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Milk fatty acids as possible biomarkers to early diagnose elevated concentrations of blood plasma nonesterified fatty acids in dairy cows

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ABSTRACT

Most cows encounter a state of negative energy balance during the periparturient period, which may lead to metabolic disorders and impaired fertility. The aim of this study was to assess the potential of milk fatty acids as diagnostic tools of detrimental levels of blood plasma nonesterified fatty acids (NEFA), defined as NEFA concentrations beyond 0.6 mmol/L, in a data set of 92 early lactating cows fed a glucogenic or lipogenic diet and subjected to 0-, 30-, or 60-d dry period before parturition. Milk was collected in wk 2, 3, 4, and 8 (n = 368) and blood was sampled weekly from wk 2 to 8 after parturition. Milk was analyzed for milk fatty acids and blood plasma for NEFA. Data were classified as "at risk of detrimental blood plasma NEFA" (NEFA ≥0.6 mmol/L) and "not at risk of detrimental blood plasma NEFA" (NEFA <0.6 mmol/L). Concentrations of 45 milk fatty acids and milk fat C18:1 *cis*-9-to-C15:0 ratio were subjected to a discriminant analysis. Milk fat C18:1 *cis*-9 revealed the most discriminating variable to identify detrimental blood plasma NEFA. A false positive rate of 10% allowed us to diagnose 46% of the detrimental blood plasma NEFA cases based on a milk fat C18:1 *cis*-9 concentration of at least 230 g/kg of milk fatty acids. Additionally, it was assessed whether the milk fat C18:1 *cis*-9 concentrations of wk 2 could be used as an early warning for detrimental blood plasma NEFA risk during the first 8 wk in lactation. Cows with at least 240 g/kg of C18:1 *cis*-9 in milk fat had about 50% chance to encounter blood plasma NEFA values of 0.6 mmol/L or more during the first 8 wk of lactation, with a false positive rate of 11.4%. Profit simulations were based on costs for cows suffering from detrimental blood plasma NEFA, and costs for preventive treatment based on daily dosing of propylene glycol for 3 wk. Given the relatively low incidence rate (8% of all observations), continuous monitoring of milk fatty acids during the first 8 wk of lactation to diagnose detrimental blood plasma NEFA does not seem cost effective. On the contrary, milk fat C18:1 *cis*-9 of the second lactation week could be an early warning of cows at risk of detrimental blood NEFA. In this case, selective treatment may be cost effective.

Key words: milk fatty acid, nonesterified fatty acid, biomarker

INTRODUCTION

Most transition dairy cows are confronted with a negative energy balance (**NEB**) in early lactation caused by 3 primary reasons: increased energy demands at calving, decreased DMI shortly before and after calving, and lagging DMI compared with energy demand for milk production (Ospina et al., 2010a). Additionally, the supply of glucogenic precursors, in particular ruminal propionate which is largely converted into glucose in the liver, is often insufficient (Heuer et al., 2000). Accordingly, milk linear odd-chain FA (i.e., C15:0 and C17:0) might provide information on the cow's glucose status as they are de novo synthesized from propionyl-CoA by rumen bacteria or in the mammary gland (Vlaeminck et al., 2006).

Glucose insufficiency in the transition period further results in low blood glucose and insulin concentrations, inducing body fat mobilization and transportation of NEFA to several organs, among them reproductive tissues and the liver. In the liver, excessive supply of NEFA might result in metabolic disorders such as fatty liver and ketosis (Grummer, 1993), which particularly take place 2 to 7 wk after parturition. Additionally, elevated plasma NEFA concentrations increase the risk for displaced abomasum, clinical ketosis, metritis, and retained placenta (Ospina et al., 2010a). The critical NEFA concentration is about 0.3 mmol/L prepartum and 0.57 mmol/L postpartum (Ospina et al., 2010b),

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whereas a concentration of 1.0 mmol/L or more postcalving has been associated with increased culling rates in the first 2 mo of lactation (LeBlanc et al., 2005; Chapinal et al., 2011; Seifi et al., 2011). Furthermore, a high blood plasma NEFA concentration seems detrimental for dairy cow fertility (Garverick et al., 2013). This has been suggested to originate from deteriorated oocyte quality (Leroy et al., 2005).

