Milk prolactin response and quarter milk yield after experimental infection with coagulase-negative staphylococci in dairy heifers

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ABSTRACT

Coagulase-negative staphylococci (CNS) are the most common bacteria involved in subclinical mastitis in dairy cows. Remarkably, CNS-infected dairy heifers produce more milk than uninfected heifers. Because the lactation hormone prolactin (PRL) is also involved in mammary gland immunity, we investigated the milk PRL response and the mammary quarter milk yield following experimental CNS challenge. Eight healthy Holstein-Friesian heifers in mid-lactation were experimentally infected using a split-udder design with 3 different CNS strains: one *Staphylococcus fleurettii* (from sawdust bedding) and 2 *Staphylococcus chromogenes* strains (one isolate from a teat apex, the other isolate from a chronic intramammary infection). Three mammary quarters per heifer were simultaneously inoculated with 1.0×10^6 cfu, whereas the remaining mammary quarter was infused with sterile phosphate-buffered saline, serving as a control. An existing radioimmunoassay was modified, validated, and used to measure PRL frozen-thawed milk at various time points until 78 h after challenge. The mean milk PRL level tended to be higher in the CNS-challenged mammary quarters compared with the control mammary quarters (7.56 and 6.85 ng/mL, respectively). The increase in PRL over time was significantly greater in the CNS-challenged mammary quarters than in the control mammary quarters. However, no difference was found in the PRL response when comparing each individual CNS strain with the control mammary quarters. The mean mammary quarter milk yield tended to be lower in the CNS-infected mammary quarters than in the control mammary quarters (1.73 and 1.98 kg per milking, respectively). The greatest milk loss occurred in the mammary quarters challenged with the intramammary strain of *S. chromogenes*. Future observational studies are needed to elucidate the relation between PRL, the

milk yield, and the inflammatory condition, or infection status, of the mammary gland.

Key words: coagulase-negative staphylococci, dairy heifer, experimental mastitis, prolactin

INTRODUCTION

Bovine mastitis, an inflammation of the mammary gland, creates a huge economic burden on the global dairy industry (Bradley, 2002). Coagulase-negative staphylococci are the predominant group of bacteria involved in subclinical mastitis (Pyörälä and Taponen, 2009) and can cause clinical mastitis with mild symptoms (Taponen et al., 2006). Thus far, more than 10 species of CNS have been isolated from bovine milk (Piessens et al., 2011) with documented species-specific differences in putative virulence (Vanderhaeghen et al., 2014), ecology, and epidemiology (Vanderhaeghen et al., 2015). Contrary to what one might expect, various studies have observed a higher test-day milk yield in CNS-infected dairy heifers and multiparous cows compared with noninfected cows (Compton et al., 2007; Schukken et al., 2009; Piepers et al., 2010). Some studies have attributed a protective effect to pre-existing CNS IMI against IMI with more virulent mastitis pathogens (e.g., Piepers et al., 2010). A meta-analysis could not confirm this finding in observational studies, but nonetheless revealed a pronounced protective effect in challenge trials (Reyher et al., 2012). Still, the positive effect on milk yield could be an indirect result of the reduced incidence of clinical mastitis observed in CNS-infected animals (Piepers et al., 2010). High-producing dairy cows might also be more susceptible to CNS IMI than low-yielding animals (Compton et al., 2007). However, even after correcting for these factors, an unexplained difference in milk yield of 2.0 kg/d remained between CNS-infected and uninfected herd mates (Piepers et al., 2013), leaving the exact mechanism to be determined.

Prolactin (PRL) has been associated with over 300 different biological actions, including lactation and mammary gland development (Bole-Feysot et al.,

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1998). In dairy cattle, PRL is required to initiate (Akers et al., 1981) and maintain the milk production after parturition (Lacasse et al., 2012). The protein hormone also acts as a cytokine on molecular and functional levels (Goffin et al., 2002). The ubiquitous PRL receptor belongs to the class I cytokine receptor superfamily, which also includes the receptors of several interleukins and hematopoietic growth factors (Bazan, 1989, 1990). The hormone promotes the activity of macrophages (Edwards et al., 1987), inhibits the apoptosis of T-lymphocytes caused by glucocorticoids (Krishnan et al., 2003), and stimulates the production of tumor necrosis factor- α and IL-12 (Brand et al., 2004). Considering the immunomodulatory actions of PRL, several studies have focused on its potential involvement in bovine mastitis. The periparturient PRL peak coincides with the principal risk period for developing mastitis (Burton et al., 2001). The hormone induces the in vitro synthesis of several cytokines in bovine mammary epithelial cells through the activation of nuclear factor kappa B (Boutet et al., 2007). Although the circulating PRL level is not affected by acute, clinical mastitis (Hockett et al., 2000; Vanselow et al., 2006), a positive correlation was found between SCC and PRL concentration in milk of chronically infected mammary quarters (Boutet et al., 2007).

