Association of peripartum plasma insulin concentration with milk production, colostrum insulin levels, and plasma metabolites of Holstein cows

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ABSTRACT

The main objective of this study was to assess associations between plasma insulin concentration around parturition and production in Holstein cows. Primiparous and multiparous cows ($n = 267$) were enrolled. Blood samples were collected within 12 h after parturition (d 0), and on d 3 and 10 after calving. In addition, blood samples were collected 7 d before (–7 d) the expected date of parturition and colostrum samples were collected within 8 h after parturition from a subset of cows to measure insulin concentration ($n = 47$). All samples were harvested from 0630 to 1100 h and were used to quantify insulin, nonesterified fatty acids (NEFA), and β -hydroxybutyrate. The plasma concentrations of insulin on d –7 and 0 were not correlated with insulin levels in colostrum. Cows were grouped according to plasma insulin concentration based on the median as low insulin (L-INS) or high insulin (H-INS) on d 0 (median = 0.35 ng/mL; range 0.2 to 1.2), 3 (median = 0.32 ng/mL; range 0.2 to 1.6), and 10 (median = 0.30 ng/mL; range 0.2 to 0.8). We detected that cows in the L-INS group on d 0 (L-INS = 0.57 ± 0.02 ; H-INS = 0.49 ± 0.02 mmol/L), d 3 (L-INS = 0.56 ± 0.02 ; H-INS = 0.49 ± 0.02 mmol/L), and d 10 (L-INS = 0.61 ± 0.03 ; H-INS = 0.55 ± 0.03 mmol/L) had higher NEFA concentrations compared with cows in the H-INS group. Compared with H-INS cows, milk yield was higher for cows classified as L-INS on d 0 (L-INS = 40.75 ± 0.69 ; H-INS = 38.41 ± 0.64 kg) and d 10 (L-INS = 40.95 ± 0.74 ; H-INS = 38.66 ± 0.64 kg). Moreover, fat-corrected milk was higher for cows classified as L-INS on d 0 (L-INS = 40.59 ± 2.36 ; H-INS = 37.73 ± 2.31 kg) and d 10 (L-INS = 41.00 ± 2.42 ; H-INS = 38.65 ± 2.28 kg) compared with H-INS cows, and energy-corrected milk was higher for L-INS cows compared with H-INS cows regardless of the day (d 0, L-INS = 44.50 ± 0.70 vs. H-INS = 41.67 ± 0.64 kg; d 3, L-INS = 43.65 ± 0.74 vs. H-INS = 40.88 ± 0.72 kg;

d 10, L-INS = 44.09 ± 0.73 vs. H-INS = 40.55 ± 0.68 kg). We conclude that low plasma insulin concentration during early lactation is associated with higher milk yield in the long term.

Key words: insulin, milk production, nonesterified fatty acid, β -hydroxybutyrate

INTRODUCTION

Insulin is an anabolic hormone secreted by β -cells of the islets of Langerhans in the pancreas in response to increased levels of glucose circulating in the blood. Binding of insulin to its cell surface receptor stimulates glucose uptake by cells, decreases gluconeogenesis, glycogenolysis, and lipolysis, increases fat and protein synthesis, and promotes cell division and cell growth (Brockman and Laarveld, 1986). However, in postpartum dairy cows, circulating concentration of insulin is extremely low to prioritize the use of glucose for milk production (Hart, 1983). In addition, it is considered that Holstein cows undergo a transient state of insulin resistance, which might increase the supply of blood glucose for lactose production by the insulin-independent mammary gland (Bell and Bauman, 1997; De Koster and Opsomer, 2013). These major endocrine adaptations are part of the homeorhetic shift that characterizes the lactating dairy cow at the beginning of lactation (Bauman and Currie, 1980; Bell and Bauman, 1997).

Several studies have investigated the effect of insulin administration on milk yield in lactating dairy cows. For instance, Schmidt (1966) showed that repeated injections of a short-acting insulin into primiparous Holstein cows resulted in decreases in milk yield and milk lactose, and increases in milk fat and protein (Schmidt, 1966). In a study by Kronfeld et al. (1963), cows that received repeated high doses of a long-acting insulin had reduced milk yields (Kronfeld et al., 1963). In both studies, milk production was restored to pre-insulin treatment after the cows were infused with exogenous glucose. These findings suggested that the observed reduction in milk yield following insulin administration was due to a reduction in blood glucose concentration

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owing to insulin's hypoglycemic effect. Nevertheless, a recent study that used a lower dose of long-acting insulin administered twice daily for a period of 10 d, and at different stage of lactation (at around 90 DIM), observed that milk yield did not differ between cows that received insulin and those that did not (Winkelman and Overton, 2013). The concentration of lactose in milk, however, was significantly lower among insulin-treated cows (Winkelman and Overton, 2013).

