



## Identification of predictive biomarkers of disease state in transition dairy cows

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### ABSTRACT

In dairy cows, periparturient disease states, such as metritis, mastitis, and laminitis, are leading to increasingly significant economic losses for the dairy industry. Treatments for these pathologies are often expensive, ineffective, or not cost-efficient, leading to production losses, high veterinary bills, or early culling of the cows. Early diagnosis or detection of these conditions before they manifest themselves could lower their incidence, level of morbidity, and the associated economic losses. In an effort to identify predictive biomarkers for postpartum or periparturient disease states in dairy cows, we undertook a cross-sectional and longitudinal metabolomics study to look at plasma metabolite levels of dairy cows during the transition period, before and after becoming ill with postpartum diseases. Specifically we employed a targeted quantitative metabolomics approach that uses direct flow injection mass spectrometry to track the metabolite changes in 120 different plasma metabolites. Blood plasma samples were collected from 12 dairy cows at 4 time points during the transition period (–4 and –1 wk before and 1 and 4 wk after parturition). Out of the 12 cows studied, 6 developed multiple periparturient disorders in the post-calving period, whereas the other 6 remained healthy during the entire experimental period. Multivariate data analysis (principal component analysis and partial least squares discriminant analysis) revealed a clear separation between healthy controls and diseased cows at all 4 time points. This analysis allowed us to identify several metabolites most responsible for separating the 2 groups, especially before parturition and the start of any postpartum disease. Three metabolites, carnitine, propionyl carnitine, and lysophosphatidylcholine acyl C14:0, were significantly elevated in diseased cows as compared with healthy controls as early as 4 wk before parturition, whereas 2 metabolites, phosphatidylcholine acyl-alkyl C42:4 and phosphatidylcholine diacyl C42:6,

could be used to discriminate healthy controls from diseased cows 1 wk before parturition. A 3-metabolite plasma biomarker profile was developed that could predict which cows would develop periparturient diseases, up to 4 wk before clinical symptoms appearing, with a sensitivity of 87% and a specificity of 85%. This is the first report showing that periparturient diseases can be predicted in dairy cattle before their development using a multimetabolite biomarker model. Further research is warranted to validate these potential predictive biomarkers.

**Key words:** predictive biomarker, plasma metabolite, periparturient disease, dairy cow

### INTRODUCTION

In dairy cows, pathologies associated with parturition, such as mastitis, metritis, retained placenta, laminitis, ketosis, and milk fever, are very common (Bigras-Poulin et al., 1990). The prevalence of these diseases is disproportionately high during the transition period, where 30 to 50% of the cows are affected by one or more of these conditions (LeBlanc, 2010). Periparturient diseases can significantly affect a dairy cow's production efficiency by reducing milk production, altering milk composition, reducing reproductive performance, increasing treatment costs, or reducing the life expectancy of the cow. Four conditions, metritis, infertility, mastitis, and laminitis, have been shown to account for the largest share of economic loss suffered by the dairy industry. For instance, early culling of dairy cows costs the Canadian dairy industry an estimated \$200 million a year, with 60% of the 160,000 cows culled in Canada in 2008 being afflicted with one or multiple of these 4 diseases (Ametaj et al., 2012).

The pathophysiology of postpartum diseases is complex, interrelated, and multifactorial, often driven by several interconnected risk factors. Despite major advances in the knowledge of their etiology, treatments for these kinds of diseases are often expensive, ineffective, or not cost-efficient. Therefore, early diagnosis or detection of those conditions before they manifest could reduce their incidence, level of morbidity, and the associated economic losses.

