Effect of Mastitis on Milk Perchlorate Concentrations in Dairy Cows

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ABSTRACT

Recent surveys have identified the presence of perchlorate, a natural compound and environmental contaminant, in forages and dairy milk. The ingestion of perchlorate is of concern because of its ability to competitively inhibit iodide uptake by the thyroid and to impair synthesis of thyroid hormones. A recent study established that milk perchlorate concentrations in cattle highly correlate with perchlorate intake. However, there is evidence that up to 80% of dietary perchlorate is metabolized in clinically healthy cows, thereby restricting the available transfer of ingested perchlorate into milk. The influence of mastitis on milk perchlorate levels, where there is an increase in mammary vascular permeability and an influx of blood-derived components into milk, remains unknown. The present study examined the effect of experimentally induced mastitis on milk perchlorate levels in cows receiving normal and perchlorate-supplemented diets. Over a 12-d period, cows were ruminally infused with 1 L/d of water or water containing 8 mg of perchlorate. Five days after the initiation of ruminal infusions, experimental mastitis was induced by the intramammary infusion of 100 µg of bacterial lipopolysaccharide (LPS). Contralateral quarters infused with phosphate-buffered saline served as controls. A significant reduction in milk perchlorate concentration was observed in the LPS-challenged glands of animals ruminally infused with either water or perchlorate. In control glands, milk perchlorate concentrations remained constant throughout the study. A strong negative correlation was identified between mammary vascular permeability and milk perchlorate concentrations in LPS-infused glands. These findings, in the context of a recently published study, suggest that an active transport process is operative in the establishment of a perchlorate concentration gradient across the blood–mammary gland interface, and that increases in mammary epithelial and vascular endothelial permeability lead to a net outflow of milk perchlorate. The overall finding that mastitis results in lower milk perchlorate concentrations suggests

that changes in udder health do not necessitate increased screening of milk for perchlorate.

Key words: dairy cow, mastitis, milk, perchlorate

INTRODUCTION

Over the past decade, there has been increasing concern about the presence of perchlorate in water and food supplies. Health concerns regarding the ingestion of perchlorate relate to its similarity in size and shape to iodide and its corresponding ability to competitively inhibit iodide uptake by the sodium-iodide symporter (NIS; Clewell et al., 2003). These properties of perchlorate result in its ability to reduce iodide uptake by the thyroid and thus impair the synthesis of thyroid hormones involved in the daily regulation of metabolism as well as physical and mental development. These concerns have led to the commissioning of a recent report establishing reference doses for perchlorate ingestion (NRC, 2005).

Perchlorate salts are used as solid oxidants in the propulsion systems of rockets and missiles and in a variety of other industrial settings, including the manufacturing of matches, flares, airbag inflation systems, batteries, rubber, and dyes (Motzer, 2001; Hogue, 2003). In addition, there is evidence that perchlorate exists naturally in the environment (Bao and Gu, 2004; Dasgupta et al., 2005). Because perchlorate salts are highly soluble, they readily diffuse in surface and groundwater (Motzer, 2001). Thus, in areas of the country with large concentrations of military installations, perchlorate contamination of regional water supplies has become ubiquitous. In particular, regional water supplies within the Colorado watershed, which provide drinking water to millions of people and food-producing animals, have been shown to contain perchlorate concentrations ranging from 8 to 30 ng/mL, although levels of up to 280 ng/mL have been reported (Urbansky and Schock, 1999; Motzer, 2001). Environmental perchlorate is not limited to this region, because perchlorate is used in the manufacture of an array of products, occurs naturally in the environment, and has correspondingly been detected in water supplies throughout the United States (Motzer, 2001; Hogue, 2003).

In addition to drinking water, another potential source of perchlorate is through the ingestion of produce grown

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in areas irrigated by perchlorate-contaminated water (Krynitsky et al., 2004; Jackson et al., 2005; Sanchez et al., 2005). The Colorado River, for example, is a major source of irrigation water for a substantial percentage of the nation's crops. The uptake of perchlorate from irrigation water has been reported for a variety of plants, including lettuce and alfalfa (Krynitsky et al., 2004; Jackson et al., 2005; Sanchez et al., 2005). Plants have the ability to concentrate perchlorate up to 2 orders of magnitude (100 $\times$) higher than that in the supplying water (Tan et al., 2004). Alfalfa, a common component of cattle feed, is reported to be particularly efficient at concentrating perchlorate, and levels of up to 2.9 mg/kg (ppm) of fresh weight have been reported (Jackson et al., 2005).

