

Original Research Paper

# The Effects of Subacute Ruminal Acidosis on Milk Fatty Acid Profile in Dairy Cattle

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**Abstract:** The objective of this experiment was to examine the effect of Subacute Ruminal Acidosis (SARA) on milk fatty acid (FA) profiles with emphasis on odd-chain and branched-chain FA. Sixteen multiparous Holstein dairy cows (618±35 kg of body weight, 221±32 days in milk) were used in a randomized complete-block design. During week 1, all cows received a high-forage diet (90% chopped grass hay, 5% grain mix, 5% protein supplement, dry-basis; HF). During weeks 2 through 4, cows either continued receiving the HF diet or received a low-fiber diet (38% chopped grass hay, 57% grain mix, 5% protein supplement, dry-basis; LF). Milk yield and dry matter intake were recorded daily, milk samples were collected twice weekly and ruminal pH was continuously recorded during the last 48 h of each week. Statistical analysis was conducted on weekly measuring Proc Mixed of SAS accounting for repeated measurement. The model included the fixed effects of diet, week, their interaction and block. Helmert contrasts were used to describe the difference between treatments over time. The dietary treatment induced a significant difference over time between treatments (diet by week 1 Vs. average week 2, 3 and 4;  $p < 0.05$ ). The treatment reduced ( $p < 0.05$ ) milk fat concentration ( $\text{g}100\text{g}^{-1}$ ) of branched-chain FA (including 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso and 17:0 iso), 18:0, trans-11 18:1, cis-9, trans-11 conjugated linoleic acid (CLA), 18:3 n-3, 20:0, cis-11 20:1, 20:4 n-3, 22:0 and 24:0. The treatment increased ( $p < 0.05$ ) milk fat concentration ( $\text{g}100\text{g}^{-1}$ ) of odd-chain FA (including 11:0 and 13:0), 12:0, 13:0 antiso, cis-9 14:1, cis-12 18:1, cis-13 18:1, trans-6-8 18:1, trans-10 18:1 and trans-12 18:1. The treatment also increased ( $p < 0.05$ ) the concentration of cis-9, cis-12 18:2; trans-9, cis-11 CLA; trans-10, cis-12 CLA; and 20:4n-6. The results indicate that SARA is associated with a shift in odd-chain and branched-chain FA, which can be utilized to diagnose a cow with SARA.

**Keywords:** Subacute Ruminal Acidosis, Milk Fatty Acid, Odd-Chain, Branched-Chain

## Introduction

Subacute Ruminal Acidosis (SARA) is a common ruminal digestive disorder in dairy cattle, which is caused by feeding rapidly fermentable carbohydrates (Plaizier *et al.*, 2009). Typically, SARA is characterized by a pH depression below 5.6 for approximately 3-5 h  $\text{day}^{-1}$  (AlZahal *et al.*, 2007). The disease is capable of affecting feed intake; rumen microflora and digestion; milk production and can cause inflammation (Plaizier *et al.*, 2009). Lactating

dairy cattle rely on a diet rich in rapidly fermentable carbohydrates in order to meet their high dietary energy needs and to increase production efficiency (Steele *et al.*, 2012).

In the United States studies have reported that approximately 19% of early lactation cows and 26% of mid lactation dairy cows have had SARA (Garrett *et al.*, 1997). It has been estimated that the economic cost of SARA is around \$1.12 per cow per day (Stone, 1999), which could add up to 1 billion dollars annually (Donovan, 1997). The economic

losses associated with SARA, include reduced milk production, decreased milk efficiency and increase death loss (Enemark, 2009). Thus early diagnosis and treatment of SARA could prevent economic losses (Enemark, 2009).

SARA does not often exhibit clear and overt clinical symptoms (Plaizier *et al.*, 2009). The most common clinical signs of SARA are reduced or erratic feed intake; which are not unique to SARA and symptoms vary in each case, therefore SARA is difficult to detect (Plaizier *et al.*, 2009). Currently, rumen pH parameters are used to diagnose SARA using stomach tubing or rumenocentesis, however these methods are expensive and invasive (Plaizier *et al.*, 2009).

A new method to diagnose SARA has surfaced, in which milk Fatty Acids (FA) profiles are monitored in pooled milk samples (Fievez *et al.*, 2012). Milk fat contains about 2 to 3% Odd- and Branched-Chain Fatty Acids (OBCFA) rising from microbial FA (Patel *et al.*, 2013). OBCFA profiles in milk can be potential markers for duodenal flow of microbes. The FA composition in cow milk can be altered through feeding (Patel *et al.*, 2013), for example changing the forage proportion are known to alter FA profiles in milk (Chilliard *et al.*, 2007); therefore OBCFAs could be used as a noninvasive tool to predict ruminal acidosis.