Because PRL is recognized as a pro-inflammatory cytokine, we hypothesize that milk PRL increases in response to an IMI with CNS. Furthermore, we hypothesize that the quarter milk yield (QMY) also increases after CNS IMI, assuming PRL stimulates the production of milk. To investigate this, an experimental challenge trial was set up using 8 clinically healthy, mid-lactating dairy heifers using 3 different CNS strains. An existing radioimmunoassay for fresh milk was modified and subsequently validated to measure bovine PRL in frozen-thawed milk samples. To assess the epithelial integrity of the blood-milk barrier, the sodium, potassium, and chloride levels were also determined in milk.

MATERIALS AND METHODS

The study is in compliance with the European Directive 2010/63/EU and was approved by the ethical committee of the Faculty of Veterinary Medicine, Ghent University (EC2012/73).

Animals

The study took place between December 2012 and May 2013 at the research dairy farm of Ghent University (Biocentrum Agri-Vet, Melle, Belgium). Eight clinically healthy Holstein-Friesian heifers in mid-lactation (78–278 DIM) were selected. Heifers with a known history

of clinical mastitis or persistent high SCC (>150,000 cells/mL) were excluded from the trial. Milk samples were cultured according to NMC guidelines 48 and 24 h before inoculation to ensure all mammary quarters were free from IMI (NMC, 1999).

CNS Strains

All heifers were inoculated with 2 different wild strains of *Staphylococcus chromogenes* and 1 *Staphylococcus fleurettii* strain. The *S. fleurettii* isolate was recovered from sawdust bedding in a dairy barn (Piessens et al., 2011; Breyne et al., 2015). The first *S. chromogenes* strain originated from a cow suffering from a persistent IMI (hereafter referred to as *S. chromogenes* IM; Supré et al., 2011; Breyne et al., 2015), whereas the second *S. chromogenes* isolate was cultured from the teat apex of a heifer (hereafter referred to as *S. chromogenes* TA; De Vlieghe et al., 2004; Breyne et al., 2015). The *S. chromogenes* TA strain has the ability to inhibit the growth of several major pathogens under laboratory conditions (De Vlieghe et al., 2004), whereas the *S. chromogenes* IM strain does not. The 2 strains also elicit a different immune response in mice (Breyne et al., 2015), and the TA strain is unable to grow in anaerobic iron-depleted medium unlike the IM strain (unpublished data). An inoculum of 1.0×10^6 cfu of each strain was prepared to induce an experimental infection. The live number of cfu was determined by plating serial dilutions of the bacterial stock on tryptic soy agar.

Experimental Study Design

A split-udder design was used. The concept of the split-udder model is grounded on within-heifer comparisons to reduce individual variation (Sipka et al., 2014). Following the morning milking, 3 mammary quarters of each heifer were instantaneously inoculated with the 3 aforementioned CNS strains (one per mammary quarter) diluted in 5 mL of PBS using a sterile catheter (Vygon, Ecouen, France). The fourth mammary quarter, serving as a control, was infused in the same manner with 5 mL of sterile, pyrogen-free PBS. Milk samples for PRL analysis and microbiological culturing were collected from each mammary quarter at 0, 4, 6, 9, 12, 18, 24, 28, 32, 36, 48, 54, 60, 72, and 78 h postinoculation (PI). Milk samples for ion analysis were collected at 0, 24, and 48 h PI. The milk SCC was determined using a DeLaval Cell Counter (DeLaval, Tumba, Sweden). Bacteriological culturing was performed according to NMC guidelines (NMC, 1999). The milk samples for the PRL and ion analysis were stored at -20°C . The cows were milked twice a day with 12-h intervals, and QMY was registered using a mammary

quarter milking device. The cow were examined clinically at each sampling. Rectal temperature, heart rate, respiration rate, rumen motility, fecal consistency, and milk appearance were registered.