Because of the presence of perchlorate in both drinking water and in forages, it is not surprising that perchlorate has been detected in dairy milk (Kirk et al., 2003, 2005; Krynitsky et al., 2004; US FDA, 2004). In a survey of milk samples collected from throughout the United States, perchlorate concentrations ranged from undetectable up to 11 $\mu\text{g/L}$ (ppb), with a mean value of 5.76 $\mu\text{g/L}$ (ppb; US FDA, 2004). A more recent survey identified a similar range of milk perchlorate levels (Kirk et al., 2005). Interestingly, the investigators involved in the latter study conducted a parallel analysis of human breast milk samples and determined that mean perchlorate concentrations were 5-fold greater in those samples than in those from cows, and that maximum values in human breast milk ranged up to 92 $\mu\text{g/L}$ (ppb; Kirk et al., 2005). Recently, we established that milk levels of perchlorate highly correlate with perchlorate intake in clinically healthy dairy cattle (Capuco et al., 2005). Further, we showed that up to 80% of dietary perchlorate is metabolized in cattle. Because bacteria possess the ability to reduce perchlorate (Coates and Achenbach, 2004; Bender et al., 2005; Krauter et al., 2005), the anaerobic environment and complex microbial flora of the rumen may contribute to the cow's ability to metabolize perchlorate and thus serve as a biological filter. The finding that milk perchlorate concentrations are higher in monogastric organisms such as humans (Kirk et al., 2005) supports the hypothesis of rumen metabolism of perchlorate in cows.

Although we established that healthy cows have the ability to reduce the transfer of dietary perchlorate to blood and milk (Capuco et al., 2005), whether mastitis or other inflammatory processes alter the transference remains unknown. During mastitis, the mammary-vascular barrier function is disrupted and serum components leak into the gland (Giri et al., 1984; Mattila and Frost, 1989). Thus, one may hypothesize that during mastitis, circulating perchlorate may accumulate in the gland and cause an increase in its concentration in milk.

Because we have just recently ascertained that the concentrations of perchlorate in milk exceed those in blood (Capuco et al., 2005), such passive transfer may be unlikely. However, decreased secretion of milk during mastitis may result in higher concentrations of perchlorate by diminishing the dilutional effect of milk. Additionally, because mastitis is accompanied by a dramatic recruitment of neutrophils to the mammary gland, biological processes analogous to the generation of hypochlorite by phagocytic neutrophils (Kalyanaraman and Sohnle, 1985; Imada et al., 1999) may provide an endogenous source of perchlorate. Conversely, perchlorate levels may be expected to decrease in milk during mastitis. Perchlorate is a well-established competitive inhibitor of the NIS (Soldin et al., 2001), which is expressed in the mammary gland (Shennan and Peaker, 2000; Perron et al., 2001). Data exist to support the ability of the NIS to transport perchlorate directly (Van Sande et al., 2003; Clewell et al., 2004). In the setting of mastitis, disruption of epithelial tight junctions and increases in mammary vascular permeability could disrupt any perchlorate gradient established by an active transporter, thus resulting in a net outflow of perchlorate from milk into the circulation and a corresponding decrease in milk perchlorate concentrations. This effect has been observed for other ions and molecules that are concentrated in milk (Wegner and Stull, 1978; McFadden et al., 1988). Therefore, the objective of the current study was to identify the effect of mastitis on milk perchlorate levels in cows receiving normal and perchlorate-supplemented diets.

MATERIALS AND METHODS

Cows

Twelve clinically healthy Holstein cows in late lactation (321 ± 12 DIM) were used for the study. Each cow had been previously fitted with a permanent 10-cm rumen cannula (BarDiamond, Inc., Parma, ID) to permit controlled ruminal administration of either the perchlorate solution or water control. Cows were housed in an environmentally controlled, tie-stall facility and fed *ad libitum* a TMR diet formulated to meet NRC requirements for lactating cows (NRC, 2001). Water was freely available by automated watering cups. Cows were provided exercise for 1 h daily after the morning milking. Cows were milked twice daily at 0700 and 1800 h. Because experimental treatments (i.e., ruminal infusion of perchlorate and intramammary infusion of bacterial LPS) were initiated just after the morning milking, total daily milk output was calculated by summing the morning and prior evening milk weights. The use and care of all animals for this study was approved by the Beltsville Agricultural Research Center's Animal Care and Use Committee.

