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The association of plasma glucose, BHBA, and NEFA with postpartum uterine diseases, fertility, and milk production of Holstein dairy cows

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The objective of this study was to investigate the association between the metabolic indicators such as nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), and glucose during the transition period and the development of uterine diseases. In total, 181 Holstein dairy cows were enrolled in the study. Plasma glucose, NEFA, and BHBA concentrations were measured at −50, −6, 3, 7, and 14 days relative to parturition. All cows enrolled in the study were evaluated for retained placenta (RP), metritis, and endometritis. Metritis and RP were diagnosed and treated by trained farm personnel. Clinical endometritis was evaluated by a veterinarian at 35 days in milk using a Metricheck device. We found plasma glucose concentration to be associated with the occurrence of metritis and clinical endometritis. Moreover, cows with an increased calving-to-conception interval (>150 days) presented higher plasma glucose concentrations than cows that became pregnant within the first 150 days, whereas BHBA and NEFA were not associated with the occurrence of any uterine disorder. Receiver operating characteristic (ROC) curves were used in an attempt to determine the cow-level critical thresholds for the occurrence of metritis, and endometritis. In addition, pairwise comparisons of area under the curve (AUC) of ROC curves for the critical thresholds for glucose, BHBA, and NEFA predicting the same uterine disease were performed. Glucose at 3 days in milk was the best predictor for metritis and endometritis diagnosis, with AUC values of 0.66 and 0.67, respectively. Multivariable logistic regressions were performed and showed that cows with higher levels of glucose at Day 3 were at 6.6 times higher odds of being diagnosed with metritis, and 3.5 times higher odds of developing clinical endometritis, compared with cows with lower glucose levels. Finally, a simple linear regression analysis demonstrated a negative correlation between daily milk yield in the first and second weeks of lactation and plasma glucose concentrations measured at Days 7 and 14, respectively. Concentrations of NEFA and BHBA were not found to be associated with milk production.

1. Introduction

Postpartum uterine diseases such as metritis, endometritis, purulent vaginal discharge and retained placenta (RP) are associated with substantially infertility, reduced milk yield, and increased culling rates [1]. These diseases have complex multifactorial causes which include exposure to bacterial pathogens [2], mineral and vitamin deficiencies [3], negative energy balance [4], and immunosuppression [5–7]. Previous studies have extensively demonstrated associations between negative energy balance markers, particularly nonesterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) in plasma, and the incidence of postpartum diseases such as clinical ketosis and displaced abomasum [8,9]. Excessive fat mobilization in dairy

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cows has also been associated with clinical endometritis and linked to immunosuppression [10–12]. The inflammation of the endometrium has been showed to have a detrimental effect on reproductive performance, reducing both the first-service conception rate and the overall pregnancy risk mainly due to dysregulated ovarian function and oocyte development [13–16].

The liver is responsible for metabolizing circulating NEFA, which can be completely oxidized for energy production, exported from the liver as lipoproteins, or partially oxidized into BHBA and other ketone bodies [17,18]. When the liver is overloaded with NEFA, hepatocytes increase the level of partial oxidation of NEFA, which leads to the accumulation of BHBA and other ketone bodies, eventually causing subclinical and clinical ketosis [19]. Therefore, hyperketonemia is a marker of liver health and has been associated with infectious diseases (e.g., mastitis, metritis) [3,20].

In addition to fat mobilization in early postpartum cows, liver gluconeogenesis increases to provide glucose for synthesis of milk lactose [21]. The large demand for glucose may lower the amount of glucose available to other tissues in the body, including those that are involved in postpartum immune defense. During the period of negative energy balance, dairy cows experience a reduction in blood glucose levels and neutrophil function. Low glucose levels observed during the transition to lactation may be associated with immunosuppression. Granulocytes depend on uptake of exogenous glucose and intracellular glycogen stores for the energy required for chemotaxis, phagocytosis, and microbial killing [22,23]. Conversely, many studies have demonstrated impaired adherence and neutrophil dysfunction associated with high glucose levels [24–26].