In an experiment conducted by Colman *et al.* (2010), diagnosis of acidosis was based on pH measurements; and OBCFA profiles within the milk (Colman *et al.*, 2010). This experiment demonstrated the potential of using milk FA profiles in diagnosing acidosis, however a general classification model is still required (Fievez *et al.*, 2012). Two other studies attempted to develop a discriminate function based on milk FA profiles (Van Nespen *et al.*, 2015; Colman *et al.*, 2010). Both studies identified C18:1 trans-10 and CLA cis-9 trans-11 as main discriminating factors; whereas the other FA may vary based on the microbiome (Van Nespen *et al.*, 2005; Colman *et al.*, 2010). Although the use of milk FA profiles is still limited to experimental treatments, it still shows some potential as a method to diagnose SARA (Fievez *et al.*, 2012). The objective of this study was to investigate the effect of SARA on milk FA profile, with an emphasis on branched- and odd-chain FA.

## Materials and Methods

The experimental procedures were detailed previously in Steele *et al.* (2012). Briefly, sixteen multiparous Holstein dairy cows (618±35 kg of BW, 221±32 DIM) were used in a randomized complete-block design. The cows were grouped by stage of lactation into 2 blocks (n = 8 each) and the cows within each block were randomly assigned into 1 of 2 dietary

treatments. During week 0 (baseline), all cows received high-forage diets (90% chopped grass hay, 5% grain mix, 5% protein supplement, dry basis; HF). The chemical composition (%) of the HF diet contained 88.2 DM, 16.2 CP, 31.8 ADF, 46.9 NDF, 2.2 EE, 10.6 Ash and 27.9 NFC and 7.4 starch (Steele *et al.*, 2012). During weeks 1 through 3, cows either continued receiving the HF diet or received a low-fibre diet (38% chopped grass hay, 57% grain mix, 5% protein supplement, % DM; LF; Steele *et al.* (2012)). The chemical composition (%) of the LF diet contained 88.2 DM, 14.5 CP, 17.6 ADF, 32.3 NDF, 2.5 EE, 7.1 Ash, 46.0 NFC and 32.1 starch (Steele *et al.*, 2012).

Milk yield and DMI were recorded daily; milk samples were collected twice weekly and ruminal pH was continuously recorded during the last 48 h of each week (AlZahal *et al.*, 2007). Milk samples were collected from each milking for the last 2 d of every experiment week and analyzed for milk composition at the CanWest DHI Milk Laboratory (Guelph, Ontario, Canada) using infrared spectroscopy (Milkoscan 400; Foss Electric, Hillerød, Denmark). Milk samples for FA analysis were collected during week 0 and week 3; and frozen immediately at -20°C until analysis. Lipids for FA analysis in this study, were extracted as explained by Or-Rashid *et al.* (2009).

Statistical analysis of FA profile was conducted using Proc Mixed of SAS. The model included the fixed effects of the week (week 0 and 3), block and their interaction (p<0.05).

## Results and Discussion

The goal of this study was to examine the effects that SARA had on milk FA profiles with an emphasis on OBCFA. To achieve this goal, cows were fed a LF diet, which contained 57% grain mix and 38% chopped grass hay. Cows receiving LF were on average 9 h/d below pH 5.6 indicating severe SARA (Steele *et al.*, 2012). LF diet compared with the HF diet had lowered DMI (p<0.01) by 1.7±0.31 kg/d; and decreased milk fat percentage (p<0.01) (Steele *et al.*, 2012). The increase in energy density of the diet led to higher milk yield (17.4±0.5 Vs. 23.4±0.9 kg/d); and higher protein yields (559±16 Vs. 782±23 g/d) (Steele *et al.*, 2012).

In agreement with ruminal pH, total Short Chain FA (SCFA) concentrations were greater (p<0.01) in HF compared to LF (Steele *et al.*, 2012). The concentrations of all SCFA were greater in HC cattle throughout the experiment, except for acetate and iso butyrate (Steele *et al.*, 2012). Ruminal butyrate displayed the largest proportional increase (p<0.01) due to treatment (6.1±1.6 mM) (Steele *et al.*, 2012).