Perchlorate Treatment

Cows were randomly assigned to 2 experimental groups that were ruminally infused with either water or perchlorate. There was no statistical difference in the stage of lactation (319 ± 20 vs. 323 ± 15 DIM) or milk production (20.39 ± 1.35 vs. 21.57 ± 2.47 kg/d) between experimental groups. The cows were subjected to continuous ruminal infusion of 1 L of distilled water per day containing either 0 or 8 mg of perchlorate, supplied as NaClO_4^- (Sigma Chemical Co., St. Louis, MO) over a 23-h infusion period (1100 h to 1000 h). The infusion of 8 mg of perchlorate per day was designed to provide an amount equivalent to the consumption of a 40 kg/d diet containing 200 ppb of perchlorate. The concentration of perchlorate in the prepared infusate solution was confirmed as described below.

Experimental Induction of Mastitis

Prior to experimental induction of mastitis, quarters were screened for the presence of IMI by aseptically plating collected milk samples on blood agar plates and incubating the plates for 24 h at 37°C. Milk SCC were also determined in aliquots of these samples (see the following section). Quarters were determined to be free of major mastitis pathogens and to have milk SCC <200,000 cells/mL. The mean ($\pm$ SE) milk SCC for all quarters prior to treatment was $50,000 \pm 9,000$ cells/mL.

Experimental mastitis was induced as described previously (Bannerman et al., 2003). Briefly, 100 μg of LPS derived from *Escherichia coli* O111:B4 (Sigma Chemical Co.) was dissolved in sterile PBS to achieve a final concentration of 20 $\mu\text{g}/\text{mL}$. The contralateral quarters of animals in each of the 2 experimental groups (i.e., animals ruminally delivered water vs. perchlorate) were infused with either 5 mL of PBS or LPS (100 $\mu\text{g}/\text{quarter}$) just after the morning milking (time 0).

Determination of Milk SCC and Whey Preparation

To quantify somatic cells, milk samples were heated to 60°C and subsequently maintained at 40°C until counted on an automated cell counter (Bentley Soma-count 150; Bentley Instruments, Inc., Chaska, MN). For the preparation of whey, milk samples were centrifuged at $44,000 \times g$ at 4°C for 30 min and the fat layer was removed with a spatula. The skimmed milk was decanted into a clean tube and centrifuged again for 30 min as just described, and the translucent supernatant was collected and stored at -70°C.

Quantification of Milk BSA and Tumor Necrosis Factor- α Concentrations.

The levels of BSA and tumor necrosis factor- α (TNF- α) in milk were determined by ELISA as previously described (Bannerman et al., 2005).

Sample Preparation for Perchlorate Analysis

Foremilk and blood samples, the latter of which were collected into heparin Vacutainers (Becton Dickinson Co., Franklin Lakes, NJ) from the coccygeal vein, were prepared for perchlorate analysis as previously described (Capuco et al., 2005). Briefly, milk and blood samples were spiked with an internal standard solution of $^{18}\text{O}_4$ -labeled perchlorate (gift of A. Krynetsky, US FDA, College Park, MD). The solution was mixed with deionized water and acetonitrile and centrifuged. The clear supernatant was passed through a Supelclean ENVI-Carb solid-phase cartridge (Supelco, Bellefonte, PA) and the eluant was collected. The volume was adjusted and a portion was filtered through a 0.45- μm pore size nylon syringe filter and analyzed by ion chromatography (IC)–triple quadrupole mass spectrometry (MS–MS). Preparation of infusate samples for analysis simply required the addition of $^{18}\text{O}_4$ -labeled perchlorate as an internal standard and filtration (0.45- μm pore size nylon syringe filter) prior to analysis by IC–MS–MS. Sodium chloride (1%) was used in blanks and spiking solutions prior to use of the Supelclean ENVI-Carb cartridges to prevent recovery loss.