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Genomics, proteomics, and metabolomics technologies are increasingly being used to help identify disease and disease risk biomarkers. Given that little evidence exists for a genetic cause to these postpartum or periparturient diseases, it stands to reason that metabolite alterations may be more suitable to developing a predictive biomarker test. More than a decade ago, studies in dairy cows established that elevated ketone bodies, such as BHBA, acetone, and acetoacetate in milk, could serve as metabolic biomarkers for subclinical ketosis (Enjalbert et al., 2001). Prepartum NEFA and postpartum BHBA were both significantly associated with development of clinical disease, whereas postpartum serum NEFA concentration was most associated with the risk of developing displaced abomasum, clinical ketosis, metritis, or retained placenta during the first 30 DIM (Ospina et al., 2010). In another study, cows developing postpartum diseases had higher mean serum NEFA and lower plasma IGF-I concentrations prepartum when compared with healthy cows (Piechotta et al., 2012). Indeed, metabolomics has been shown to be a particularly effective platform for investigating the pathophysiology of pregnancy-associated diseases in humans, such as pre-eclampsia and pregnancy-induced diabetes, and identifying predictive biomarkers for those conditions (Bahado-Singh et al., 2012, 2013). In a previous study using a metabolomics approach, we reported unhealthy alterations of rumen metabolites with increasing proportions of cereal grains in the diet of dairy cows (Ametaj et al., 2010). Extending on those observations, our group used multiple metabolomics platforms to identify and quantify rumen metabolites, confirming detailed rumen metabolite alterations with increased grain proportions in the diet (0, 15, 30, and 45% barley grain on a DM basis), as well as temporal changes in the metabolite concentrations during each dietary period (Saleem et al., 2012). Moreover, feeding dairy cows diets rich in rumen-degradable carbohydrates and low in fiber led to lower rumen pH and large accumulation of rumen endotoxin; the latter was correlated with perturbations of plasma metabolites allied to carbohydrate and lipid metabolism (Zebeli et al., 2011). Nuclear magnetic resonance-based metabolomics elucidated the relationship between the metabolite profile and technological properties of bovine milk from 2 dairy breeds (Sundekilde et al., 2011) and revealed strong association between milk metabolites and SCC in bovine (Sundekilde et al., 2013). Moreover, Klein et al. (2012) reported that high concentrations of milk glycerophosphocholine along with a high ratio of glycerophosphocholine to phosphocholine could be used to aid in the reliable selection of healthy and metabolically stable cows for breeding purposes. Building from the success of these efforts, we decided to perform a

longitudinal and cross-sectional metabolomic study to look at plasma metabolite levels of dairy cows during the transition period, before and after becoming ill with different postpartum pathologies. This was done to investigate whether changes in plasma metabolite concentrations could be predictive for the development of periparturient or postpartum diseases in dairy cows.

Specifically, we employed a targeted quantitative metabolomics approach that uses direct flow injection MS/MS to track the metabolite changes in 120 different plasma metabolites. Blood plasma samples were collected from 12 dairy cows at 4 time points during the transition period (−4 and −1 wk before and 1 and 4 wk after parturition). Out of the 12 cows studied, 6 developed multiple periparturient diseases during the post-calving period, whereas the other 6 remained healthy during the entire experimental period. From this work, we were able to identify at least 5 plasma metabolites that can be used as potential predictive biomarkers for the early diagnosis and monitoring of multiple periparturient diseases in transition dairy cows.

## MATERIALS AND METHODS

### *Pre- and Postpartum Diets*

All cows were fed the same close-up diet starting at 3 wk before the expected day of parturition. The close-up diet is usually offered to the dairy cows when they are close to parturition and contained approximately 20% concentrate on a DM basis (Table 1). After parturition, cows were gradually switched during the first 7 d to a fresh lactation diet with a higher proportion of grain (up to 50% on a DM basis) to meet the energy demands for high milk production (Table 2). Daily ration was offered as TMR for ad libitum intake to allow approximately 10% feed refusals throughout the experiment. All cows were fed once daily in the morning at 0800 h.

### *Sample Collection*

Blood samples were collected from the coccygeal veins of 12 transition Holstein dairy cows at 4 time points: during 4 (25–31 d) and 1 wk (4–10 d) before calving (d 0) and during 1 (4–10 d) and 4 wk (25–31 d) after calving. Multiparous cows, with parities ranging from 2 to 4, were used in the study. Samples were collected before the morning feeding at 0800 h using 10-mL Vacutainer tubes (Becton Dickinson, Franklin Lake, NJ) containing a sodium heparin anticoagulant. Blood samples were stored on ice and centrifuged within 20 min at 4°C for 20 min at 3,000 × *g* in a Rotanta 460 R centrifuge (Hettich Zentrifugan, Tuttlingen, Germany) to separate the plasma. Plasma samples were stored at

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