A strong association between decreased immune response and a greater degree of negative energy has been reported in cows that developed uterine disease compared with healthy cows [10,12,27,28]. For instance, cows that developed uterine disease experienced a greater degree of negative energy balance, increased serum levels of inflammatory markers, greater blood glucose concentration at calving, and had lower intracellular neutrophil glycogen levels [20,28]. Moreover, cows with metritis and cows with cystic ovaries had increased levels of ketone bodies than unaffected cows [29].

Besides, relationships between blood metabolites and the reproductive performance of dairy cows have been reported earlier [30,31]. For instance, in a multivariate description of factors that influence fertility in dairy cows, Westwood et al. (2002) showed that increased concentrations of plasma glucose were associated with greater probability of estrous expression at first ovulation, whereas higher serum concentrations of NEFA lowered the probability of conception by 150 days [31], supporting previous observations where more mobilization of body tissue delayed the resumption of ovarian activity [32], However, in another study, days-to-conception was not associated with glucose levels but was inversely related to milk production [33].

Thus far, most current research has focused on the evaluation of the markers of negative energy balance, NEFA and BHBA as predictors of inflammation and postpartum diseases [8,34,35], whereas few studies have evaluated the importance of glucose as a potentially significant risk factor for the development of uterine diseases. Therefore, the objective of this study was to investigate associations between the metabolic indicators NEFA, BHBA, and glucose during the transition period and the occurrence of uterine diseases and the subsequent effect on fertility. Because uterine diseases have negative effect on milk production, and milk production is accompanied by changes in glucose and energy metabolism, milk yield was also evaluated.

2. Materials and methods

2.1. Farm, management, and sample collection

This study was conducted from October 2012 until January 2013 on a dairy farm located near Ithaca, New York. In total, 181 Holstein dairy cows (108 dry cows and 73 pregnant heifers) were enrolled in the study. The farm milked 3300 Holstein cows 3 times daily in a double 52-stall parallel milking parlor. The cows were housed in freestall barns, with concrete stalls covered with mattresses and bedded with composted manure solids. All cows were offered a total mixed ration consisting of approximately 55% forage (corn silage, haylage, and wheat straw) and 45% concentrate (corn meal, soybean meal, canola, cottonseed, and citrus pulp) on a dry matter basis of the diet. The diet was formulated to meet or exceed the National Resarch Council nutrient requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% fat-corrected milk (National Resarch Council 2001). The farm reproductive management used a combination of Presynch, Resynch, and detection of estrus, with 25% to 30% of cows bred via timed AI and the remainder bred after detection of estrus solely by activity monitors (Alpro; DeLaval, Kansas City, MO, USA). Pregnancy was diagnosed by rectal palpation at 39 $\pm$ 3 days since the last insemination. All study cows were followed until 300 days postpartum or the date of culling (if $<$300 days) from the herd. Data regarding health traits, reproductive performance (the cow being diagnosed pregnant within 150 days postpartum), and milk yield during the subsequent lactation were obtained from DairyComp (DairyComp 305, Tulare, CA, USA) records for the herd, and descriptive statistics were calculated (version 9.3, SAS Institute Inc., Cary, NC, USA).

Blood was sampled from all study subjects at -50 ($\pm$3), -6 ($\pm$3), 3 ($\pm$3), 7 ($\pm$3), and 14 ($\pm$3) days relative to parturition. Blood collection was performed via the coccygeal vein/artery using a 10-mL vacuum tube with lithium heparin, and a 20-gauge $\times$ 2.54-cm needle (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). After collection, all blood samples were transported to the laboratory on ice, and plasma was harvested after centrifugation at $\times 2000g$ for 15 minutes at 4 °C. Plasma was frozen at -80 °C. Body condition scores (BCS) were determined for all study cows at blood collection time by a single investigator using a five-point scale with a quarter-point system as previously described [36].