Perchlorate Analysis

All perchlorate analyses used the IC–MS–MS method as described previously (Capuco et al., 2005). Ion chromatography separation utilized a Waters IC-Pak Anion HR HPLC column, 4.6×75 mm (Waters, Milford, MA), and a Waters IC-Pak Anion Guard-Pak guard column on a Waters 2690 XE separations module operated at 50°C. The mobile phase was 100 mM ammonium acetate:acetonitrile (50:50 vol/vol) with a flow rate of 0.3 mL/min; all of the eluant was allowed into the MS. The injection volume was 50 μL . Total run time was 15 min, with elution of perchlorate occurring at ~9 min.

The atmospheric pressure ionization–tandem mass spectrometry analysis was performed on a benchtop triple-quadrupole mass spectrometer (Quattro LC; Micromass Ltd., Manchester, UK). The instrument was operated in negative electrospray ionization mode with settings as described previously (Capuco et al., 2005).

Analyte concentrations were calculated by the internal standard method using $^{18}\text{O}_4$ -labeled perchlorate as the internal standard. The ion pairs for the native perchlorate and $^{18}\text{O}_4$ -labeled perchlorate were 99 m/z transi-

tion to 83 m/z and 107 m/z transition to 89 m/z , respectively. Calibration curves were prepared in the mobile-phase solvents using concentrations from 0.05 to 50 ng/mL of perchlorate. Peak integration and quantitation were performed using MassLynx 4.0 software (Micro-mass Ltd.). Matrix spikes, duplicates, and blanks were analyzed at a rate of 1 per 20 samples. Spike recoveries for milk and blood averaged 89 and 102%, respectively, and the relative percentage differences for duplicate pairs for milk and blood averaged 4.6 ($n = 14$ pairs) and 24% ($n = 9$ pairs), respectively. Standard curves were linear with an $r^2 = 0.996$.

Statistical Methods

Repeated-measures ANOVA was performed using PROC MIXED (SAS version 8.2; SAS Institute, Cary, NC) to compare the mean responses of treatment groups. For statistical analysis of milk SCC, data were transformed to $\log_{10}$ values. A P -value of <0.05 was considered significant.

RESULTS

Stable Elevation of Circulating Blood Perchlorate Concentrations with Increased Dietary Consumption of Perchlorate

Five days before the induction of experimental mastitis (d -5), 6 cows were initiated on a regimen of ruminal infusion of 8 mg/d of perchlorate delivered continuously as a 1-L solution over a 23-h period. A control group of 6 additional cows was ruminally infused with 1 L of water/d. The actual amount of infusate delivered was determined by calculating the difference in the daily weight of individual flasks supplying the prepared solution pumped into the rumen of each cow, before and at the end of each 23-h infusion period. The weight of solution delivered to the rumen was consistent throughout the study, and calculated daily values of infusate volume were within 5% (Figure 1). Correspondingly, in those cows ruminally infused with the perchlorate solution, the amount of perchlorate delivered was within 5% of 8 mg on each day of the study.

Prior to initiation of ruminal infusion, the mean ($\pm$ SE) blood perchlorate concentration of all animals was 0.423 ± 0.047 ng/mL. In cows ruminally infused with 1 L of water per day, blood perchlorate values remained consistent with preinfusion levels throughout the study (Figure 2). In cows ruminally infused with 8 mg of perchlorate per day, blood perchlorate concentrations reached a steady-state value of 3.201 ± 0.321 ng/mL just prior to induction of experimental mastitis at time 0. Blood concentrations of perchlorate remained elevated and constant throughout the 168-h study period follow-

Figure 1. Daily delivery of rumen infusate. Five days prior to intramammary infusion of PBS or bacterial LPS, 6 cows were started on a continuous ruminal infusion of 1 L of water per day containing 8 mg of perchlorate (ClO_4^-). As a control group, 6 additional cows were started on a continuous ruminal infusion of 1 L of water per day. Immediately following the morning milking on d 0, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 μg) to induce experimental mastitis. Cows continued to be ruminally infused with either water or perchlorate until 168 h after the LPS intramammary infusion (i.e., the end of the d-6 infusion period). Mean ($\pm$ SE) ruminal infusion volumes of water or perchlorate for all cows ($n = 12$) are shown (striped bars; left y-axis). For those cows in the experimental group receiving perchlorate ($n = 6$), the mean ($\pm$ SE) amount of daily perchlorate infused is shown (solid bars; right y-axis).

ing induction of experimental mastitis. After collection of the 168-h time point samples, all ruminal infusions were stopped and a final (postinfusion) sample was collected 48 h later. By that time, all cows infused with either water or perchlorate had blood perchlorate concentrations comparable to those observed prior to the initiation of ruminal infusions.

