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# Relationship between glucocorticoids and prolactin during mammary gland stimulation in dairy cows

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## ABSTRACT

The objectives of this study were to determine the role of glucocorticoids in the regulation of prolactin (PRL) release induced by mammary gland stimulation and to investigate whether the milk depression induced by glucocorticoids in dairy cows is due to a decrease in PRL release. In experiment 1, 8 dairy cows were used in a 4 × 4 Latin square design. Four hours after the morning milking, the cows received 1 of the following treatments: (1) a 5-min manual stimulation of the mammary gland; (2) an i.v. injection of 1 mg of dexamethasone; (3) 2 infusions of 2.5 g of metyrapone (an inhibitor of cortisol biosynthesis) in the omasum 4 and 2 h before a 5-min stimulation of the mammary gland; or (4) no treatment. Sixty minutes later, the mammary gland of each cow was stimulated for 5 min. Blood samples were collected from 20 min before to 120 min after the start of the treatment. When the mammary gland was stimulated twice in 60 min, less PRL and cortisol were released during the second stimulation. Metyrapone did not affect PRL or cortisol release. Dexamethasone decreased serum cortisol concentration but did not affect PRL concentration. In experiment 2, 16 cows were used in a crossover experimental design consisting of 2 experimental weeks separated by 1 resting week. During the first week, cows were treated as follows: (1) 4 cows were injected with 0.5 g of domperidone (a PRL secretagogue) in canola oil on d 1 and 2 and 20 mg of dexamethasone on d 1; (2) 4 cows were injected with 0.5 g of domperidone on d 1 and 2; (3) 4 cows were injected with canola oil on d 1 and 2 and with 20 mg of dexamethasone on d 1; and (4) 4 cows were injected with canola oil on d 1 and 2. During the second experimental week, the same 4 treatments were repeated, except the cows that did not receive dexamethasone in the first week received it on d 1 of the second week, and cows that did receive it in the first week did not receive it in the second week. On d 1 and 2 of each week, blood samples were collected during morning milking for PRL determination. Dexamethasone reduced milk production and decreased both basal and milking-induced PRL release. It also increased milk fat and protein percentages and decreased milk lactose content. Domperidone increased basal PRL levels in serum and milk but did not affect milk yield. Although we cannot rule out the possibility that inhibition of PRL secretion or reduction of mammary gland PRL responsiveness play a role in the inhibition of milk production by glucocorticoids, the fact that enhancement of PRL secretion by domperidone could not prevent the depression of milk yield suggests that other mechanisms are involved.
**Key words:** milking, dexamethasone, milk production

## INTRODUCTION

Prolactin (**PRL**) is one of the major hormones involved in the control of mammary gland functions, including galactopoiesis (Lacasse et al., 2016); this hormone is released during milking and nursing in response to mammary gland stimulation. The regulation of PRL secretion is not completely understood, however. As lactation advances, basal PRL concentration and the amount of PRL released during milking are both reduced (Koprowski and Tucker, 1973; Miller et al., 2000; Bernier-Dodier et al., 2011). As well, milking-induced PRL release in cows decreases as the interval between milkings or manual stimulations decreases (Lacasse and Ollier, 2014). The reason for reduction in PRL release during lactation or after multiple mammary gland stimulations is not known. It could be due to decreased sensitivity of the mammary skin to tactile stimulus, or to reduced responsiveness at the level of the hypothalamus or the pituitary gland.

Besides PRL, milking also induces the release of glucocorticoids, which are steroid hormones synthesized by the adrenal glands. Interestingly, adrenalectomy prevented the usual decrease in PRL levels during the second half of lactation in rats (van der Schoot and de Greef, 1983). Furthermore, female rats treated with dexamethasone, a glucocorticoid analog, exhibited a lower suckling-induced PRL response and lower milk