However, limited evidence exists regarding the associations of periparturient plasma insulin and milk yield of Holstein cows. Thus, our hypothesis was that low plasma insulin levels around calving and during early lactation would be positively associated with milk production. Our primary objective was to explore the association of plasma insulin at different DIM during the immediate postpartum with milk production. We also explored the association between postpartum plasma insulin and NEFA and BHB concentrations.

MATERIALS AND METHODS

Farm and Management

An observational study was conducted in a large commercial dairy farm located in Cayuga County near Ithaca, New York. The farm milked ~3,600 Holstein cows thrice daily in a rotary parlor with integrated milk meters that recorded individual production at every milking (DeLaval, Tumba, Sweden). The cows were housed in freestall barns, with concrete stalls bedded with composted manure solids. After calving, all cows were offered a TMR of approximately 55% forage (corn silage, haylage, and wheat straw) and 45% concentrate (corn meal, soybean meal, canola, cottonseed, and citrus pulp) on a DM basis. The TMR was fed at approximately 0800 h and feed was pushed up 6 to 8 times a day. The diet was formulated to meet or exceed the NRC nutrient requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% FCM (NRC, 2001). As cows demonstrated signs of calving, they were moved to individual maternity pens for delivery, where trained farm personnel assisted with parturition as needed. Calves were removed from their dams immediately after birth. The Presynch-Ovysynch protocol, in combination with estrus detection and AI, was used for the first service (Pursley et al., 1995; Moreira et al., 2001). A combination of heat detection and the Resynch (Fricke et al., 2003) protocol was used to inseminate cows after the first service. A voluntary waiting period of 50 d was used. Estrus was detected based only on electronic activity sensors (Alpro, DeLaval) worn around the neck.

Study Design and Animal Sampling

In total, 267 Holstein dairy cows (primiparous, $n = 98$; multiparous, $n = 169$) were enrolled in the study from October to December 2015. Blood samples, collected from 0630 to 1100 h, were obtained from all study cows from the coccygeal vein or artery using 10-mL K_2 EDTA blood collection tubes (BD Vacutainer, Franklin Lakes, NJ) within 12 h (d 0), and at 3 (2 to 5 d) and 10 (8 to 11 d) d relative to parturition. After collection, samples were placed on ice, and plasma was obtained within 3 h by centrifugation at $2,000 \times g$ for 15 min at 4°C, and frozen at -20°C. A total of 18 blood samples were not collected on d 3 and 1 sample was not collected on d 7. Additionally, blood and colostrum samples from a subset cows (primiparous, $n = 20$; multiparous, $n = 27$) were collected at -7 ± 3 d (blood) and within 8 h after calving (colostrum). Colostrum was collected by trained farm personnel following recommendations of the research group. Briefly, teats were cleaned and disinfected, the initial 2 streams were discarded, and approximately 10 mL of colostrum from each quarter was collected into a sterile 50-mL falcon tube without preservative (VWR, Chicago, IL). Colostrum samples were transported on ice to the laboratory, homogenized by vortex mixing, and aliquots of 2 mL were centrifuged at $10,000 \times g$ for 15 min at 4°C, and supernatant was stored at -20°C for further analysis. Body condition scores were determined at 0 and at 35 (32 to 38 d) DIM using a 5-scale, quarter-point system as previously described (Edmonson et al., 1989). Weekly milk yield average was recorded for 12 wk after calving. Four cows were culled on wk 7, 3 on wk 9, and 2 on wk 11.

Blood and Colostrum Analysis

Insulin concentration was determined using a commercial ELISA kit for bovines (Bovine Insulin ELISA, ALPCO, Salem, NH). Plasma concentrations of NEFA (NEFA-C kit, Wako Pure Chemical Industries, Richmond, VA) and BHB (Williamson and Mellanby, 1974; Sigma-Aldrich, St. Louis, MO) were determined by colorimetric methods. The intra- and interassay coefficients of variation for the assays were <8.5 and <10%, respectively.