Induction of Clinical Mastitis via Intramammary Infusion of Bacterial LPS

Five days after initiation of ruminal infusions, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 μg) to induce experimental mastitis (time 0). Regardless of the ruminal infusion treatment, intramammary infusion of LPS induced a marked transient decrease ($\sim 40\%$) in milk production over the 24-h period immediately following the LPS challenge (Figure 3A). In addition to changes in milk production, intramammary infusion of LPS elicited a systemic response characterized by the induction of fever. Rectal temperatures initially increased within 4 h of the LPS challenge, reached a maximum at 6 h, and returned to baseline (time 0) levels by 10 h (Figure 3B). There was no difference in the febrile response between water- and perchlorate-infused animals, and peak temperatures 6 h after the LPS challenge were comparable (41.27 ± 0.18

Figure 2. Effect of increased dietary levels of perchlorate on blood perchlorate concentrations. Prior to initiation of ruminal infusion of either perchlorate or water, blood samples were collected to determine basal concentrations of circulating perchlorate (prelevels). Cows were then ruminally infused with either perchlorate (ClO_4^- ; 8 mg/d) or water. Five days after initiation of the perchlorate treatment, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 μg) to induce experimental mastitis (time 0). Blood samples were collected just prior to intramammary infusion and throughout the 168-h study period. At the end of this study period, ruminal infusion of perchlorate was ceased and a final blood sample was collected 48 h later (postlevels). Mean ($\pm$ SE) blood perchlorate concentrations are reported in nanograms per milliliter for each experimental group ($n = 6$). *Significantly increased compared with preruminally infused levels of perchlorate ($P < 0.05$).

and $41.54 \pm 0.16^\circ\text{C}$, respectively). Ruminal infusion of either water or perchlorate had no effect on milk production or rectal temperatures throughout the study.

Intramammary challenge with LPS elicited a localized inflammatory response characterized by increases in milk SCC (Figure 4A) and the induction of $\text{TNF-}\alpha$ (Figure 4B). Within 2 h of the challenge, milk SCC were elevated in LPS-challenged quarters regardless of the ruminal infusion treatment. Milk SCC reached a mean ($\pm$ SE) $\log_{10}$ peak of 7.845 ± 0.146 (arithmetic mean $\pm$ SE of $96.56 \times 10^6 \pm 39.07 \times 10^6$) and 7.641 ± 0.087 (arithmetic mean $\pm$ SE of $48.35 \times 10^6 \pm 9.75 \times 10^6$) cells/mL in the LPS-infused quarters of animals ruminally infused with water or perchlorate, respectively (Figure 4A). Milk SCC remained elevated in the LPS-challenged quarters of animals ruminally infused with water or perchlorate for >96 and 168 h after the challenge, respectively. Similar to milk SCC, increases in $\text{TNF-}\alpha$ concentrations were observed 2 h following the LPS challenge regardless of the ruminal infusion treatment. Tumor necrosis factor- α concentrations peaked 2 to 4 h later and did not return to basal (time 0) levels until 24 h after the initial challenge. Milk SCC and $\text{TNF-}\alpha$ concentrations in PBS-in-

fused quarters remained unchanged from baseline (time 0) levels throughout the study. Relative to water infusate controls, there was no significant treatment effect of perchlorate on either milk SCC or $\text{TNF-}\alpha$ concentrations throughout the study.

Breakdown of the Blood–Milk Barrier in LPS-Infused Glands

Elevated concentrations of milk BSA, an indicator of increased permeability of the mammary vasculature and epithelium, were evident within 4 h of the LPS challenge (Figure 5). Milk BSA concentrations remained elevated until >12 h after the challenge. Twenty-four hours after the LPS infusion, BSA concentrations returned to levels that were not statistically distinguishable from basal (time 0) levels. Bovine serum albumin concentrations in milk from PBS-infused quarters remained unchanged from baseline (time 0) levels throughout the study. Relative to water infusate controls, there was no significant treatment effect of perchlorate on milk BSA levels throughout the study.