Excessive amounts of NEFA, released during body fat mobilization, are also transferred to the milk. As these NEFA are particularly rich in long-chain FA, such as C18:1 *cis*-9 and C18:0 (Hostens et al., 2012), concentrations in milk fat of those FA might be linked to severity of NEB. Therefore, the objective of the current study was to assess whether milk FA are potential biomarkers for detrimental levels of blood plasma NEFA. For this objective, milk and blood plasma sampled during the first 8 wk after parturition were used. Samples were obtained from a large-scale experiment with 92 early lactating cows receiving either a glucogenic or a lipogenic diet in early lactation after a normal (60 d) or shortened (30 d) dry period or without dry period. The objective was approached in 4 steps: (1) measurement of FA in milk and NEFA in blood plasma; (2) discriminant analysis to determine the milk FA with the greatest biomarker potential; (3) assessment of the potential value of these milk FA as diagnostic and early warning biomarkers; and (4) economic validation.

MATERIALS AND METHODS

Experimental Setup, Animals, and Housing

The Institutional Animal Care and Use Committee of Wageningen University and Research Center approved the experimental protocol. Details about the experimental design have been reported earlier (van Knegsel et al., 2014). In short, Holstein-Friesian dairy cows (n = 108) were selected from the Dairy Campus Research dairy herd (Wageningen University and Research Center Livestock Research, Lelystad, the Netherlands) for an experiment on the effect of dry period length (0, 30, and 60 d) and early lactation diet (glucogenic or lipogenic) on metabolic health (assessed through blood plasma BHBA, NEFA, glucose, IGF-1, and insulin). Cows were blocked for parity (primiparous or multiparous), expected calving date, milk production in the previous lactation, and BCS and randomly assigned to treatments (0, 30, or 60 d) and early lactation ration (glucogenic or lipogenic), resulting in a 3 × 2 experimental design. Cows were housed in a freestall with a slatted floor and cubicles. During lactation, cows were milked twice daily (0500 and 1630 h). The drying-off protocol for cows with the 30- and 60-d dry period consisted of a transition to the far-off ration at d 7 before drying off, and milking once daily at d 4 before drying off cows. At drying off, cows were treated with an intramammary antibiotic (Supermastidol, Virbac Animal Health, Barneveld, the Netherlands). Milk yield was recorded daily. From the original 108 cows in the experiment, 3 cows showed health problems postcalving and were not considered further. The health status of the cows was checked regularly by the animal care workers, but no further signs of health problems were observed in the 105 cows. Hence, from 105 cows of the experiment (17 or 18 in each of the 6 treatment groups), milk and blood samples were obtained. Blood samples were taken weekly from wk 3 precalving till wk 8 postcalving. Blood was sampled from the coccygeal vein into heparinized tubes. Blood plasma was obtained by centrifugation for 15 min at 3,000 × *g* at 4°C. Blood plasma samples were kept at −20°C until analysis for plasma metabolites. Milk sampling for FA analysis took place every Friday morning in wk 2, 3, 4, and 8 postcalving. Milk samples were collected in 10-mL tubes and were stored at −20°C. Frozen milk samples were sent to the Laboratory for Animal Nutrition and Animal Product Quality (Faculty of Bioscience Engineering, Ghent University, Belgium). From the 105 cows, 13 cows were excluded because the data were incomplete (missing milk FA of wk 2, 3, 4, and 8 or blood NEFA from wk 2 to 8 of lactation). Hence, only 92 of the 105 cows were used in data analysis.

Prepartum, dry cows received a dry cow ration, lactating cows received a lactating cow ration supporting 25 kg of milk. From 3 wk prepartum until 100 DIM, all cows were fed 1 of the experimental concentrates (lipogenic or glucogenic); lactating cows received 1 kg/d of standard lactation concentrate in the milking parlor. Forage composition consisted prepartum of grass silage, corn silage, wheat straw, and a protein source (rapeseed meal or soybean meal) in a ratio of 39:25:25:11 (DM basis). Postpartum to 100 DIM, forage consisted of grass silage, corn silage, straw, and a protein source (rapeseed meal or soybean meal) in a ratio 51:34:2:13 (DM basis; Tables 1 and 2).

Milk Analysis

The FA profile was obtained after milk fat extraction (mini Röse-Gottlieb method, adapted from Chouinard et al., 1997), methylation (Stefanov et al., 2010), and gas chromatographic analysis of FAME (Agilent Technologies 7890A GC System equipped with a flame ionization detector, Agilent Technologies, Santa Clara, CA). Samples were injected by split injection (split ratio 1:50). The carrier gas was hydrogen, inlet pressure 246.38 kPa. Fatty acid peaks were identified based on