The treatment reduced (p<0.05) milk fat concentration (g/100g) of branched chain FA (including

14:0 iso, 15:0 iso, 16:0 iso and 17:0 iso; Fig. 1), 15:0 anteiso, 18:0, 18:3 n-3, 20:0 cis-11, 20:4 n-3, 22:0 and 24:0. The reduced branched chain FAs is likely due to a decline in the forage: Concentrate ratio. Higher concentrations of branched chain FA in milk are typically a result of high forage diets, due to a larger population of cellulolytic bacteria that can produce acetate (Patel *et al.*, 2013). Acetate can be elongated into branched-chain FAs, which will eventually be deposited into the milk (Patel *et al.*, 2013). In SARA, there are more amylolytic bacteria in the rumen, which produce propionate, leading to less branched-chain FAs being produced (Fievez *et al.*, 2012). Therefore, the

decreased branched-chain FAs in the milk, is likely a result of an acidic rumen changing the microbiome. Shingfield *et al.* (2005) conducted a study comparing two levels of concentrate with oil supplement on a diet based on grass silage. The study discovered that there was a higher concentration of iso C17:0 and iso C:14 with a higher amount of forage in the diet (Shingfield *et al.*, 2005). Vlaeminck *et al.* (2006) found similar results where iso C15:0 increased in proportion to grass silage. Another study by Patel *et al.* (2013) also discovered that augmented proportions of grass silage in the diet increased the branched-chain FAs iso C15:0 and iso C17:0.

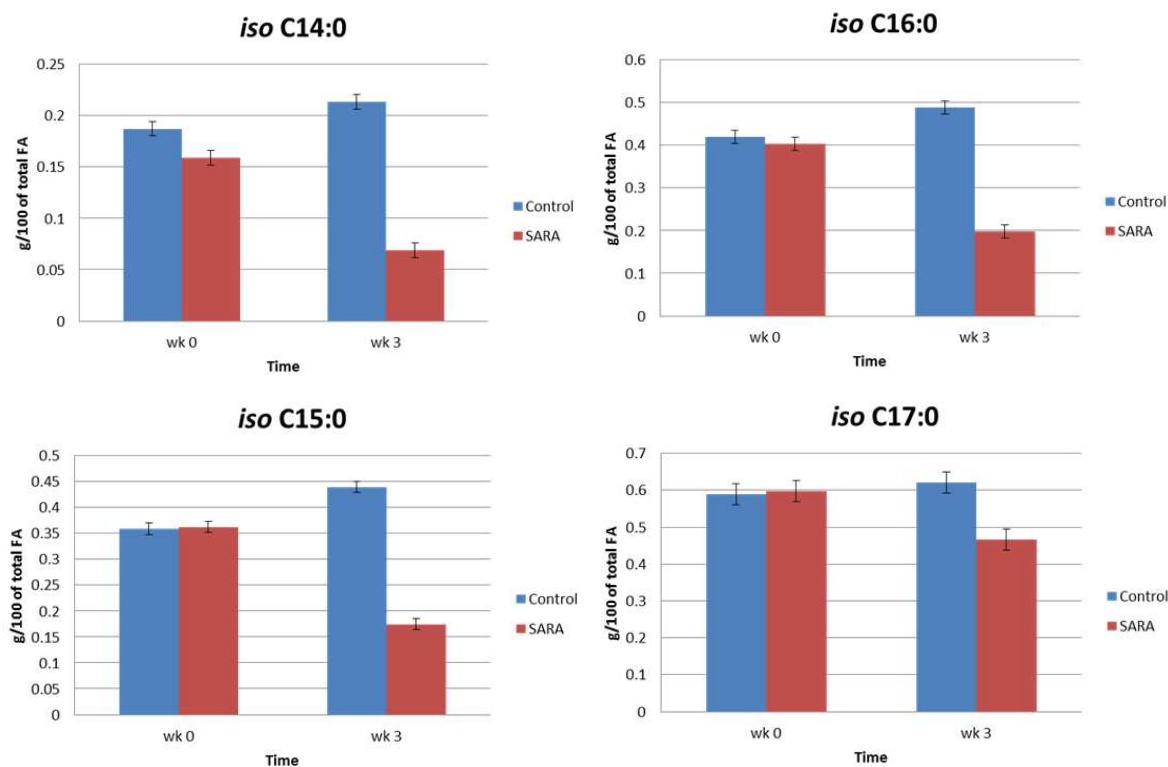


Fig. 1. The treatment reduced ( $p < 0.05$ ) milk concentration of branched chain FA iso C15:0, iso C16:0, iso C14:0 and iso C17:0 as seen in figure a-d