Decreased Milk Concentrations of Perchlorate During Mastitis

Prior to initiation of ruminal infusion, the mean ($\pm$ SE) perchlorate concentration in the milk of all quarters was 4.36 ± 0.35 ng/mL. In cows ruminally infused with 1 L of water per day, there was no difference in preinfusion levels of milk perchlorate and those present 5 d later just prior to the intramammary challenge at time 0 (Figure 6). In contrast, milk perchlorate concentrations in cows ruminally infused with perchlorate were significantly elevated at time 0 (23.96 ± 2.07 ng/mL) relative to preinfused basal milk levels. Milk perchlorate concentrations remained elevated throughout the remainder of the infusion period (i.e., 168 h post-LPS intramammary challenge) in all quarters of animals ruminally infused with perchlorate, and they returned to baseline (preinfusion) levels within 48 h of ceasing the infusion.

A marked decrease in milk perchlorate concentrations was observed 4 to 12 h after the intramammary infusion in LPS-challenged glands (Figure 6). This decrease was apparent in the LPS-challenged quarters of animals ruminally infused with either water or perchlorate, and the duration of the decrease was identical for both groups. In the PBS-infused quarters of all animals, milk perchlorate concentrations remained similar to those observed at time 0 throughout the 168-h study period following intramammary infusion. A strong negative correlation existed between permeability of the blood–milk barrier (Figure 5) and milk perchlorate concentrations in the LPS-challenged glands of animals ruminally infused

Figure 3. Effect of LPS-induced mastitis on milk production and core body temperature. Prior to initiating the ruminal infusion of perchlorate or water, (A) total daily milk weights (sum of morning and previous evening outputs) and (B) rectal temperatures were recorded to establish baseline measurements (prelevels). Cows were then ruminally infused with either perchlorate (ClO₄⁻; 8 mg/d) or water. Five days after initiating the perchlorate treatment, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 µg) to induce experimental mastitis (time 0). Intramammary infusions were performed immediately following the morning milking. Milk weights and rectal temperatures were recorded throughout the 168-h study period. At the end of this study period, ruminal infusion of perchlorate was ceased and the final milk weight and rectal temperature data were collected 48 h later (postlevels). Mean (± SE) milk weights and temperatures are reported in kilograms and degrees Celsius, respectively, for each experimental group (n = 6). *Significantly different from time 0 levels in cows ruminally infused with perchlorate ($P < 0.05$). #Significantly different from time 0 levels in cows ruminally infused with water ($P < 0.05$).

with either perchlorate ($r = -0.849$; $P = 0.0005$) or water ($r = -0.786$; $P = 0.0024$).

DISCUSSION

We recently reported on the fate of dietary perchlorate in clinically healthy dairy cows and measured the distribution of ingested perchlorate in milk, blood, urine, and feces (Capuco et al., 2005). In that study, we established that the output of perchlorate was well below the amount ingested, and that up to 80% of dietary perchlorate may be metabolized in the rumen. From a human health perspective, one implication of this finding is that if sources of ingested perchlorate are from drinking water, then regional sources of dairy milk would be expected to contain lower levels of perchlorate than corresponding water supplies. Because that investigation was performed with clinically healthy cattle, we conducted a follow-up study to determine whether mastitis could influence the concentrations of perchlorate in milk.

In the present study, cows were ruminally infused with 1 L/d of either water or 8 mg of perchlorate to assess changes in milk perchlorate in cows exposed to normal or high-perchlorate diets. The infusion of 8 mg of perchlorate/d provided an amount equivalent to the consumption of a 40 kg/d diet containing 200 ng/mL (ppb) of perchlorate. The quantity of perchlorate that was infused established milk concentrations that fell within

detection ranges that provided maximal leeway for the detection of increases or decreases in milk perchlorate concentrations that might occur following experimental mastitis. Although the amount infused provided a relatively high level of perchlorate, there have been limited reports of water supplies and forages that contain similar or higher concentrations (Motzer, 2001; Jackson et al., 2005).