Statistical Analysis

Sample size calculation was conducted using JMP PRO (version 12, SAS Institute Inc., Cary, NC). Considering an average standard deviation of 3.7 kg/d for milk yield (Douglas et al., 2006; Smith et al., 2009;

Greco et al., 2015) and assuming a type I error rate of 5% and a power of 80%, a total of 110 cows per group were deemed necessary to detect a significant difference of 2 kg/d between groups. However, an additional ~20% of cows were enrolled in the study to compensate for cows removed from the herd during the study period beyond the control of the investigators.

Descriptive statistical analysis was performed using the UNIVARIATE procedure in SAS (version 9.4, SAS/STAT, SAS Institute Inc., Cary, NC). Cows were classified into high plasma insulin (**H-INS**) or low plasma insulin (**L-INS**) based on the median plasma insulin levels at d 0, 3, and 10 relative to parturition. Continuous variables evaluated over time [insulin, NEFA, BHB, weekly average milk yield, monthly test day milk (TDM), 3.5% FCM, ECM, percent fat, and percent protein] were analyzed using general linear repeated measures mixed models with the MIXED procedure of SAS. To account appropriately for within-cow correlation, the error term was modeled by imposing a first-order autoregressive covariance structure for all statistical models. Fixed effects of group (H-INS/L-INS at d 0, 3, or 10), time, parity, BCS at calving, dystocia, twins, stillbirth, and the 2-way interaction terms group by parity, and group by time were offered to the models. Tukey's honest significance test for multiple comparisons was used. Data are reported as least squares means $\pm$ standard error of the mean. Differences in sire predicted transmitting ability for milk yield between cows classified as low or high insulin concentration at different time points was evaluated using the GLIMMIX procedure of SAS. The relationship between plasma insulin at d -7 and 0 after calving and the concentration of insulin in colostrum was analyzed with the Spearman correlation test using the multivariate method of JMP PRO.

For all models, variables and their respective interaction terms were retained in the model when $P \leq 0.15$. Significance was considered when $P \leq 0.05$ or a tendency if $0.05 < P \leq 0.10$.

RESULTS

Descriptive Data

The median plasma insulin concentration on d 0 was 0.35 ng/mL (range 0.20 to 1.20) and 133 cows (primiparous, $n = 29$; multiparous, $n = 104$) were identified as L-INS (range 0.20 to 0.35), whereas 134 cows (primiparous, $n = 69$; multiparous, $n = 65$) were identified as H-INS (range 0.36 to 1.20). The median plasma insulin concentration on d 3 was 0.32 ng/mL (range 0.20 to 1.60) and 125 cows (primiparous, $n = 37$; multiparous, $n = 88$) were identified as L-INS

(range 0.20 to 0.32), whereas 124 cows (primiparous, $n = 57$; multiparous, $n = 67$) were identified as H-INS (range 0.33 to 1.60). Last, the median plasma insulin concentration on d 10 was 0.30 ng/mL (range 0.20 to 0.80) and 133 cows (primiparous, $n = 33$; multiparous, $n = 100$) were identified as L-INS (range 0.20 to 0.30), whereas 133 cows (primiparous, $n = 64$; multiparous, $n = 69$) were identified as H-INS (range 0.31 to 0.80).

Supplemental Table S1 (<https://doi.org/10.3168/jds.2017-14029>) illustrates the frequency distribution of each insulin classification group (L-INS or H-INS) for sample collection d 0, 3, and 10.

Colostrum and Plasma Insulin Levels Around Parturition

Figure 1 illustrates the insulin plasma concentrations at d -7, 0, 3, and 10 relative to parturition, and the colostrum insulin levels by parity from a subset of cows ($n = 47$). Primiparous cows had lower ($P < 0.05$) plasma insulin levels on d 0, 3, and 10 compared with -7 d (Figure 1A). Multiparous cows had lower ($P < 0.05$) plasma insulin levels on d 3 and 10 after calving compared with d -7 (Figure 1B). The mean $\pm$ standard error of the mean of colostrum insulin concentration for primiparous cows was 106.74 ± 14.9 ng/mL (Figure 1A) and for multiparous cows it was 53.99 ± 6.12 ng/mL (Figure 1B).

