows; †tendency ($P < 0.10$) to differ between control and bolus cows.

tion; the results herein, however, indicate that anionic salt supplementation before dry-off had no effect on milk production in the following lactation.

A total of 104 cows (52 in each treatment group) were monitored for udder health during the first 200 d after calving. Of these cows, 29 (27%) showed at least 1 case of IMI. The incidence of IMI was not affected ($P = 0.79$) by treatment, with control cows having a 27.5% incidence of IMI and bolus cows having a 26.4% incidence of IMI. The lack of effect on the incidence of ML, which is a risk factor for IMI, was probably one of the reasons for the lack of differences in udder health after calving.

Lying Behavior. Total daily lying time was affected by an interaction ($P < 0.001$) between treatments and day relative to dry-off, with cows in the bolus group lying for an additional 85 min during the first 24 h after dry-off compared with control cows (Figure 6). This difference in daily lying time between control and bolus cows early after dry-off could be attributed to the greater udder pressure in control cows than in bolus cows. Previous studies have also reported negative associations between udder pressure and daily lying time (Leitner et al., 2007; Bach et al., 2015). Furthermore, several authors (Zobel et al., 2013; Chapinal et al., 2014; Rajala-Schultz et al., 2018) have described negative relationships between milk production before dry-off and lying time or duration of lying bouts after milking cessation.

Frequency of lying bouts was affected by an interaction ($P = 0.02$) between treatment and time, with bolus cows having fewer lying bouts (9.5 ± 0.55 bouts/d) on d 2 after dry-off than control cows (10.8 ± 0.54 bouts/d). The more frequent lying bouts in control cows relative to bolus cows could also be an indication of some discomfort due to udder pressure that may have forced control cows to stand up. Cows in the bolus group had longer ($P < 0.001$) lying bouts during the second day after dry-off (82.3 ± 4.81 min/d) than control cows (72.7 ± 4.81 min/d).

Blood Metabolites. Blood PRL concentration, which is positively related to milk production in dairy cows (Koprowski and Tucker, 1973; Lacasse et al., 2012, 2016), tended to be greater in control cows than in bolus cows (Table 2). Furthermore, as expected, it decreased with time in both treatment groups after dry-off. In line with the results herein, other authors (Ollier et al., 2014) reported a depression in blood PRL concentration after dry-off.

Reported effects of dietary anionic salts on concentrations of blood Ca and P have been inconsistent in dairy cows. For instance, lowering dietary DCAD in nonlactating prepartum cows has been reported to increase total serum Ca (Joyce et al., 1997) and ionized