The basal concentration of milk perchlorate in all cows prior to initiation of the study was 4.36 ± 0.35 ng/mL, an amount well within the range of that reported in surveys of commercial milk supplies throughout the United States (Kirk et al., 2003; US FDA, 2004; Kirk et al., 2005). Ruminal infusion of 8 mg/d of perchlorate increased these levels ~5.5-fold to a concentration of 23.96 ± 2.07 ng/mL at time 0 (Figure 6), just prior to the induction of mastitis. These milk perchlorate concentrations are approximately twice the maximum values observed in a survey of commercial milk (Kirk et al., 2005) and are reflective of the high-perchlorate diet to which cows were subjected in the current investigation. The ~5.5-fold increase in milk perchlorate concentration following ruminal infusion reflected increased availability of perchlorate in the circulation, where blood perchlorate concentrations increased ~7.5-fold following ruminal infusion (Figure 2). Consistent with our previous study, the milk and blood concentrations reported here (following ruminal infusion of 8 mg/d of perchlorate) were between

Figure 4. Effect of LPS-induced mastitis on milk SCC and tumor necrosis factor- α (TNF- α) production. Prior to initiating the ruminal infusion of perchlorate or water, milk SCC (A) were quantified and milk TNF- α levels (B) were assayed to establish baseline measurements (prelevels). Cows were then ruminally infused with either perchlorate (ClO_4^- ; 8 mg/d) or water. Five days after initiating the perchlorate treatment, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 μg) to induce experimental mastitis (time 0). Milk SCC and TNF- α concentrations were monitored throughout the 168-h study period. At the end of this study period, ruminal infusion of perchlorate was ceased and the final milk SCC and TNF- α levels were measured in samples collected 48 h later (postlevels). Mean ($\pm$ SE) milk SCC and TNF- α concentrations are reported in millions per milliliter and nanograms per milliliter, respectively, for each experimental group ($n = 6$). *Significantly increased compared with time 0 levels in cows ruminally infused with perchlorate ($P < 0.05$). #Significantly increased compared with time 0 levels in cows ruminally infused with water ($P < 0.05$).

the corresponding range of milk and blood values detected in cows ruminally infused with 4 and 40 mg/d of perchlorate (Capuco et al., 2005).

To investigate the effect of mastitis on milk perchlorate concentrations, experimental mastitis was induced by the intramammary infusion of bacterial LPS. Consis-

Figure 5. Effect of LPS-induced mastitis on permeability of the blood-milk barrier. As an indicator of changes in the integrity of the blood-milk barrier, BSA concentrations in milk were assayed throughout the study by ELISA. Prior to initiating the ruminal infusion of perchlorate or water, milk BSA concentrations were quantified to establish baseline measurements (prelevels). Cows were then ruminally infused with either perchlorate (ClO_4^- ; 8 mg/d) or water. Five days after initiating the perchlorate treatment, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 μg) to induce experimental mastitis (time 0). Milk BSA levels were monitored throughout the 168-h study period. At the end of this study period, ruminal infusion of perchlorate was ceased and final milk BSA levels were measured in samples collected 48 h later (postlevels). Mean ($\pm$ SE) BSA concentrations are reported in micrograms per milliliter for each experimental group ($n = 6$). *Significantly increased compared with time 0 levels in cows ruminally infused with perchlorate ($P < 0.05$). #Significantly increased compared with time 0 levels in cows ruminally infused with water ($P < 0.05$).

tent with previous reports (Shuster et al., 1991; Bannerman et al., 2003), LPS elicited a systemic response characterized by the induction of fever and decreased milk production (Figure 3). Bacterial LPS evoked a localized inflammatory response as evidenced by an increase in milk SCC (Figure 4A), the induction of TNF- α (Figure 4B), and perturbation of the blood-milk barrier function (Figure 5). None of these indicators of inflammation were evident in control quarters infused with PBS. Albeit on a faster kinetic scale, the responses elicited by intramammary infusion of LPS mirrored those observed during gram-negative bacterial infection (Bannerman et al., 2004; Vangroenweghe et al., 2004) and reflect the prominent role of this molecule in inducing host innate immune responses (Hogan and Smith, 2003).

