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Short communication: Effect of estrogen supplemented at dry-off on temporal changes in concentrations of lactose in blood plasma of Holstein cows

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ABSTRACT

The objective was to determine the effect of supplemental estrogen (estradiol cypionate, ECP) at dry-off on temporal changes in concentrations of lactose in blood plasma of Holstein cows as an indicator of rate of mammary involution. Thirty-two Holstein cows (8/group) were assigned randomly to 4 treatment groups: 30-d dry, 30-d dry + ECP, 60-d dry, and 60-d dry + ECP. A single injection (7.5 mL) of cottonseed oil (30- and 60-d dry) or ECP (15 mg) in oil (30- and 60-d dry + ECP) was administered intramuscularly at dry-off. Blood samples were collected from the coccygeal vein of all cows 24 h before dry-off and at dry-off, and then 8 samples were collected throughout the subsequent 48 h to monitor concentrations of lactose in blood plasma. No significant effects of ECP on the overall mean concentrations of lactose were detected. Concentrations of lactose increased and were greatest in blood collected 20 h (520.4 ± 54.1, 268.1 ± 48.2, 345.0 ± 52.3, 418.4 ± 49.8 μ*M*, for the 4 treatment groups respective to the order listed above) after supplemental ECP and final milk removal. At 40 h, concentrations approached those observed 24 h before dry-off (140.5 ± 52.1, 57.6 ± 47.1, 90.1 ± 51.4, 61.2 ± 48.4 μ*M*, respectively). Concentrations of lactose at 20 h were positively correlated with milk yield of cows at dry-off. Similar temporal profiles of lactose in blood plasma of cows supplemented or not with ECP indicated that ECP at dry-off did not markedly alter the course of tight junction leakage that typically occurs in mammary epithelial tissue during progressive early involution when milk removal is discontinued.
Key words: dry period, estrogen, plasma lactose

A dry period between consecutive lactations is required to achieve optimal milk production during the ensuing lactation (Makuza and McDaniel, 1996). Determination of the minimum length of dry period needed to allow mammary tissue to undergo the remodeling that is required for optimal and profitable milk production is an ongoing research interest and goal (Bachman and Schairer, 2003; Kuhn and Hutchison, 2005; Rastani et al., 2005). To date it appears that dry periods shortened to 28 to 40 d did not decrease subsequent milk production (Bachman and Schairer, 2003; Gulay et al., 2003; Watters et al., 2008) or affect the health status and apparent reproductive efficiency of dairy cows (Gümen et al., 2005; Watters et al., 2008).

Dry periods are initiated by the cessation of milk removal which, in turn, initiates further mammary tissue involution and remodeling (Hurley, 1989), changes that, in part, are characterized by the opening of tight junctions located between adjacent mammary epithelial cells (Stelwagen et al., 1994). The relatively impermeable barrier created by tight junctions, located in the apical aspect of the lateral membranes of epithelial cells, is further degraded with the activation of plasminogen in milk and the increase in plasmin activity that occurs within 3 d of dry-off (Politis et al., 1990).

Estrogen administered at cessation of milk removal accelerates the involution process in mammary tissue (Athie et al., 1996). The mechanism involves induction of plasmin (serine protease) activity within the mammary gland (Athie et al., 1997), suggesting that supplementation of estrogen at dry-off may decrease the length of the dry period needed for optimum milk production during the subsequent lactation (Athie et al., 1996). Yet, subsequent studies did not detect beneficial effects of supplemental estrogen on subsequent milk yield (**MY**) when it was provided at the onset of dry periods shortened to 30 d (Bachman, 2002; Gulay et al., 2003).

In lactating mammals large quantities of lactose are synthesized from glucose in mammary tissue, but not in other organs or tissues. Typically, lactose was in low concentrations in mammalian blood (e.g., cow = 66.9 μ*M*, Delamaire and Guinard-Flament, 2006; ewe = 11.9 μ*M*, Castillo et al., 2008; goat = 64.5 μ*M*, Stelwagen et al., 1994). Still, upon dry-off and during progressive mammary involution the passage of ions and solutes such as lactose into blood occurred as the integrity of

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tight junctions decreased (Stelwagen et al., 1994, 1997). The hypothesis of the present study was that supplementation of estrogen at dry-off will cause increases in blood plasma lactose more rapidly through acceleration of the involution process than will occur in nonsupplemented cows.