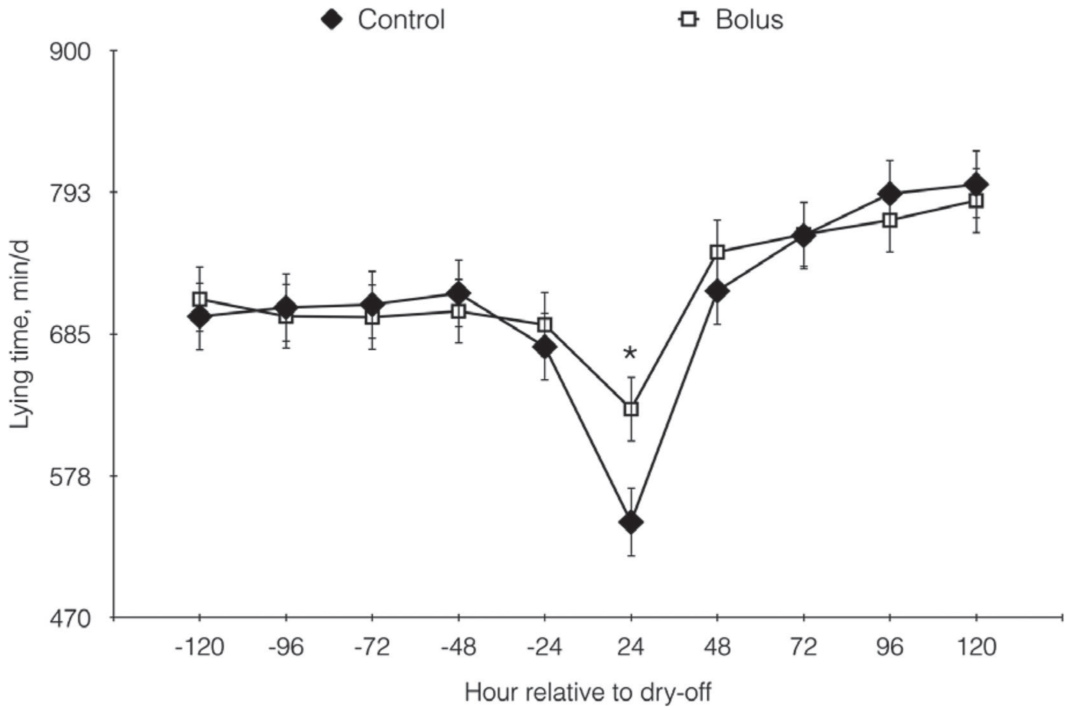

Figure 6. Experiment 3. Time devoted to lying (min/d) as affected by bolus application. Error bars depict SEM at each time point. Asterisk depicts difference ($P < 0.05$) between control and bolus cows.

Ca (Moore et al., 2000; Charbonneau et al., 2006), but no changes have been found in other studies (Oetzel et al., 1991; Vagnoni and Oetzel, 1998). Interestingly, in this study, blood Ca concentration was unaffected by bolus application (Table 2), although serum Ca progressively increased after dry-off (0 h = 9.14; 8 h = 9.28; 24 h = 9.62; 48 h = 9.81 ± 0.067) independently of treatments. By contrast, blood P concentration, which has been found to be less strictly controlled by homeostatic systems in dairy cows (Horst, 1986), was lower in supplemented cows than in control cows (Table 2). Nevertheless, and despite this reduction, mean blood P concentrations observed herein remained within physiological P concentration in blood (4–8 mg/dL) in adult cows (Goff, 1999) regardless of treatment. Thus, overall, the results herein suggested that administration of acidogenic boluses at dry-off elicits a normal physiological response to a mild metabolic acidosis without altering calcemia or phosphatemia.

The application of bolus had no effect on plasma NEFA concentrations (Table 2), but it resulted in a lower blood BHB concentration compared with control cows (Table 2). The reduction in blood BHB in bolus cows could probably be explained by lower amounts of

Table 2. Blood prolactin, calcium, phosphorus, BHB, and nonesterified fatty acids (NEFA) concentrations as affected by treatment and time relative to dry-off (experiment 3)[1]

	Treatment[2]			P-value[3]		
Item	Control	Bolus	SE	T	t	T × t
Prolactin, ng/mL	15.2	11.9	1.41	0.10	<0.001	0.20
Calcium, mg/dL	9.50	9.39	0.07	0.31	<0.001	0.54
Phosphorous, mg/dL	6.79	6.31	0.14	0.02	<0.001	0.38
BHB, m*M*	0.53	0.44	0.02	<0.001	<0.001	0.95
NEFA, m*M*	0.35	0.35	0.03	0.94	<0.001	0.08

[1]Blood was collected from 25 cows per treatment group at 0, 8, 24, and 48 h after dry-off.

[2]Control = no treatment; bolus = received 2 boluses (5 min apart) about 12 to 8 h before last milking preceding dry-off.

[3]T = effect of treatment; t = effect of time of sampling: T × t = interaction between treatment and time.

butyrate being metabolized in the rumen wall of bolus cows as a possible consequence of the reduced DMI, as described in experiment 2.

CONCLUSIONS

Data from the present study indicated that supplementation with approximately 40 g of NH_4Cl combined with $CaCl_2$ and $CaSO_4$, via 2 oral boluses, induces a slight metabolic acidosis in the cow (as indicated by a decrease in urine pH) and reduced milk production in pregnant dairy cows at the end of lactation for at least 48 h following bolus administration. The decrease in milk yield could partially be explained by a reduction in DMI. Also, when administered 8 to 12 h before dry-off, acidogenic boluses reduced udder pressure during the first 2 d after dry-off and increased daily lying time on the first day after dry-off. Application of anionic salts at dry-off via oral boluses could be an interesting approach to facilitating the drying-off of dairy cows.

ACKNOWLEDGMENTS

This research was sponsored by Boehringer Ingelheim Vetmedica GmbH (Ingelheim am Rhein, Germany). We acknowledge the CERCA program from Generalitat de Catalunya (Barcelona, Spain) for supporting this project.

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