The induction of mastitis resulted in decreased concentrations of milk perchlorate relative to noninflamed glands (Figure 6). The decrease in milk perchlorate levels in LPS-infused glands was evident in animals subjected to control or perchlorate treatments. In fact, the duration of decreased milk perchlorate concentrations was identical (4 to 12 h postintramammary infusion of LPS), and the mean percentage decreases during this time period

Figure 6. Effect of LPS-induced mastitis on milk perchlorate concentrations. Prior to initiating ruminal infusion of either perchlorate or water, milk samples were collected to determine basal levels (pre-levels) of perchlorate in milk. Cows were then ruminally infused with either perchlorate (ClO_4^- ; 8 mg/d) or water. Five days after initiating the perchlorate treatment, the contralateral quarters of all cows were infused with either PBS or bacterial LPS (100 μg) to induce experimental mastitis (time 0). Milk samples were collected just prior to intramammary infusion and throughout the 168-h study period. At the end of this study period, ruminal infusion of perchlorate was ceased and a final milk sample was collected 48 h later (postlevels). Mean ($\pm$ SE) milk perchlorate levels are reported in nanograms per milliliter for each experimental group ($n = 6$). *Significantly increased compared with preruminally infused levels of perchlorate ($P < 0.05$). ^a, ^cSignificantly decreased compared with quarters infused with PBS in cows ruminally infused with perchlorate or water, respectively ($P < 0.05$).

were comparable in animals experimentally infused with water alone or water supplemented with perchlorate ($41 \pm 5\%$ vs. $50.6 \pm 4.2\%$, respectively; $P = 0.155$).

Milk concentrations of perchlorate exceeded those in the blood of both control and perchlorate-treated cows. Because perchlorate is a known competitive inhibitor of iodide and is transported by the NIS (Soldin et al., 2001; Van Sande et al., 2003; Clewell et al., 2004), this concentration gradient is presumably established through the active transport of perchlorate into mammary tissue via the NIS or a similar active transporter. During mastitis, a hallmark of localized inflammation is the breakdown in integrity of the mammary vascular endothelial-epithelial barrier (Giri et al., 1984; Mattila and Frost, 1989). Based on the observed decrease in perchlorate concentrations in milk from glands with mastitis (Figure 6), we hypothesize that disruption of the mammary-vascular barrier function during inflammation disrupts the ability of the mammary gland to maintain the perchlorate concentration gradient generated by active transport. Consistent with this hypothesis, a strong in-

verse relationship was identified between decreases in milk perchlorate levels and increases in mammary vascular permeability.

Blood perchlorate concentrations were measured every 24 h to establish that circulating levels remained constant throughout the experiment (Figure 2) and were reflective of consistent rumen delivery (Figure 1). This frequency of sampling did not enable measurements of blood perchlorate levels during the 4- to 12-h post-LPS challenge time period when milk perchlorate levels decreased. Considering the dilutional effect of blood, we estimate that a compensatory increase in blood concentration during this period would be <0.01 ng/mL, a difference much too small to detect with existing methodologies.

The current study investigated changes in milk perchlorate levels in cows that were in late lactation (321 ± 12 DIM). The milk perchlorate concentrations observed here following ruminal infusion of 8 mg/d of perchlorate were between those previously reported for cows ruminally infused with 4 and 40 mg/d of perchlorate (Capuco et al., 2005). Because the cows in the previous study were in midlactation (153 ± 15 DIM), perchlorate secretion into milk appears to be rather constant from mid to late lactation. Whether perchlorate secretion occurs to a similar extent in early, postpartum lactating cows remains unknown. There is evidence that the inflammatory response to bacterial LPS or *E. coli* IMI is influenced by the stage of lactation (Burvenich et al., 2003; Lehtolainen et al., 2003, 2004). Depending on the inflammatory parameter assayed, the response can be heightened in early lactating animals. Further, early lactating cows are less likely than those in later stages of lactation to clear intramammary *E. coli* infections rapidly and therefore mount an inflammatory response that often persists. Thus, based on the findings of the present study, one might expect that greater or prolonged inflammation, whether because of the stage of lactation or other effects, would result in more pronounced or lengthier decreases in milk perchlorate levels.

The present report is the first to examine the effects of mastitis on milk perchlorate levels in dairy cattle. In the context of a previous report (Capuco et al., 2005), the current investigation provides support for the hypothesis that the elevated levels of perchlorate in milk relative to those in blood can be attributed to active transport processes, and that increases in permeability of the blood-milk barrier disrupt the maintenance of an established concentration gradient. The finding that localized inflammation in the gland can actually result in lower milk perchlorate concentrations suggests that additional screening of milk for perchlorate based solely on changes in udder health status is unwarranted.

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Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

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