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Metabotypes with elevated protein and lipid catabolism and inflammation precede clinical mastitis in prepartal transition dairy cows

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ABSTRACT

Clinical mastitis (CM), the most prevalent and costly disease in dairy cows, is diagnosed most commonly shortly after calving. Current indicators do not satisfactorily predict CM. This study aimed to develop a robust and comprehensive mass spectrometry-based metabolomic and lipidomic workflow using untargeted ultra-performance liquid chromatography high-resolution mass spectrometry for predictive biomarker detection. Using a nested case-control design, we measured weekly during the prepartal transition period differences in serum metabolites, lipids, inflammation markers, and minerals between clinically healthy Holstein dairy cows diagnosed with mastitis postcalving (CMP; n = 8; CM diagnosis d 1 = 3 cows, d 2 = 2 cows, d 4 = 1 cow; d 25 = 1 cow, and d 43 = 1 cow that had subclinical mastitis since d 3) or not (control; n = 9). The largest fold differences between CMP and control cows during the prepartal transition period were observed for 3′-sialyllactose in serum. Seven metabolites (*N*-methylethanolamine phosphate, choline, phosphorylcholine, free carnitine, trimethyl lysine, tyrosine, and proline) and 3 metabolite groups (carnitines, AA metabolites, and water-soluble phospholipid metabolites) could correctly classify cows for their future CM status at both 21 and 14 d before calving. Biochemical analysis using lipid and metabolite-specific commercial diagnostic kits supported our mass spectrometry-based omics results and additionally showed elevated inflammatory markers (serum amyloid A and visfatin) in CMP cows. In conclusion, metabolic phenotypes (i.e., metabotype) with elevated protein and lipid metabolism and inflammation may precede CM in prepartal transition dairy cows. The discovered serum metabolites and lipids may assist in predictive diagnostics, prevention strategies, and early treatment intervention against CM, and thereby improve cow health and welfare.
Key words: clinical mastitis, lipidomic, metabolomic, transition period, 3′-sialyllactose

INTRODUCTION

Bovine mastitis is an inflammatory response of the mammary gland, which is primarily caused by bacterial infections (Eberhardt, 1996; Viguier et al., 2009). Bovine mastitis can be subdivided into clinical (**CM**) and subclinical mastitis (**SCM**); CM can be diagnosed by visible changes in milk consistency and mammary gland appearance (redness, swelling, heat, or pain) or both, and SCM can be diagnosed by elevated SCC in milk (>200,000 cells/mL milk) in the absence of visible changes in milk and udder appearance (Eberhardt, 1996; Sharma et al., 2011). Bovine CM is one of the most prevalent and costly clinical diseases in dairy cows, which makes it economically important to the dairy industry. In the United States, about 16.5% of the dairy cows have CM in the first 30 d of lactation (USDA, 2009) and the incurred cost is about $440 per case (Kelton et al., 1998; Rollin et al., 2015); therefore, prevention and early treatment of CM are a priority.

Traditional CM indicator studies focus on indicators in milk at the onset of CM, including SCC, serum proteins, enzymes, electrolytes, degradation products of milk proteins, and acute phase proteins (Lai et al., 2009; Sundekilde et al., 2013). In recent years, this research has been extended to metabolomics approaches to discover indicators in infected bovine milk, which can aid in the detection, differential diagnosis of CM based on pathogen, and to examine the pathophysiology of CM (Mansor et al., 2013; Thomas et al., 2016; Xi et al., 2017). Dairy cows are most susceptible to naturally occurring CM within the first weeks after calving; however, the infection may occur during the close-up period or around calving (Rollin et al., 2015), when milk samples are not available (Hurley and Theil,

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2011). Multiple studies have become available during recent years that used blood samples collected during the last 2 mo before and after calving for the discovery of predictive biomarker of various diseases in early-lactation dairy cows (Imhasly et al., 2015; Dervishi et al., 2017; Zhang et al., 2017); however, to the best of our knowledge, none looked at predictive serum indicators of CM. Dervishi et al. (2015, 2017) reported that cows subsequently developing SCM had elevated serum concentrations of inflammatory markers, monosaccharides, and AA and their metabolites 4 wk before calving.