Milk Analysis

Prolactin RIA. Milk PRL was determined by a double antibody, homologous RIA adapted from Malven and McMurtry (1974). Because this protocol was developed for fresh, whole milk, we first modified and validated the RIA for frozen milk samples in particular. For the validation, mammary quarter milk samples ($n = 4$) were collected from randomly selected, multiparous Holstein-Friesian cows at a Belgian commercial dairy farm. The samples were then stored at -20°C for 96 h. Afterward, the milk samples were thawed in a warm water bath at 40°C for 30 min (Chew et al., 1977) and centrifuged at $1,800 \times g$ for 15 min at 20°C (Sigma 2-16K, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Bovine PRL (NIH-B5) was used both as a standard and tracer. To generate the tracer, bovine PRL was radiolabeled with ^{125}I using the lactoperoxidase technique (Thorell and Johansson, 1971). A standard curve ranging from 0.8 to 100 ng/mL was prepared in Tris buffer (25 mM Tris; 0.01 mM MgCl_2 ; 1.5 mM NaN_3) containing 0.1% (wt/vol) BSA at pH 7.5. To analyze each standard concentration, 100 μL was added to duplicate tubes containing 200 μL of Tris-BSA buffer. A sample volume of 50 μL was used for milk to minimize potential incubation damage to the labeled antigen (Malven and McMurtry, 1974). Afterward, 100 μL of tracer of approximately 30,000 cpm was added, followed by 100 μL of antiserum (R#144). This antiserum was collected from a rabbit after injection with biological bovine PRL (NIH-B5; Boutet et al., 2007). An antibody titer of 1:120,000 was previously selected for this assay, resulting in the binding of circa 30 to 40% of the total amount of ^{125}I -PRL. The amount of radiolabelled bPRL bound to the antiserum in tubes containing no bPRL (B_0) was designated as 100% ^{125}I bound. After an overnight incubation at room temperature, the antibody-bound fraction of PRL was precipitated using a secondary antibody polyethylene glycol solution (Ayad et al., 2007). The radioactivity of the precipitated ^{125}I -PRL was measured with a gamma counter (LKB Wallac 1261 Multigamma automatic counter, Breda, the Netherlands).

Five parameters were taken into account to validate the RIA: (1) inter- and intraassay coefficient of variation, (2) minimal detection limit (MDL), (3) parallelism, (4) accuracy, and (5) specificity. Three milk samples with a low (2.4 ng/mL), medium (12.9 ng/mL), and high (19.7 ng/mL) PRL concentration were used to

calculate the inter- and intraassay coefficient of variation. To assess the intraassay coefficient of variation, the samples were assayed 10 times within the same run. The interassay coefficient of variation was evaluated by measuring each sample in 10 consecutive assays. The MDL was calculated by measuring the mean concentration of 20 B_0 (zero standard) replicates minus twice the standard deviation within the same assay. For the evaluation of parallelism, a sample dilution curve of a frozen-thawed sample was generated to detect any potential interference of the sample matrix. The accuracy or spike-recovery of the assay was studied by adding a series of known amounts of PRL to a sample with a low endogenous PRL concentration. To evaluate potential cross-reactivity (specificity), 2 structurally similar compounds were tested: native ovine growth hormone (oGH) and bovine placental lactogen (bPL). The validated RIA was then applied to all mammary quarter milk samples from the 8 heifers in the present experimental trial.

Indicators of Epithelial Integrity. The milk samples were thawed and subsequently centrifuged at $16,000 \times g$ for 30 min at 4°C . The sodium, potassium, and chloride concentrations were analyzed in the fat-depleted fraction using an ion-selective electrode analyzer (Roche, Basel, Switzerland).

Statistical Analyses

The response of mammary quarter milk PRL after CNS challenge was evaluated using a linear mixed regression model (SPSS 22.0, Chicago, IL), with the time of sampling, quadratic term of time of sampling, and inoculum as predictor variables. Time of sampling and its quadratic term were included as a continuous variable, whereas inoculum was considered as a categorical variable. The interaction between inoculum and time of sampling was also tested, but only kept in the model when significant. A similar model was constructed to assess the association between QMY, and time of sampling, its quadratic term, and inoculum as predictor variables. For both outcome variables, the inoculum was initially considered a dichotomous variable (challenged versus control). In a second approach, the effect of all different CNS strains was examined (*S. chromogenes* IM, *S. chromogenes* TA, and *S. fleurettii*). To determine the relationship between inoculation (challenge versus control) and the milk ion concentration, comparable linear mixed regression models were constructed for sodium, potassium, and chloride. Sampling time was included as a 3-level categorical variable (0, 24, and 48 h). A natural logarithmic transformation of sodium and chloride was performed to obtain a normalized distribution of the residuals. In all aforementioned analyses,

heifer and mammary quarter were included as random effects to account for the correlated nature of the data. Compound symmetry was selected as a covariance pattern to account for the clustering of repeated samplings within mammary quarter. Statistical significance was set at $P \leq 0.05$.