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**Table 1**. Summary of the treatments of experiment 1

| | Hours after morning milking | | |
|---|---|---|---|
| Treatment | 0 and 2 | 4 | 5 |
| STIM | | 5-min manual stimulation of the mammary gland | 5-min manual stimulation of the mammary gland |
| DEXA | | Intravenous injection of 1 mg of dexamethasone | 5-min manual stimulation of the mammary gland |
| METY | Infusion of 2.5 g of metyrapone in omasum | 5-min manual stimulation of the mammary gland | 5-min manual stimulation of the mammary gland |
| CTL | | No treatment | 5-min manual stimulation of the mammary gland |

secretion compared with control animals (Horváth et al., 2001). Although glucocorticoids act in synergy with PRL to activate the gene expression of caseins (Skarda et al., 1982), their administration has also been reported to inhibit milk production in both rodents and cows (Shamay et al., 2000; Vilela and Giusti-Paiva, 2011). The mechanism by which glucocorticoids inhibit milk secretion is not known. Given the recent body of evidence supporting the galactopoietic role of PRL in ruminants (Lacasse et al., 2016), the negative effect of glucocorticoids on milk secretion may be mediated in part by the inhibition of PRL release or the reduction of mammary gland responsiveness to this hormone. However, dexamethasone has also been shown to decrease plasma IGF-1 concentrations in cows (Maciel et al., 2001). Given that IGF-1 stimulates milk yield in goats (Prosser et al., 1990), glucocorticoid-induced milk inhibition may also involve an action on IGF-1 secretion or clearance.

The objectives of this study were to evaluate the effects of glucocorticoids on milking-induced PRL release and to test whether glucocorticoid-induced inhibition of milk production is due to a reduction in PRL secretion.

## MATERIALS AND METHODS

### *Animals and Experimental Design*

The experiments were conducted at Agriculture and Agri-Food Canada's Sherbrooke Research and Development Centre (Sherbrooke, QC, Canada) in accordance with the guidelines of the Canadian Council on Animal Care. The animals were housed in a tie-stall barn and were milked twice daily at 12-h milking intervals.

### *Experiment 1*

We used 8 multiparous fistulated Holstein dairy cows in mid-lactation (196 ± 11 DIM) in a 4 × 4 Latin square design for this experiment. Cows were allocated to 1 of 4 groups that were balanced in terms of cows' parity, milk production, and DIM. Three days before the start of treatments, a Silastic catheter (i.d. 1.02 mm, o.d. 2.16 mm; Dow Corning Corp., Midland, MI) was inserted into each cow's jugular vein. Each experimental day was separated by at least 2 resting days.

On each experimental day, the cows received 1 of the following treatments: (1) a 5-min manual stimulation of the mammary gland 4 h after the morning milking (**STIM**); (2) an i.v. injection of 1 mg of the glucocorticoid analog dexamethasone (Vetoquinol, Lavaltrie, QC, Canada) 4 h after the morning milking (**DEXA**); (3) infusions of 2.5 g of metyrapone (Sigma-Aldrich, Oakville, ON, Canada), an inhibitor of cortisol biosynthesis, in the omasum 4 and 2 h before a 5-min manual stimulation of the mammary gland 4 h after the morning milking (**METY**); or (4) no treatment 4 h after the morning milking (**CTL**). Sixty minutes after treatment, the mammary gland of each cow was manually stimulated for 5 min. A summary of the treatments is presented in Table 1.

Blood samples were collected before, during, and after the first manual stimulation or injection (−20, −10, 0, 3, 5, 7, 10, 15, 20, 25, 30, 40, 60 [start of the second stimulation], 63, 65, 67, 70, 75, 80, 85, 90, 100, and 120 min relative to the start of the first manual stimulation or injection) in Vacutainer tubes without additives (BD, Mississauga, ON, Canada). The blood tubes were left overnight at 4°C to allow clotting before centrifugation (1,900 × *g*, 4°C, 15 min). Serum samples were then kept at −20°C until determination of PRL and cortisol concentrations. Additional blood samples were collected in EDTA-coated Vacutainer tubes (BD) at 60, 63, 65, and 67 min relative to the start of the first manual stimulation or injection. These tubes were immediately placed on ice and centrifuged (1,900 × *g*, 4°C, 15 min). The plasma samples were then stored at −80°C until determination of ACTH.