Relationships Between Colostrum Insulin and Plasma Insulin

The relationships between insulin concentration in colostrum and plasma at d -7 and 0 relative to parturition are depicted in Figure 2. Colostrum insulin concentration was not associated with plasma insulin levels, irrespective of the day. The correlation between insulin level in plasma versus that in colostrum on d -7 and 0 relative to parturition was $r = -0.08$ ($P = 0.57$; Figure 2A) and $r = 0.12$ ($P = 0.42$; Figure 2B), respectively.

Association Between Insulin Classification and NEFA and BHB Concentrations

Cows Classified into L-INS or H-INS at d 0. Plasma concentrations of insulin, NEFA, and BHB are illustrated in Figure 3A. Cows grouped as L-INS had lower ($P < 0.001$) plasma insulin when compared with H-INS cows (L-INS = 0.30 ± 0.007 ; H-INS = 0.42 ± 0.006 ng/mL). Interestingly, cows in the L-INS group had higher ($P = 0.003$) NEFA concentrations compared with cows in the H-INS group (L-INS = 0.57 ± 0.02 ; H-INS = 0.49 ± 0.02 mmol/L). We observed an insulin-

Figure 1. Concentration of insulin in colostrum and plasma of primiparous ($n = 20$; A) and multiparous ($n = 27$; B) cows at -7 , 0 (within 12 h after calving), 3 , and 10 d relative to parturition. Different letters (a,b) indicate a significant difference ($P < 0.05$). Error bars represent the SEM.

group by parity interaction ($P = 0.007$) for BHB. The plasma levels of BHB did not differ between primiparous H-INS and L-INS cows (Figure 4A). Nevertheless, multiparous L-INS cows tended to have higher ($P < 0.10$) BHB concentrations at d 0 and 10 than H-INS cows (Figure 4B).

Cows Classified into L-INS or H-INS at d 3.

Plasma concentrations of Insulin, NEFA, and BHB are illustrated in Figure 3B. Cows grouped as L-INS had lower ($P < 0.001$) plasma insulin when compared with H-INS cows (L-INS = 0.31 ± 0.007 ; H-INS = 0.42 ± 0.007 ng/mL). Moreover, L-INS cows had higher ($P = 0.008$) NEFA concentrations compared with H-INS cows (L-INS = 0.56 ± 0.02 ; H-INS = 0.49 ± 0.02 mmol/L). The plasma concentration of BHB did not differ ($P = 0.96$) between L-INS and H-INS cows (L-INS = 0.73 ± 0.02 ; H-INS = 0.73 ± 0.02 mmol/L).

Cows Classified into L-INS or H-INS at d 10.

Plasma concentrations of Insulin, NEFA, and BHB are illustrated in Figure 3C. Cows classified as L-INS had lower ($P < 0.001$) plasma insulin levels compared with H-INS cows (L-INS = 0.31 ± 0.007 ; H-INS = 0.41 ± 0.007 ng/mL). Furthermore, cows in the L-INS group had higher ($P = 0.01$) NEFA concentrations compared with cows in the H-INS group (L-INS = 0.61 ± 0.03 ; H-INS = 0.55 ± 0.03 mmol/L). Differences in BHB plasma levels were not observed ($P = 0.54$) between groups (L-INS = 0.74 ± 0.02 ; H-INS = 0.73 ± 0.02 mmol/L).

Milk Yield and Composition

To test for the genetic merit of milk production cows grouped as L-INS or H-INS at all sample collec-

Figure 2. Relationship between plasma insulin concentration on d -7 (A) or 0 (B) and colostrum insulin level ($n = 47$). Spearman's correlation coefficient and P -value are illustrated in each graph.

Figure 3. Plasma concentration of insulin, nonesterified fatty acids (NEFA), and BHB of cows classified as low insulin (L-INS) or high insulin (H-INS) based on the median of plasma insulin measured within 12 h (panel A), at 3 d (panel B), and 10 d (panel C) after calving. P -values for the fixed effect of group, time, and group $\times$ time are included in each graph. Results are reported as LSM $\pm$ SEM. *** $P \leq 0.001$, * $P \leq 0.05$, † $P \leq 0.1$.

tion time points (d 0, 3, and 10), PTA for milk yield was evaluated. No significant differences were observed among groups at any time point ($P > 0.51$). Test day milk, FCM, ECM, percent fat, and percent protein at the first, second, and third DHI tests of cows classified as L-INS or H-INS on d 0, 3, and 10 are presented in Table 1 and Supplemental Figures S1 and S2 (<https://doi.org/10.3168/jds.2017-14029>).