RESULTS

Experimental Infection

The data of one mammary quarter were omitted from the analysis due to a naturally occurring IMI. Another mammary quarter was also excluded from the analysis for the same reason, but only 12 h after inoculation. An increase in SCC was observed in all challenged mammary quarters, indicating the establishment of IMI, whereas the SCC in the control mammary quarters remained low [geometric mean of 47,000 cells/mL; interquartile range (IQR) of 31,000–71,000 cells/mL]. Twenty-four hours PI, the *S. fleurettii*, *S. chromogenes* TA, and *S. chromogenes* IM challenged mammary quarters had a geometric mean SCC of 2,400,000 cells/mL (IQR: 442,000–3,420,000 cells/mL), 669,000 cells/mL (IQR: 441,000–1,681,000 cells/mL), and 2,596,000 cells/mL (IQR: 1,662,000–4,775,000 cells/mL), respectively. The challenged mammary quarters did not show any visual signs of clinical mastitis. However, 3 heifers did experience a short bout of fever ($>39.5^{\circ}\text{C}$) between 9 and 12 h PI. The *S. chromogenes* IM and TA strains were recovered from milk up to 78 h PI. In all mammary quarters, the *S. fleurettii* strain was eliminated within 9 h PI. No pathogens other than CNS were isolated during the study. The PBS-infused mammary quarters remained free from infection during the entire study period as demonstrated by bacteriological culture.

Prolactin

RIA Validation. The intraassay coefficient of variation for samples with a low, medium, and high PRL concentration was 9.7, 8.3, and 8.1%, respectively. The interassay coefficient of variation for the corresponding samples was 11.9, 14.4, and 16.1%, respectively. The MDL of the assay was 0.536 ng/mL. Serial dilutions showed a dose-response curve parallel to the standard curve (data not shown). The recovery ratio of the spiked PRL was consistently $>85\%$. The binding of radiolabelled PRL remained unaltered when testing the cross-reactivity, except for very high concentrations of oGH (starting at 6.2×10^3 ng/mL). This slightly interfered with the assay ($B/B_0 < 0.80$), resulting in an apparent increase of the PRL concentration.

Milk PRL. The overall mean PRL concentration tended to be higher in the challenged mammary quarters than in the control mammary quarters (LSM = 7.56 and 6.85 ng/mL, respectively) throughout the study ($P = 0.10$, Table 1). The PRL level varied over time in both challenged and control mammary quarters, although not in a linear manner ($P < 0.001$). The evolution of PRL over time was different between the challenged and the control mammary quarters (interaction inoculum $\times$ time of sampling: $P = 0.05$, Figure 1). No significant difference was observed in the PRL response between each of the 3 CNS strains and the control mammary quarters ($P = 0.77$), nor in the evolution over time between strains (interaction inoculum $\times$ time of sampling: $P = 0.14$).

Quarter Milk Yield

The overall mean QMY per milking tended to be lower in the CNS challenged mammary quarters than in the control mammary quarters (LSM = 1.73 and 1.98 kg, respectively) throughout the study ($P = 0.06$, Table 2a). The decline of the QMY over time was greater in the CNS-challenged mammary quarters than in the control mammary quarters (interaction inoculum $\times$ time of sampling: $P < 0.001$, Figure 2). The difference in QMY compared with the control mammary quarters was more pronounced in the mammary quarters challenged with *S. chromogenes* IM (-0.38 kg per milking) than with *S. chromogenes* TA (-0.21 kg per milking) or *S. fleurettii* (-0.17 kg per milking; Table 2).

Blood-Milk Barrier

The concentration of potassium in milk was not significantly influenced by the challenge with CNS strains ($P = 0.37$). A small, but significant increase in the natural logarithmic of sodium and chloride was noted in the challenged quarters (interaction inoculum $\times$ time of sampling $P < 0.05$). However, the sodium and chloride levels did not exceed the upper limit of the normal reference ranges (Figure 3; Gaucheron, 2005).

