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Grain-based versus alfalfa-based subacute ruminal acidosis induction experiments: Similarities and differences between changes in milk fatty acids

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ABSTRACT

Subacute ruminal acidosis (SARA) is one of the most important metabolic disorders, traditionally characterized by low rumen pH, which might be induced by an increase in the dietary proportion of grains as well as by a reduction of structural fiber. Both approaches were used in earlier published experiments in which SARA was induced by replacing part of the ration by a grain mixture or alfalfa hay by alfalfa pellets. The main differences between both experiments were the presence of blood lipopolysaccharide and *Escherichia coli* and associated effects on the rumen microbial population in the rumen of grain-based induced SARA animals as well as a great amount of quickly fermentable carbohydrates in the grain-based SARA induction experiment. Both induction approaches changed rumen pH although the pH decrease was more substantial in the alfalfa-based SARA induction protocol. The goal of the current analysis was to assess whether both acidosis induction approaches provoked similar shifts in the milk fatty acid (FA) profile. Similar changes of the odd- and branched-chain FA and the C18 biohydrogenation intermediates were observed in the alfalfa-based SARA induction experiment and the grain-based SARA induction experiment, although they were more pronounced in the former. The proportion of *trans*-10 C18:1 in the last week of the alfalfa-based induction experiment was 6 times higher than the proportion measured during the control week. The main difference between both induction experiments under similar rumen pH changes was the decreasing sum of *iso* FA during the grain-based SARA induction experiment whereas the sum of *iso* FA remained stable during the alfalfa-based SARA induction experiment. The cellulolytic bacterial community seemed to be negatively affected by either the presence of *E. coli* and the associated lipopolysaccharide accumulation in the rumen or by the amount of starch and quickly fermentable carbohydrates in the diet. In general, changes in the milk FA profile were related to changes in rumen pH. Nevertheless, feed characteristics (low in structural fiber vs. high in starch) also affected the milk FA profile and, as such, both effects should be taken into account when subacute acidosis occurs.
Key words: milk fatty acid, subacute ruminal acidosis

INTRODUCTION

Subacute ruminal acidosis is one of the most important metabolic disorders in intensive dairy farms and affects rumen fermentation, animal welfare, productivity, and farm profitability (e.g., increased veterinary costs and decreased fertility and productivity; Morgante et al., 2007). Several studies have investigated the etiology and pathophysiology of SARA (Gozho et al., 2005; Krause and Oetzel, 2005; Dohme et al., 2008). The main SARA induction protocol used in these studies relied on increasing the amount of quickly fermentable carbohydrates through increasing dietary proportions of grain. However, SARA can also be provoked by an insufficient amount of physically effective fiber in the diet (Mertens, 1997; Kleen et al., 2009). An experiment in which SARA was induced by the reduction of physically effective fiber was adopted by Khafipour et al. (2009b), in which alfalfa hay was gradually replaced by pellets consisting of ground alfalfa hay. Differences in blood parameters and rumen microbial population between the grain-induced and the alfalfa-induced SARA experiments have been described by Khafipour et al. (2009a,b,c). The main metabolic differences between the two protocols were the presence of blood LPS and higher numbers of *Escherichia coli* in the rumen of animals suffering from SARA in the grain-based SARA induction experiment. The lowest rumen pH levels were recorded in the alfalfa-induced acidotic animals, but disease-associated blood parameters, such as blood LPS, were not elevated in the latter situation.

Recent research has focused on the identification of biomarkers in milk for the detection of SARA. Milk fat depression has been associated with a decrease in rumen pH and the milk FA profile also showed potential as *trans* C18:1 FA and odd- and branched-chain

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FA (**OBCFA**) were related to SARA (Enjalbert et al., 2008; Colman et al., 2010). As the concentration in milk fat of these milk fatty acids depends on the ruminal microbial population, which differed between both acidosis induction experiments (Khafipour et al., 2009a, 2009b, 2009c), the goal of the current analysis was to assess whether these different microbial populations were associated with differences in the milk fatty acid profile.

