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Effect of replacing maize silage with red clover silage in the diet on milk fatty acid composition in cows

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ABSTRACT

This study aimed to evaluate the effect of replacing maize silage plus soybean meal with red clover silage (RCS) plus wheat on the fatty acid (FA) profile in the milk fat of cows. Forty-four lactating German Holstein cows were used in a 4 × 4 Latin square design with 21-d periods composed of 13 d of adaptation to diets followed by an 8-d sampling phase. Experimental diets offered as total mixed ration consisted of a constant forage-to-concentrate ratio (75:25) with target proportions of RCS to maize silage of 15:60 (RCS_{15}), 30:45 (RCS_{30}), 45:30 (RCS_{45}), and 60:15 (RCS_{60}) on a dry matter basis. Increasing the level of RCS in the diet was accompanied by a reduction of linoleic acid content in the diet and decreased linearly the proportions of linoleic acid in the milk up to 4%. Proportions of α-linolenic acid in milk increased 2-fold with RCS_{60} compared with RCS_{15}, which resulted from the linear increase in α-linolenic acid intake with incremental levels of RCS. Vaccenic acid in the milk fat was reduced by 24%. Rumenic acid, a conjugated linoleic acid (*cis*-9,*trans*-11 conjugated linoleic acid) considered to be a human health promoter, was also decreased by 22%. Reduced rumenic acid in the milk fat was probably due to a reduced amount of vaccenic acid produced in the rumen and, consequently, to the low amount of vaccenic acid to be desaturated to rumenic acid in the mammary gland by Δ^9-desaturase. Oleic acid was enriched in the milk fat, although the dietary concentration of oleic acid decreased. Stearic acid proportions remained constant with increasing levels of RCS. The proportions of total polyunsaturated FA were increased by 12%, and the long-chain FA proportions increased linearly with increasing levels of RCS. Myristic acid was reduced linearly, but palmitic acid remained constant. Saturated FA was reduced linearly by 2%. Branched-chain FA, which are presumed to possess anticarcinogenic properties, were reduced to a small extent only (quadratic effect). We conclude that replacing maize silage with RCS appears to alter milk FA composition by reducing linoleic acid intake and ruminal biohydrogenation. Feeding RCS represents a strategy to increase intake of α-linolenic acid in dairy cows. However, because changes in the FA profile show positive as well as negative effects, no distinct conclusions can be drawn with regard to human health benefits.

Key words: red clover, biohydrogenation, polyunsaturated fatty acid, fatty acid profile

INTRODUCTION

Cow milk fat is the most variable milk component, in concentration and composition (Palmquist, 2006), and consists predominantly of triglycerides (Bauman and Griinari, 2003). The fatty acids (**FA**) in milk fat are either synthesized de novo in the mammary gland or taken up from the blood; FA in the blood originate largely from the diet and partially from microbial lipid metabolism in the rumen as well as from mobilized body fat reserves (Bauman and Griinari, 2003). Although diets of dairy cows are generally high in UFA, milk fat consists mainly of SFA, which is partly due to extensive microbial biohydrogenation (**BH**) of dietary UFA in the rumen (Shingfield et al., 2010). From a human health point of view, it would be desirable to decrease the SFA content, especially concentrations of medium-chain SFA, and to increase the PUFA content in milk fat (Lock and Bauman, 2004; Dewhurst et al., 2006). The PUFA of particular interest are linoleic acid (**LA**, *cis*-9,*cis*-12 C18:2), α-linolenic acid (**α-LNA**, *cis*-9,*cis*-12,*cis*-15 C18:3), and rumenic acid (**RA**, *cis*-9,*trans*-11 C18:2) that represents the primary isomer of CLA in milk.

Because BH of PUFA in the rumen decisively influences the composition of milk fat, reducing to a certain extent the BH in the rumen or increasing the supply of PUFA to improve the amount that escape BH represent

