J. Dairy Sci. 96:3599–3610
http://dx.doi.org/10.3168/jds.2012-5900

Identification of hepatic biomarkers for physiological imbalance of dairy cows in early and mid lactation using proteomic technology

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ABSTRACT

Identification of biomarkers for degree of physiological imbalance (PI), a situation in which physiological parameters deviate from normal, is needed to reduce disease risk and improve production and reproduction performance of cows. The objective was to describe the liver proteome in early and mid lactation for cows with different degrees of PI with a special focus on biomarkers and pathways involved in periparturient disease complexes. Twenty-nine cows in early [49 ± 22 d in milk (DIM); n = 14] and mid (159 ± 39 DIM; n = 15) lactation were nutrient restricted for 4 d to increase PI by supplementing the ration with 60% wheat straw. Liver biopsies were collected −1 and 3 d relative to restriction. Before restriction, an index for PI was calculated based on plasma nonesterified fatty acids, β-hydroxybutyrate, and glucose concentrations. Within E and M cows, a subsets of 6 cow was classified as having either the greatest (PI) or least (normal; N) degree of PI and were used for isobaric tags for relative and absolute quantitation (iTRAQ)-based quantitative profiling in liver using liquid chromatography-tandem mass spectrometry. We identified pyruvate carboxylase and isocitrate dehydrogenase as potential hepatic biomarkers for PI for cows during early lactation and alcohol dehydrogenase-4 and methylmalonate-semialdehyde dehydrogenase for cows in mid lactation. This preliminary study identified new biomarkers in liver for PI and provided a better understanding of the differences in coping strategies used for cows in PI. Despite the small sample size (n = 3/group), the results lay a foundation for future research focused on the usefulness of the hepatic biomarkers for predicting PI and thereby cows at risk for disease during lactation.

Key words: physiological imbalance, liver, biomarker, proteome

INTRODUCTION

Despite improvements in genetics and management in the dairy industry over the past decades, the incidence of metabolic diseases (e.g., ketosis and hepatic lipidosis) and reproductive problems is still high (Ingvartsen, 2006). Around parturition, the changing hormonal and metabolic environment, coupled with changes in the nervous, immune, and digestive systems, increase the risk of disease for dairy cows during lactation (Ingvartsen, 2006). The health problems in the dairy industry are associated with reduced animal welfare and economic outcome to the farmer; therefore, the incentive to prevent diseases is strong. As herd size steadily increases, the number of animals that need surveillance per farm staff increases. Consequently, the need exists to develop automated in-line and real-time surveillance systems for use on farm for early detection of at-risk animals based on biomarkers; that is, physiological imbalance (**PI**). Physiological imbalance has been defined as cows whose physiological parameters deviate from the normal and who consequently have an increased risk of developing production diseases (clinical or subclinical) and reduced production or reproduction (Ingvartsen, 2006). If PI can be prevented through feeding and management, early detection of PI may reduce the risk of certain diseases, particularly in early lactation, as previously proposed (Bjerre-Harpøth et al., 2012; Ingvartsen and Moyes, 2013; Moyes et al., 2013).

Recent evidence suggests that individual concentrations of circulating metabolites, such as NEFA and BHBA, are associated with multiple diseases such as ketosis and displacement of the abomasum (Seifi et al., 2011). Because most diseases in the dairy industry are multi-faceted (Ingvartsen, 2006; Ingvartsen and Moyes, 2013), the use of individual metabolites (e.g., urine or milk BHBA for ketosis, and liver triacylglycerol content for hepatic lipidosis) may not be the optimal method to predict risk of clinical disease in lactating dairy cows. Recent investigations in our laboratory showed that (1) nutrient restriction to experimentally increase PI resulted in marked changes in plasma NEFA, BHBA, and glucose; (2) stage of lactation plays a pivotal role with

Received July 3, 2012.
Accepted January 29, 2013.
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regard to the degree of change in individual metabolites (Bjerre-Harpøth et al., 2012); and (3) an index for PI based on plasma NEFA, BHBA, and glucose was a better indicator for risk of disease in early lactation than calculated energy balance or individual metabolites alone (Moyes et al., 2013). However, it is of major interest to identify alternative biomarkers for PI and for early identification of specific subclinical diseases such as ketosis and hepatic lipidosis.