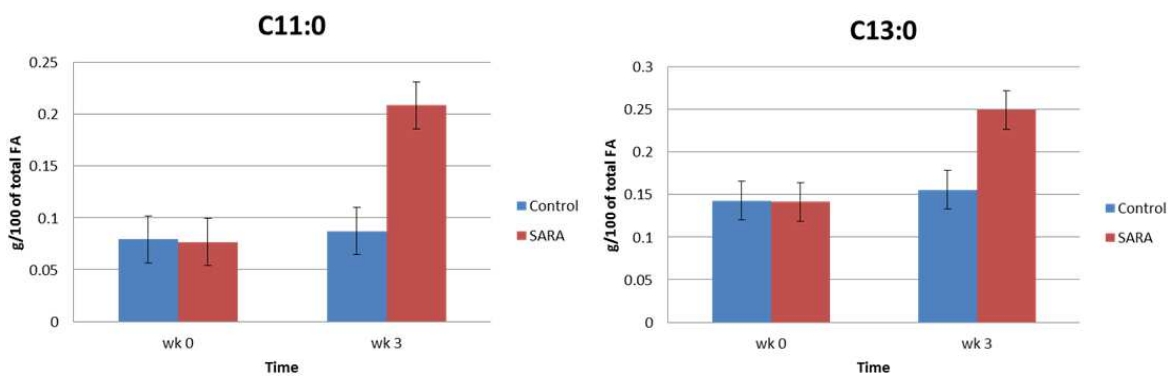


Fig. 2. The treatment increased ( $p < 0.05$ ) milk fat concentration of odd-chain FA C11:0 and C13:0 as seen in figure a-b

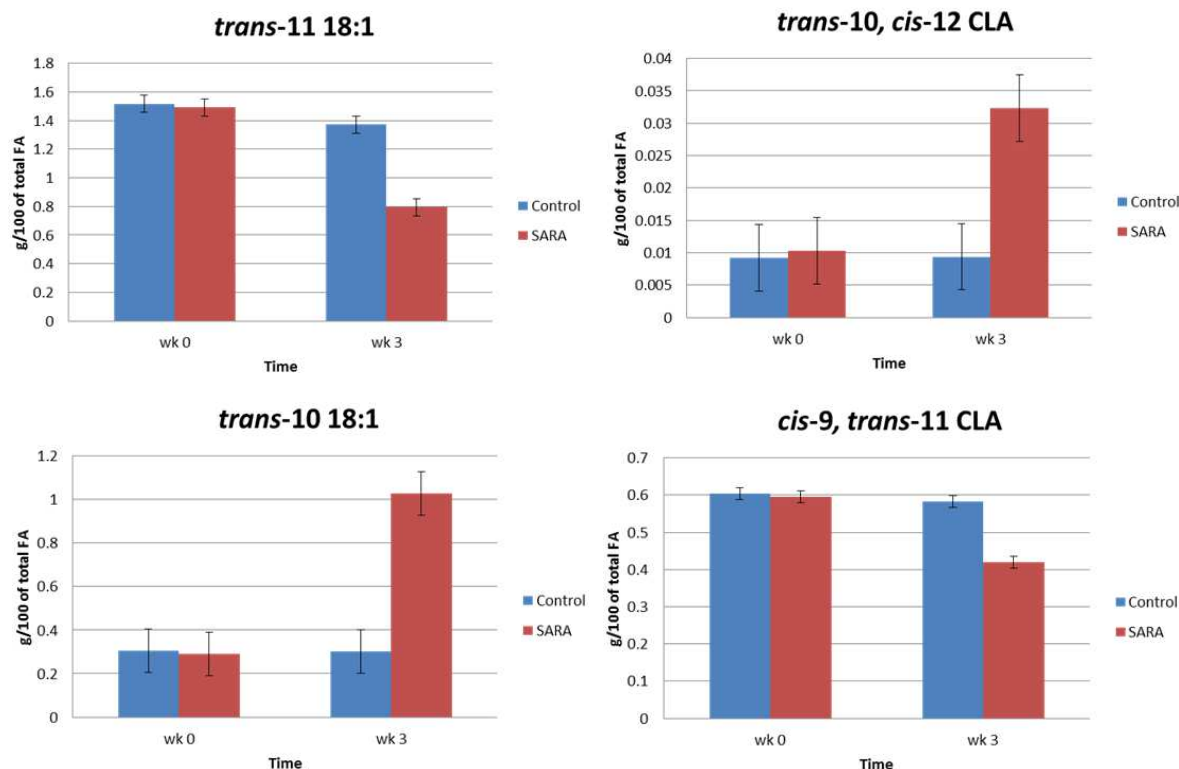


Fig. 3. The LF diet decreased *trans*-11 18:1 (a) and *cis*-1, *trans*-11 CLA (b); and increased *trans*-10 18:1 (c) and *trans*-10, *cis*-12 CLA (d)