Cows Classified into L-INS or H-INS at d 0. Cows classified as L-INS on d 0 had higher TDM ($P = 0.01$), FCM ($P = 0.005$), and ECM ($P = 0.004$) yields compared with H-INS cows. For TDM, FCM, and ECM, we detected an insulin-group by test number interaction ($P < 0.05$), whereby L-INS cows produced higher yields of TDM, FCM, and ECM than H-INS cows on test 1. No differences were observed for percent fat between groups. However, percent protein was higher ($P = 0.02$) for H-INS cows when compared with L-INS cows.

For weekly milk yields, we observed that cows classified as L-INS produced 2.04 kg/d more milk ($P = 0.02$) during the first 12 wk after calving compared with H-INS cows (L-INS = 41.93 ± 0.64 , H-INS = 39.89 ± 0.59 kg; Figure 5A).

Cows Classified into L-INS or H-INS at d 3. Test day milk yield was not affected by insulin group at d 3. However, FCM tended ($P = 0.09$) to be higher in L-INS cows when compared with H-INS cows. Fur-

thermore, relative to H-INS cows, ECM was higher ($P = 0.007$) for L-INS cows. Percent fat was similar among groups. However, percent protein tended to be higher ($P = 0.09$) for H-INS cows when compared with L-INS cows. Additionally, a group by test number interaction ($P = 0.05$) was observed for percent protein, whereby H-INS cows tended ($P = 0.07$) to have a higher percent protein than L-INS cows on test 1.

No differences were observed between L-INS and H-INS cows for weekly milk yields (L-INS = 41.26 ± 0.64 , H-INS = 40.43 ± 0.62 kg; Figure 5B).

Cows Classified into L-INS or H-INS at d 10. For TDM and FCM, we observed an insulin-group by parity interaction ($P < 0.05$). Primiparous L-INS cows had higher TDM and FCM yields compared with primiparous H-INS cows. No differences were observed among multiparous L-INS and H-INS cows. Relative to H-INS cows, ECM was higher ($P = 0.005$) for L-INS cows. No differences were observed for percent fat between groups. However, percent protein was higher ($P = 0.02$) for H-INS cows compared with L-INS cows.

For weekly milk yields, we observed an insulin-group by parity interaction ($P = 0.002$), in which primiparous L-INS cows produced 4.87 kg/d more milk than primiparous H-INS cows (L-INS = 38.22 ± 1.17 , H-INS = 33.35 ± 0.84 kg; Figure 6A). No differences were observed among multiparous L-INS and H-INS cows (Figure 6B).

Figure 4. Plasma concentration of BHB of primiparous (A) and multiparous (B) cows classified as low insulin (L-INS) or high insulin (H-INS) based on the median plasma insulin concentration from blood samples collected within 12 h (d 0) after calving. Results are reported as LSM $\pm$ SEM. $\dagger P \leq 0.1$.

BCS Loss

We evaluated BCS loss in L-INS and H-INS cows on d 0, 3, and 10. The L-INS cows lost more ($P < 0.001$)

Figure 5. Weekly milk yields of cows classified as low insulin (L-INS) or high insulin (H-INS) based on the median plasma insulin from blood samples collected within 12 h (d 0) of parturition, and on d 3 (B), and d 10 (C) after calving. P -values for the fixed effect of group, time, group $\times$ time, and group $\times$ parity are included in each graph. Results are reported as LSM $\pm$ SEM.

Table 1. Milk yield and composition from cows classified as low insulin (L-INS) or high insulin (H-INS) based on the median plasma insulin concentration from blood samples collected within 12 h (d 0) of parturition, and on d 3 and 10 after calving at first, second, and third DHI test