DISCUSSION

Because CNS-infected cows appear to produce more milk than noninfected cows (Compton et al., 2007; Schukken et al., 2009; Piepers et al., 2010), we investigated the milk yield and the response of milk PRL after CNS challenge in clinically healthy dairy heifers. A plethora of evidence implicates PRL as an immunomodulating factor (Edwards et al., 1987; Krishnan et al., 2003; Brand et al., 2004; Boutet et al., 2007).

Figure 1. The average prolactin (PRL; $\pm$ SEM) concentration in milk following experimental inoculation in challenged quarters versus control quarters. The challenged quarters were inoculated with *Staphylococcus fleurettii*, the teat apex strain of *Staphylococcus chromogenes*, and the intramammary strain of *Staphylococcus chromogenes*.

Therefore, we hypothesized that PRL increases in the milk after CNS infection. Milk PRL might then simultaneously stimulate the secretion of milk as a galactopoietic hormone (Lacasse et al., 2012) in a paracrine or autocrine manner (Clevenger et al., 1995), potentially explaining the aforementioned milk yield increase in CNS-infected cows.

First, the modified PRL RIA for frozen-thawed milk samples proved to be reproducible, accurate, and specific. Only high concentrations of GH interfere with the assay, resulting in an apparent increase of PRL. This might be due either to cross-specificity or to the pres-

ence of trace amounts of pituitary PRL in the native GH preparation. Either way, any cross-reactivity is irrelevant because the endogenous GH amount is limited in bovine milk (Burton et al., 1994).

This study confirmed that milk PRL indeed increases in challenged mammary quarters compared with control mammary quarters after CNS inoculation. This corresponds with the results from Boutet et al. (2007), demonstrating that the PRL level is elevated in chronically infected mammary quarters with high SCC (Boutet et al., 2007). Even though others have observed an increased milk production in naturally CNS-infected cows (Schukken et al., 2009; Piepers et al., 2013), this study reported a substantial milk loss in both challenged and control mammary quarters. Then again, this observation is not entirely unexpected. Unlike the previous observational studies, our experimental trial only monitored the milk yield for a short period of time during the acute phase of inflammation. This experimental challenge might not necessarily reflect a natural infection, based on the high infection dose and the direct intracisternal inoculation of CNS. Also, the aforementioned observational studies (Schukken et al., 2009; Piepers et al., 2013) focused on milk production at animal level, where uninfected glands could compensate for the production loss in CNS-infected mammary quarters (Leitner et al., 2004). Our study measured the production at the mammary quarter level and found transitory milk loss in all mammary quarters. The extent of production loss in the unchallenged mammary quarters has previously been used to score the severity of experimental mastitis (Burvenich et al., 2003). Because the production of the control mammary

Table 1. Linear mixed regression model for milk prolactin after experimental infection, including all 3 CNS strains combined (model 1; left) and with *Staphylococcus chromogenes* TA¹, *S. chromogenes* IM², and *S. fleurettii* considered separately (model 2; right)

Predictor variable	Model 1 Prolactin (ng/mL)				Model 2 Prolactin (ng/mL)			
	β^3	SE	LSM	P-value ⁴	β	SE	LSM	P-value
Intercept	7.56	0.01	—	—	7.56	0.71	—	—
Inoculum				0.10				0.77
Control	Ref. ⁵	—	6.85	—	Ref.	—	6.85	—
Challenge ⁶	0.21	0.48	7.56	—	—	—	—	—
<i>S. fleurettii</i>	—	—	—	—	0.38	0.59	7.60	—
<i>S. chromogenes</i> TA	—	—	—	—	-0.11	0.59	7.45	—
<i>S. chromogenes</i> IM	—	—	—	—	0.37	0.59	7.62	—
Time of sampling	0.005	0.01	—	0.28	0.018	0.013	—	0.17
Quadratic term of time of sampling	-0.001	<0.001	—	<0.001	-0.001	<0.001	—	<0.001
Inoculum $\times$ time of sampling	—	—	—	0.05	—	—	—	0.14

¹Teat apex strain of *S. chromogenes*.

²Intramammary strain of *S. chromogenes*.

³Regression coefficient.

⁴P-value for overall effect.

⁵Referent.

⁶Quarters challenged with *S. chromogenes* TA, *S. chromogenes* IM, and *S. fleurettii* combined.