Our hypothesis was that untargeted ultra-performance liquid chromatography high resolution mass spectrometry (**UPLC-MS^E**) can be used to discover serum metabolites and lipids that precede CM during the close-up period in dairy cows. To accomplish our goal, our major objectives were (1) to develop a robust and comprehensive, untargeted MS-based metabolomic and lipidomic workflow for biomarker detection in serum, which could be also applied to other biological fluids; (2) to identify serum metabolites, lipids, minerals, and inflammatory markers, individually or as group, that can classify during the close-up period dairy cows that develop CM in early lactation; and (3) to describe temporal changes in serum metabolites, lipids, minerals, and inflammatory markers during the prepartal transition period.

MATERIALS AND METHODS

This study was part of a prospective study designed to identify predictive serum indicators of periparturient diseases in dairy cows. All procedures involving animals were approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP Number 3991).

Study Design and Animal Management

The study cohort consisted of 161 purebred Holstein cows from a 1,000-head commercial dairy farm in Oregon's Central Willamette Valley; cows had 1 to 6 completed lactations and were free of clinical diseases, including abnormal mammary gland appearance (tender, painful or warm to touch, swelling, hardness, or skin redness), 4 wk before expected calving date. Using a nested case-control design, we identified 8 cows that developed CM postcalving (**CMP**) and matched them by parity (mean ± SD, range; CMP =1.88 ± 0.64, 1–3 completed lactations; control = 1.44 ± 0.73, 1–3 completed lactations), BCS (control = 3.68 ± 0.19, 3.3–3.9; CMP = 3.75 ± 0.29, 3.3–4.1), and calving season with 9 cows that remained free of clinical diseases (control) as well as subclinical ketosis and hypocalcemia during the first 49 d after calving. Clinical mastitis was diagnosed based on daily test for abnormal milk consistency (e.g., flakes, clots) and mammary gland appearance (redness, swelling, heat, or pain). If abnormal milk consistency, mammary gland appearance, or both were detected, a milk sample was collected and on-farm culturing with blood agar was performed. All CMP cows except 2 were diagnosed with CM within 4 d after calving: d 1 = 3 cows, d 2 = 2 cows, and d 4 = 1 cow. Another CMP cow had SCM (SCC >1,000,000 cells/mL) directly after calving and was diagnosed with CM 43 d after calving. The only CMP cow without elevated SCC at the beginning of lactation was diagnosed with CM at 25 d after calving. To identify serum indicators that could precede CM from various naturally occurring pathogens rather than indicators specific to 1 type of pathogens, we selected cows that differed in pathogens based on cultured growth (gram-positive = 3 cows; gram-negative = 3 cows; no cultured growth = 2 cows). Within 1 d of CM diagnosis, cows were treated based on their cultured growth. Three CMP cows (6, 9, and 10 mo prior) and 1 control cow (11 mo prior) had an episode of CM in the previous 12 mo. Based on the cultured growth, the current CM was from a different pathogen than the prior CM. To avoid confounding with other diseases, cows that developed other concurrent clinical diseases besides CM were excluded from this nested case-control study.

Management of the cows and animal health surveillance and disease treatment has been described in detail previously (Qu et al., 2014); additional information is provided in Supplemental Information S1 (https://doi.org/10.3168/jds.2017-13977). Starting 28 d before the expected calving date, BCS of cows were scored once weekly by 3 trained independent evaluators until 4 wk after calving (Edmonson et al., 1989). Before calving, cows were housed in a straw-bedded freestall barn and were fed once in the morning (0730 h), a TMR based on corn, corn silage, and alfalfa and triticale hay that met NRC guidelines (NRC, 2001), as summarized in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-13977). After calving, cows were housed in freestall pens with slatted floors and were fed in head gates around 0800 and 1330 h a TMR based on corn, corn silage, and alfalfa hay (Supplemental Table S1) that met NRC guidelines (NRC, 2001).

Sample Collection

Blood samples were taken from the coccygeal vein or artery at 3 (−24 to −18 d), 2 (−17 to −11 d), and 1 wk (−10 to −4 d) before calving and the morning after calving within 10 min after morning feeding. We chose serum rather than plasma as biological matrix because