Item ¹	Insulin d 0			Insulin d 3			Insulin d 10		
	L-INS (LSM $\pm$ SEM)	H-INS (LSM $\pm$ SEM)	P -value ²	L-INS (LSM $\pm$ SEM)	H-INS (LSM $\pm$ SEM)	P -value ²	L-INS (LSM $\pm$ SEM)	H-INS (LSM $\pm$ SEM)	P -value ²
Milk yield (kg/d), TDM	40.75 $\pm$ 0.69	38.41 $\pm$ 0.64	0.01	39.98 $\pm$ 0.69	38.95 $\pm$ 0.67	0.28	40.95 $\pm$ 0.74	38.66 $\pm$ 0.64	0.02 ³
Primiparous	—	—	—	—	—	—	37.01 $\pm$ 1.28	32.26 $\pm$ 0.92	0.01
Multiparous	—	—	—	—	—	—	44.91 $\pm$ 1.28	45.06 $\pm$ 0.92	—
FCM (kg/d), TDM	40.59 $\pm$ 2.36	37.73 $\pm$ 2.31	0.005	40.11 $\pm$ 2.36	38.40 $\pm$ 2.30	0.09	41.00 $\pm$ 2.42	38.65 $\pm$ 2.28	0.006 ³
Primiparous	—	—	—	—	—	—	35.95 $\pm$ 2.65	30.95 $\pm$ 2.48	0.04
Multiparous	—	—	—	—	—	—	46.16 $\pm$ 2.41	45.39 $\pm$ 2.28	—
ECM (kg/d), TDM	44.50 $\pm$ 0.70	41.67 $\pm$ 0.64	0.004	43.65 $\pm$ 0.74	40.88 $\pm$ 0.72	0.007	44.09 $\pm$ 0.73	40.55 $\pm$ 0.68	0.005
Fat (%), TDM	3.81 $\pm$ 0.16	3.77 $\pm$ 0.16	0.56	3.83 $\pm$ 0.16	3.76 $\pm$ 0.15	0.39	3.85 $\pm$ 0.16	3.77 $\pm$ 0.15	0.30
Protein (%), TDM	2.99 $\pm$ 0.02	3.07 $\pm$ 0.02	0.02	3.01 $\pm$ 0.02	3.06 $\pm$ 0.02	0.09	2.98 $\pm$ 0.02	3.07 $\pm$ 0.02	0.003

¹TDM = test day milk; FCM = 3.5% FCM.

² P -value indicates overall group effect.

³ P -value for the interaction term group $\times$ parity was <0.05.

BCS, regardless of the day, compared with H-INS cows (Figure 7A).

DISCUSSION

Our main hypothesis was that low plasma insulin during early postpartum would be associated with increased milk yield. In support of our hypothesis, we observed that cows with low plasma insulin (L-INS cows) produced more milk, FCM, and ECM when compared with cows with high plasma insulin levels (H-INS cows).

Due to its anabolic action, insulin stimulates the uptake of glucose, AA, and fatty acids into body tissues, whereas it inhibits metabolic pathways for glucose production (Hart, 1983; De Koster and Opsomer, 2013). It is well known that in postpartum dairy cows, insulin secretion is diminished to prioritize the use of glucose for milk production; this is considered one of the major

endocrine adaptations of dairy cows from late pregnancy to early lactation (Hart, 1983; Bell, 1995; Bell and Bauman, 1997). Here, we described the plasma insulin concentrations before calving and during early lactation. In agreement with the literature, we observed that circulating insulin is suppressed after parturition to ensure glucose availability to the insulin-independent mammary gland for production of copious amounts of milk.

Previous studies have shown that administration of insulin decreases milk yield in Holstein cows (Kronfeld et al., 1963; Schmidt, 1966; Winkelman and Overton, 2013). Schmidt (1966) observed that primiparous cows that received repeated administrations of a short-acting insulin had reduced milk yield and lactose content of milk. Similarly, Kronfeld et al. (1963) observed a decrease in milk yield in cows given repeated injections of long-acting insulin. In the present study, we observed that cows with low plasma insulin concentrations produced significantly more milk and higher FCM and ECM compared with cows with high plasma insulin. Moreover, our results are in accordance with the study conducted by Lucy et al. (2009). In that study, North American Holstein cows produced more milk when compared with New Zealand Holstein-Friesian cows. They observed that New Zealand Holstein-Friesian cows tended to have higher plasma insulin concentration 1 wk before calving and numerically higher on the first week postpartum than North American Holstein cows (Lucy et al., 2009). Therefore, the aforementioned studies support our findings, that low insulin is associated with higher milk production. We also observed a group by parity interaction when milk yield was analyzed from cows classified as L-INS or H-INS on d 10, where the increase in milk yield was driven by primiparous cows. An explanation for this observation may be the differences in the control of nutrient partitioning that exist between primiparous and multiparous cows, in which first-parity animals promote nutrient utilization into growth (Wathes et al., 2011). Thus, primiparous cows that still have high circulating insulin on d 10 might redirect the use of glucose toward growth.