For the present study, we generated an index for PI based on plasma concentrations of NEFA, BHBA, and glucose, as previously described (Moyes et al., 2013), for cows in early and mid lactation that were subjected to dietary nutrient restriction to experimentally increase PI (Bjerre-Harpøth et al., 2012) and thus to understand the biological basis for PI that will help reduce risk of production diseases such as fatty liver and ketosis. The isobaric tags for relative and absolute quantitation (**iTRAQ**) method (Gygi et al., 1999; Ross et al., 2004), when combined with mass spectrometry, is ideally suited for the identification of biomarkers via its comparative and quantitative multiplexing analysis (Song et al., 2008). For this study, liver samples from a subset of cows with the greatest (i.e., PI) and least (i.e., normal) degree of PI were compared by iTRAQ-based proteomic profiling. Our objectives were to describe the liver proteome in early and mid lactation for cows at different degrees of PI with a special focus on biomarkers and pathways involved in periparturient disease complexes. This study identifies preliminary biomarkers for cows in early and mid lactation and describes changes in relevant pathways during a nutrient restriction period. The results lay a foundation for future studies examining the usefulness of the biomarkers as predictors of PI that provide a better understanding of the biological bases for PI and risk of disease for cows during lactation.

MATERIALS AND METHODS

All procedures involving animals were evaluated and approved by the Danish Animal Experiments Inspectorate and complied with the Danish Laws concerning animal experimentation and care of experimental animals.

Animals and Housing

Data for this study originated from a larger experiment that included 47 healthy Holstein cows from the resident herd (Danish Cattle Research Centre, Tjele, Denmark). Descriptions of experimental design, diet composition, and data collection may be found in a previous publication (Bjerre-Harpøth et al., 2012). In short, a subset of 29 cows in early (49 ± 22 DIM; n = 14) and mid (159 ± 39 DIM; n = 15) lactation were selected to identify biomarkers for PI. Of these, 13 cows were primiparous and 16 cows were multiparous (≥2 second lactation). Cows in early lactation had an average milk yield of 42.0 ± 7.0 kg/d and weighed 647 ± 58 kg; cows in mid lactation had an average milk yield of 29.0 ± 7.3 kg/d and weighed 623 ± 57 kg at the start of the study.

Cows were housed in a single group in a loose housing system containing individual automatic feeding stations (Roughage Intake System, Insentec BV, Marknesse, the Netherlands) and milked automatically by an automatic milking system (**VMS**, Voluntary Milking System, DeLaval, Tumba, Sweden). Freestalls contained mats of hard rubber material and sawdust as bedding. Cows had free access to water and the VMS at all times throughout the study period. Cows were weighed automatically at each milking via an electronic scale placed in the VMS and average daily BW was calculated.

Experimental Design and Diets

The 19-d experiment consisted of 3 periods (i.e., 1 = before restriction, 2 = during restriction, and 3 = recovery). Period 1 was an 8-d control period where feed intake, BW, daily milk production, and milk composition were measured. During period 1 (i.e., −120 to 0 h relative to nutrient restriction), all cows were fed the same standard control diet and were allowed 3 kg/d of VMS concentrates (Bjerre-Harpøth et al., 2012). During period 2 (0–96 h relative to nutrient restriction; 4 d), dietary intake for all cows was restricted to provide ~40% of requirements recommended by the NRC (2001) for NE_L based on BW, milk production, and milk composition by substituting 60% of the control TMR with wheat straw. This feeding strategy was designed to allow for ad libitum intake and maintain gut fill while limiting nutrient intake. To avoid negative effects due to limited protein supply, the mix was adjusted to maintain a dietary protein content of 12%. To prevent separation of the straw and control TMR, 300 mL of water/kg of feed was added during mixing. During nutrient restriction, cows were allowed only 300 g of concentrates per visit in the VMS, with a maximum intake of 1.0 kg/d. During period 3, cows returned to the control diet and were monitored for an additional 7 d (i.e., recovery period; 97–264 h relative to restriction). Energy balance was calculated according to NRC recommendations, as described by Bjerre-Harpøth et al. (2012).