MATERIALS AND METHODS

Sample Collection

The current study is based on milk samples collected during 2 different acidosis induction experiments, which were described by Khafipour et al. (2009a) and Khafipour et al. (2009b). Some of the data reported in the earlier publications were reanalyzed. Additionally, milk FA analyses were carried out. As the aim was to relate rumen parameters and milk FA, only the 4 rumen-fistulated animals were used in this manuscript, whereas data from the other 4 non-rumen-fistulated cows were discarded. This might result in a slight deviation from some of the data reported previously. Data from wk 5 and 6 of the grain-based SARA induction study were used for further analysis. In addition, rumen pH parameters of 1 d were linked to the pooled milk sample of that same day and not to the previously reported average values. A brief description of each experimental protocol is provided below.

Experiment 1. Four Holstein cows were used during 2 consecutive 6-wk periods. During wk 1 to 5 of each 6-wk period, cows received a TMR ad libitum with a forage-to-concentrate ratio of 50:50 (wt/wt on a DM basis). During wk 6 of both periods, a SARA challenge was conducted by replacing 21% of the DM of the TMR with pellets containing 50% ground wheat and 50% ground barley, resulting in a forage-to-concentrate ratio of 40:60 (wt/wt on a DM basis). Rumen pH was monitored continuously for 4 consecutive days during wk 5 and 6 of both experiments using indwelling pH probes. Cows were milked twice daily and milk samples were collected during 4 consecutive milkings during both sampling weeks. No preservative was used and milk samples were immediately frozen.

Experiment 2. Four Holstein cows were used in a 6-wk study. During wk 1, cows received a TMR that contained 50% of DM as concentrate and 50% of DM as chopped alfalfa hay. From wk 2 to 6, alfalfa hay was gradually replaced by alfalfa pellets at a rate of 8% per week to induce SARA. Rumen pH was monitored continuously for 5 consecutive days during each week of the experiment using indwelling pH probes. Cows were milked twice daily and milk samples were collected during 4 consecutive milkings during each sampling week. No preservative was used and milk samples were immediately frozen.

Sample Measurements

Milk Analysis. Milk samples of the evening and the morning after the pH registration day were sampled and pooled by volume for further analysis. In the first experiment, milk from 2 d in wk 5 (control) and wk 6 (SARA) of both periods was analyzed (n = 32). In the second experiment, milk was sampled weekly on 2 d (n = 48). Milk samples were stored at −20°C until being analyzed for FA composition. Milk FA were quantified by GC after extraction (Chouinard et al., 1997) and methylation (Stefanov et al., 2010) and were expressed as grams per 100 g of FA methyl esters. Tridecanoic acid (as triacylglyceride; Sigma, Bornem, Belgium) was added as internal standard to assess the accuracy of the chromatograms.

GC Analysis. Analysis of the FA was done by GC (HP 7890A; Agilent Technologies Belgium NV, Diegem, Belgium) equipped with a 75-m SP-2560 capillary column (i.d.: 0.18 mm; film thickness: 0.14 μm; Supelco Analytical, Bellefonte, PA) and a flame ionization detector. A combination of 2 oven temperature programs was used in this study to achieve separation of most *cis* and *trans* C16:1 and C18:1 isomers according to the method of Kramer et al. (2008) with modifications. A first temperature program was as follows: at the time of sample injection, the column temperature was 70°C for 2 min, which was then increased at 15°C/min to 150°C, followed by a second increase of 1°C/min to 165°C, which was maintained for 12 min, followed by a third increase at 2°C/min to 170°C, which was maintained for 5 min, and a final increase at 5°C/min to 215°C, which was maintained for 10 min. A second temperature program was used to separate most of the coeluting isomers: at the time of sample injection, the column temperature was 70°C, which was then increased at 50°C/min to 175°C and maintained isothermal for 13 min, followed by a second increase at 5°C/min to 215°C, which was maintained for 10 min. For both programs, inlet and detector temperatures were 250 and 255°C, respectively. The split ratio was 100:1. The flow rate for the hydrogen carrier gas was 1 mL/min. Most FA peaks were identified using quantitative mixtures of methyl ester standards (BR2 and BR3, Larodan Fine Chemicals AB, Malmö, Sweden; Supelco 37, Supelco Analytical; PUFA-3, Matreya LLC, Pleasant Gap, PA). Fatty acids for which no standards were available commercially were identified by order of elution according to Precht et al. (2001) and Kramer et al. (2008).