Table 2. Linear mixed regression model for quarter milk yield after experimental infection, including all 3 CNS strains combined (model 1; left) and with *Staphylococcus chromogenes* TA¹, *S. chromogenes* IM², and *S. fleurettii* considered separately (model 2; right)

Predictor variable	Model 1 Quarter milk yield (kg)				Model 2 Quarter milk yield (kg)			
	β ³	SE ⁴	LSM	P-value	β	SE	LSM	P-value
Intercept	1.86	0.18	—	—	1.86	0.18	—	—
Inoculum				0.06				0.44
Control	Ref. ⁵	—	1.98	—	Ref.	—	1.98	—
Challenge ⁶	0.15	0.17	1.73	—	—	—	—	—
<i>S. fleurettii</i>	—	—	—	—	0.29	0.20	1.81	—
<i>S. chromogenes</i> TA	—	—	—	—	0.15	0.20	1.77	—
<i>S. chromogenes</i> IM	—	—	—	—	0.02	0.20	1.60	—
Time of sampling	−0.02	0.004	—	<0.001	−0.02	0.005	—	0.001
Quadratic term of time of sampling	<0.001	<0.001	—	<0.001	<0.001	<0.001	—	<0.001
Inoculum × time of sampling	—	—	—	<0.001	—	—	—	0.001

¹Teat apex strain of *S. chromogenes*.
²Intramammary strain of *S. chromogenes*.
³Regression coefficient.
⁴P-value for overall effect.
⁵Referent.
⁶Quarters challenged with *S. chromogenes* TA, *S. chromogenes* IM, and *S. fleurettii* combined.

quarters was practically restored in all cows after 48 h, we conclude that the systemic and long-term effects of the induced CNS IMI, even when using a high inoculum dose, were limited. As seen in other experimental infection trials, the CNS in this study evoke a mild inflammatory response despite the high infectious dose (Simojoki et al., 2009, 2011). It should, however, be noted that the overall milk yield during the experimental trial was unexpectedly low in all mammary quarters for Holstein-Friesian heifers. We believe this could be

related to the different housing conditions and milking routines during the experimental trial.

Under physiological conditions, circulating pituitary PRL is transported from the bloodstream to the milk compartment via transcytosis. After binding on the membrane receptor, PRL is internalized by the mammary epithelial cell and subsequently released into the milk through secretory vesicles (Ollivier-Bousquet, 1998). Mastitis increases tight junction permeability, hereby enabling the paracellular transport of blood-borne components (Nguyen and Neville, 1998). Changes in milk ion concentration can indicate the disruption of the blood-milk barrier (Stelwagen et al., 1994). In this study, the sodium and chloride levels increased significantly in the challenged mammary quarters. This might imply that the PRL merely leaks from the bloodstream into the milk compartment, as a result of an increased tight junction permeability. Whether the milk PRL increase is entirely due to passive diffusion can neither be confirmed nor denied in this experimental setup. The biological significance of plasma-borne proteins in the milk could be questioned. Future research should address the origin of PRL in milk. Other ruminants, such as sheep and goats (Le Provost et al., 1994), are able to produce extra-pituitary PRL in the mammary gland. So far, this has never been demonstrated in cattle, but it would support the hypothesis of PRL as a pro-inflammatory cytokine and autocrine lactation hormone. Although Boutet et al. (2007) found no association between bacterial species and PRL concentration, we also wonder if the release of PRL during IMI is pathogen dependent. Additional longitudinal studies on mammary quarter level will shed more light on the

Figure 2. Mean quarter milk yield (±SEM) following experimental inoculation in challenged quarters versus control quarters. The challenged quarters were inoculated with *Staphylococcus fleurettii*, the teat apex strain of *Staphylococcus chromogenes*, and the intramammary strain of *Staphylococcus chromogenes*.

Figure 3. The average ($\pm$ SEM) milk Na⁺, K⁺, and Cl⁻ levels in the control and challenged quarters. The dashed horizontal lines represent the upper and lower reference range of the respective ions in bovine milk from noninfected mammary glands. The challenged quarters were inoculated with *Staphylococcus fleurettii*, the teat apex strain of *Staphylococcus chromogenes* and the intramammary strain of *Staphylococcus chromogenes*.

true association between QMY and CNS infections, and clarify the role of PRL in IMI.

CONCLUSIONS

This study demonstrated that milk PRL increases after an experimental intramammary CNS challenge in dairy heifers. The mechanism behind this PRL response and its biological relevance remain to be determined.

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