The treatment increased ( $p < 0.05$ ) milk fat concentration (g/100g) of odd chain FA (including 11:0 and 13:0; Fig. 2), 12:0, 13:0 antiso, *cis*-9 14:1, *cis*-12 18:1, *cis*-13 18:1, *trans* 6-8 18:1, *trans*-12 18:1, *cis*-9, *cis*-12 18:2 and 20:4 n-6 (Fig. 2). In this study, they were correlated with a decline in milk fat from 3.89% or 0.76 kg/d to 3.25 or 0.73 (Steele *et al.*, 2012). The milk FA profile, likely reflects the decrease concentrate: Forage ratio. During SARA, amyolytic bacteria, which are typically at low levels in the rumen, begin to thrive as the pH begins to drop (Fievez *et al.*, 2012). Amyolytic bacteria contain low levels of branched chain FA, however they are also enriched with linear-odd chain FA and anteiso branched chain FA (Fievez *et al.*, 2012). Odd-chain FAs are formed through elongation of propionate, which is produced by the increased amyolytic bacteria (Fievez *et al.*, 2012). Colman *et al.* (2010) demonstrated that odd-chain FAs, such as C15:0 and C17:0, increased with a more concentrated diet, mirroring our results. AlZahal *et al.* (2009), found that cows receiving lower forage diets also had greater milk concentrations of odd chain FA like C11:0 and C13:0. Furthermore, another study by Craninx *et al.* (2008), demonstrated that cows with acidosis have more odd-chain FA in their milk compared to branched-chain FA, mimicking patterns similar to our experiment.

The treatment reduced *trans*-11 18:1 and *cis*-9, *trans*-11 CLA; and increased *trans*-10 18:1; *trans*-10, *cis*-12 CLA and *trans*-9, *cis*-11 CLA (Fig. 3). In this study the *trans*-10 isomers were associated with a decline in milk fat (%) from 3.89 to 3.25 (Steele *et al.*, 2012). During acidosis, these FA are a result of intermediates from a secondary biohydrogenation pathway of C18:2 n-6 (Fuentes *et al.*, 2009). An increase in ruminally derived *trans*-10 isomer is closely linked with milk fat depression, (Bauman and Griinari, 2003). *Trans*-10 C18:1 reacts to dietary factors and its concentrations have been negatively associated with milk fat yield in cows (Bernard *et al.*, 2008). This could explain why our SARA induced cows experienced a decrease in milk fat production.

A study by Griinari *et al.* (1998) observed an increase in *trans* octadecenoic acids and *trans*-10 isomers of *trans*-octadecenoic acid, resulting in milk fat depression. Therefore, they supported the theory that low rumen pH, resulting from feeding low fiber diets, results in the incomplete biohydrogenation of FA (Griinari *et al.*, 1998). AlZahal *et al.* (2010) also demonstrated that cows being fed moderate forage: Concentrate had a greater increase in *trans*-10 FA and *trans*-9, *cis*-11 conjugated linoleic acid than high forage: Concentrate. Additionally, the levels of the isomers were also related to the polyunsaturated FA levels (AlZahal *et al.*, 2010). Baumgard *et al.* (2000)

detected a relationship in milk fat depression and increases in trans-10 cis-12 and C18:1 trans-10. In addition Fuentes *et al.* (2009), also demonstrated that a pH of 5.6 decreased the trans-11 C18:1 and cis-9, trans-11 CLA; and increased the concentration of trans-10 C18:1, trans-10 cis 15 C18:2 and trans-10, cis-12 CLA. These studies indicate that the biohydrogenation intermediated FA in milk could be indicators the presence of SARA, as they are closely linked to rumen pH.

## Conclusion

In conclusion, our study demonstrated that SARA was associated with shifts in OBCFAs concentration in milk, due likely to shifts in the rumen microbiome. These shifts could be used to detect SARA, by pooling milk samples and avoiding invasive tests. Currently, the use of milk FA profile in detecting SARA is limited to experimental treatment, but there is potential for clinical application.

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## Author's Contributions

**Courtney Mitchell:** Contributed to data interpretation and writing of the manuscript.

**Ousama AlZahal:** Conducted experiment, analyzed data, and contributed to writing of manuscript.

**Mamun Or-Rashid:** Conducted FA analysis and contributed to data interpretation.

**Michael Alexander Steele:** Conducted experiment and reviewed manuscript.

**Brian William McBride:** The principal investigator of the study.

## Ethics

This study was approved by the Animal Care Committee at the University of Guelph, under the guidelines of the Canadian Council for Animal Care.

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