Thirty-two Holstein cows from the University of Florida Dairy Research Unit herd were used. Treatments were in a 2 × 2 factorial arrangement. The cows were assigned randomly about 70 d before expected calving dates to 1 of 2 treatment groups, 60- or 30-d dry. Cows assigned to the longer dry period were supplemented with 7.5 mL (i.m.) of cottonseed oil (**D60**) or oil containing estradiol cypionate (**ECP**; 15 mg; **D60ECP**) at the time of dry-off and their transfer from the milking herd to the dry cow herd. Cows scheduled for a 30-d dry period remained in the milking herd until ~30 d before expected calving dates at which time they were dried-off and received 7.5 mL (i.m.) of either cottonseed oil (**D30**) or oil containing 15 mg of ECP (**D30ECP**) before they were transferred to the dry cow herd. There were 8 cows/treatment; a total of 16 cows were supplemented with estrogen at dry-off. The age of cows ranged from 3 to 8 yr and from 1 to 6 completed lactations.

Blood samples were collected from the coccygeal vein of all cows at 24 h before dry-off and a second sample was collected at dry-off (i.e., at time of final milk removal). Following dry-off, blood samples were collected 8 times during the subsequent 48 h at the following intervals: 12, 8, 4, 4, 4, 4, 4, and 8 h after the previous sample. Blood samples were collected using 20-gauge Vacutainer needles (2.54 cm) and 10- × 100-mm tubes containing sodium heparin (Becton Dickinson, Fairlawn, NJ). All blood samples were centrifuged within 10 min of collection at 2,619 × g for 20 min at 5°C (Jouan GR 412 centrifuge, Winchester, VA). Plasma was harvested and stored in capped polypropylene tubes at −20°C until analyzed for lactose. Lactose assays were performed according to Stelwagen et al. (1994) in 96-well microtiter plates. Data were analyzed by PROC MIXED (SAS Institute, 2007). The statistical model included hour of blood sample collection relative to dry-off, fixed effect of treatment, and random effect of cow nested within treatment. PROC REG (SAS Institute, 2007) was used to analyze the association between concentrations of lactose in blood plasma (average of 2 highest values) and milk yield at dry-off. Because it was reasonable to assume that the length of the dry period that followed dry-off did not influence the concentration of lactose in blood plasma during the 48 h after dry-off, the concentrations of lactose in plasma were compared between cows supplemented or not supplemented with ECP at dry-off, irrespective of dry period treatment.

Actual dry period lengths for cows in D30, D30ECP, D60, and D60ECP were 26.8 ± 2.2, 29.1 ± 2.2, 54.2 ± 2.2, and 59.1 ± 2.1 d, respectively. Supplementing ECP at dry-off did not affect actual length of the subsequent dry period within the 30- and 60-d dry period groups. The overall mean MY of cows in D30, D30ECP, D60, and D60ECP did not differ and were 17.0 ± 4.3, 14.3 ± 4.3, 14.0 ± 4.2, and 14.4 ± 4.4 kg/d, respectively. The overall mean concentrations of lactose in blood plasma of cows in D30, D30ECP, D60, and D60ECP were 282.7 ± 52.2, 189.5 ± 48.9, 191.0 ± 51.6, 196.6 ± 52.9 μM, respectively. Overall, cows in the 4 treatment groups had similar temporal profiles of lactose (Figure 1). Concentrations of lactose increased after dry-off and reached a peak about 20 h after dry-off and approached initial concentrations at 40 h after dry-off (Figure 1). Importantly, no difference (P = 0.59) was detected in mean concentrations of lactose in blood plasma of ECP-supplemented and nonsupplemented cows (198.4 ± 44.9 vs. 231.5 ± 46.4 μM, respectively). A positive association was detected (R^2 = 0.52; Figure 2; P < 0.01) between the average of the 2 greatest concentrations of lactose in plasma observed for individual cows and their MY at dry-off. Moreover, including MY as a covariate in statistical analyses did not result in the detection of significant differences in mean concentrations of lactose among treatment groups.

The results of the current study support in vivo studies and confirm that lactose in blood plasma can be used as an indicator of the permeability of tight junctions (Stelwagen et al., 1997, 1998). In the lactating mammary gland the permeability of tight junctions increased following 18 h of milk accumulation in cows (Stelwagen et al., 1997). As mammary dry-off was initiated and involution progressed the tight junctions opened; there was an increase in mammary epithelial permeability with the subsequent passage of ions such as Na^+ and K^+ and lactose into blood (Delamaire and Guinard-Flament, 2006). Because of increased permeability following dry-off, the passage of lactose from milk into blood appeared a noninvasive way to follow the time course of mammary involution following dry-off.

The amount of lactose in blood plasma was affected by its rate of synthesis in the mammary epithelial cells, rate of passage through tight junctions, and rate of clearance from blood (elimination half-life = 44 min for lactose; Stelwagen et al., 1997). Cessation of milk removal increased the permeability of tight junctions after 18 h in cows (Stelwagen et al., 1997) and after 21