Increasing milk protein production has been observed in dairy cows that received an exogenous administration of insulin (Kronfeld et al., 1963; Schmidt, 1966; Winkelman and Overton, 2013). Our results on milk protein content are in accordance with the published studies. We observed that cows classified as H-INS produced significantly more protein in milk compared with L-INS cows. Although the observed reduction in milk protein of L-INS cows might be due in part to a dilution effect, it has also been demonstrated that insulin can promote milk protein synthesis (Grinari et al., 1997; Mackle et al., 1999).

Figure 6. Weekly milk yields of primiparous (A) and multiparous (B) cows classified as low insulin (L-INS) or high insulin (H-INS) based on the median of plasma insulin on d 10 after calving. Results are reported as LSM $\pm$ SEM.

Figure 7. Body condition score loss from calving until 35 DIM of cows classified as low insulin (L-INS) or high insulin (H-INS) based on the median of plasma insulin within 12 h of parturition (A), on d 3 (B), and d 10 (C) after calving. Results are reported as LSM $\pm$ SEM. *** $P \leq 0.001$.

As mentioned above, insulin signaling normally promotes triglyceride synthesis in adipose tissue by increasing lipoprotein lipase activity, thus favoring the uptake of fatty acids into adipocytes from circulating lipoproteins (Hayirli, 2006). Here, the described lipogenic and anti-lipolytic effects of insulin were reflected by plasma NEFA levels. We observed that cows with low plasma insulin had significantly higher concentrations of circulating NEFA. In support, L-INS cows lost significantly more BCS than did H-INS cows. Interestingly, BHB plasma levels did not differ between H-INS and L-INS cows. However, it has been shown that the blood concentrations of NEFA and BHB during early lactation have a weak correlation (Ospina et al., 2013; McCarthy et al., 2015). In the study conducted by McCarthy et al. (2015), the authors suggested that other sources of carbon such as lactate or ketogenic AA might contribute to BHB synthesis.

Last, we measured the relationship between insulin concentrations in colostrum and in plasma at 7 d before the expected day of calving and within 12 h relative to parturition. Insulin in colostrum is known to play a role in postnatal intestinal development (Shehadeh et al., 2006; Hammon et al., 2013). Ronge and Blum (1988) observed that the colostrum insulin concentration was ~ 20 times higher than in prepartum plasma and ~ 35 times higher than in milk (Ronge and Blum, 1988). In addition, a recent study shown that the colostrum insulin concentration was ~ 80 times higher than in prepartum plasma (Mann et al., 2016). In the present study, we observed that the colostrum insulin concentration was ~ 100 times higher than in prepartum plasma. However, the transport mechanism of circulating insulin into the mammary gland is not understood. The dramatic difference in insulin concentration between plasma and colostrum suggests that insulin is actively and not passively transported from blood to the mammary gland.

Here, we observed no relationship between plasma insulin and colostrum insulin levels, either at -7 d from the expected day of parturition or within 12 h after parturition. However, the study conducted by Mann et al. (2016) revealed a moderate positive correlation between plasma insulin and colostrum insulin on d 1 to 3 prepartum (Mann et al., 2016). Further investigation is needed to elucidate the exact mechanism of insulin uptake by the mammary gland.

The limitations in the present study need to be pointed out. One limitation is that the study was limited to a single dairy farm, which restricts the external generalization of the results. Because plasma insulin concentration significantly varies during the day (Lefcourt et al., 1999), it is possible that median of plasma insulin observed in the current study and used to group cows as L-INS or H-INS might have been different if samples were collected at a different time of the day. However, according to the results presented by Lefcourt et al. (1999), between 0630 to 1100 h (range of sample collection time of our study) seems to be the most stable period of time during the day for plasma insulin concentration. In addition, in that study, cows were fed a TMR at 0900 h, which is similar feeding time for the cows in the present study (~ 0800 h). Nevertheless, because our study was conducted in a commercial dairy farm, the exact time of feeding could have varied during the study, thus altering the concentration of insulin in plasma. Therefore, the results presented here need to be interpreted in light of these limitations.

CONCLUSIONS

In summary, we observed that cows with low plasma insulin during the early postpartum produced more milk and had higher FCM and ECM compared with cows with high plasma insulin. Cows with high insulin

concentrations had a higher percentage of milk protein compared with cows with low insulin levels. The results presented here highlight the importance of the depression of postpartum insulin secretion as a key endocrine adaptation to support high milk production.

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