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alternative approaches to enhance the nutritional quality of milk fat of cows for human diets. Red clover (*Trifolium pratense* L.) is one of the most important forage legumes for silage production in northern Europe and North America (Moorby et al., 2016). Several studies have shown enhanced concentrations of total PUFA, LA, and α-LNA in milk fat of cows when feeding red clover silage (**RCS**) compared with grass silage (Dewhurst et al., 2003a; Moorby et al., 2009; Halmemies-Beauchet-Filleau et al., 2014). This effect is generally linked to the polyphenol oxidase (**PPO**) activity present in red clover (Van Ranst et al., 2011; Lee, 2014). Because of a reduction in lipolysis and subsequent BH in the rumen, a higher escape of dietary PUFA from the rumen may occur when feeding RCS (Halmemies-Beauchet-Filleau et al., 2013). However, RCS is characterized by relatively high-CP and low-energy contents. Maize silage (**MZS**) is a significant source of rapidly fermentable carbohydrates, has low CP content, and, therefore, is a good complement to RCS. In line with this, some studies demonstrated improvements in feed intake and milk production when feeding RCS–MZS mixtures compared with RCS as the sole forage source (Broderick et al., 2001; Hoffman and Bauman, 2003). In contrast, Schulz et al. (2018) found reduced DMI and milk yield when replacing MZS with RCS in diets of cows. However, to our knowledge, no published study so far exists that examined the effect of feeding different dietary ratios of RCS to MZS on the milk FA profile of cows. Red clover silage provides a source of homegrown protein, MZS is highly important as a forage on dairy farms, and consumer awareness of the potential health benefits of some milk FA is increasing; therefore, an evaluation of the effects of different proportions of RCS and MZS on FA profile of cow milk is required.

The aim of the present study was to evaluate the effect of replacing MZS with RCS in the diet on milk FA composition of cows, with special focus on the FA related to ruminal BH and promoters of human health: LA, α-LNA, RA, vaccenic acid (**VA**, *trans*-11 C18:1), stearic acid (**SA**, C18:0), myristic acid (**MA**, C14:0), and oleic acid (**OA**, *cis*-9 C18:1). We hypothesized that increasing levels of RCS in diets of cows would improve the quality of milk FA profile.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The study reported herein was performed in accordance with the German Animal Welfare Act (Federal Republic of Germany, 2014) and approved by the Animal Welfare Commission of the Ministry of Energy, Agriculture, the Environment and Rural Areas of the federal state of Schleswig-Holstein, Germany (V 242–72241.123–5). The feeding experiment was carried out with 44 German Holstein cows at the experimental farm Schädtbek of the Max Rubner-Institute (Dobersdorf, Germany). Cows were randomly assigned to 4 groups according to milk yield, DIM, lactation number, and BW, averaging (mean ± SD) 38.7 ± 7.3 kg/d, 149 ± 103 d, 1.9 ± 1.1, and 624 ± 52 kg at the start of the experiment, respectively.

Red clover ('Harmonie') and maize (*Zea mays* 'Amagrano,' 'LG 30.222,' and 'Saludo') were grown as pure stands. Detailed information about the preparation of RCS and MZS are described by Schulz et al. (2018). The 4 experimental diets offered as TMR consisted of a constant forage-to-concentrate ratio (75:25 on a DM basis) with targeted proportions of RCS to MZS in diet DM of 15:60 (**RCS_{15}**), 30:45 (**RCS_{30}**), 45:30 (**RCS_{45}**), and 60:15 (**RCS_{60}**). All diets contained (on a DM basis) on average 8.8% ground lupine seeds and 16% soybean meal plus ground wheat in different ratios to obtain isonitrogenous diets. The proportion of lupine seeds was similar across diets to avoid an effect on the milk FA profile due to its relatively high content of ether extract. Ingredients and chemical composition of the 4 experimental diets are presented in Table 1. Diets were offered once daily at approximately 0600 h for ad libitum consumption. Cows were kept in a freestall barn equipped with cubicles bedded with chopped straw and had free access to water. Cows were housed and fed per group (4 groups of 11 cows each). Animal:feeding place ratio and animal:cubicle ratio were both 1:1. The design of the experiment was a 4 × 4 Latin square. Each experimental period comprised 21 d with a 13-d adaption phase followed by an 8-d-sampling phase.

Sampling, Recordings, and Chemical Analyses

Diets. Details on diet sampling and analysis of DM, crude ash, ether extract, CP, starch, sugar, NDF, ADF, and ADL concentrations are described by Schulz et al. (2018). Fatty acid compositions of diets were analyzed according to the official analytical methods in Germany (DGF, 2015) by LUFA-ITL GmbH (Kiel, Germany). Fatty acid methyl esters were obtained by transesterification (method C-VI 11a) without prior fat extraction. For this purpose 1 to 2 g of feed sample and 4 mL of a 0.5-mol/L solution of potassium hydroxide in methanol were mixed in a 50-mL round-bottom flask and heated under reflux to the boiling point. After addition of 5 mL of a solution of boron trifluoride (wt = 12–15 g/100 g) in methanol, the sample was boiled for 3 min. After cooling to room temperature, 5 mL of isooctane