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Effects of forage type, forage to concentrate ratio, and crushed linseed supplementation on milk fatty acid profile in lactating dairy cows

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ABSTRACT

The effects of an increasing proportion of crushed linseed (CL) in combination with varying forage type (grass or corn silage) and forage to concentrate ratio (F:C), and their interactions on milk fatty acid (FA) profile of high-producing dairy cows was studied using a 3-factor Box-Behnken design. Sixteen Holstein and 20 Swedish Red cows were blocked according to breed, parity, and milk yield, and randomly assigned to 4 groups. Groups were fed different treatment diets formulated from combinations of the 3 main factors each containing 3 levels. Forage type (fraction of total forage dry matter, DM) included 20, 50, and 80% grass silage, with the remainder being corn silage. The F:C (DM basis) were 35:65, 50:50, and 65:35, and CL was supplied at 1, 3, and 5% of diet DM. Starch and neutral detergent fiber content (DM basis) of the treatment diets ranged from 117 to 209 g/kg and 311 to 388 g/kg, respectively. Thirteen treatment diets were formulated according to the Box-Behnken design. During 4 experimental periods of 21 d each, all treatment diets were fed, including a repetition of the center point treatment (50% grass silage, 50:50 F:C, 3% CL) during every period. Intake, production performance, and milk FA profile were measured, and response surface equations were derived for these variables. Shifting from 80% grass silage to 80% corn silage in the diet linearly increased dry matter intake (DMI), net energy for lactation (NE_L) intake, *cis*-9,*cis*-12-C18:2 (C18:2n-6) intake, and milk yield, and linearly decreased *cis*-9,*cis*-12,*cis*-15-C18:3 (C18:3n-3) intake and milk fat content. Shifting from a high forage to a high concentrate diet linearly increased DMI, NE_L intake, C18:2n-6 intake, and milk yield, and decreased milk fat content. Supplementation of CL linearly increased C18:3n-3 intake, but had no effect on DMI, NE_L intake, milk yield, or milk fat content. Shifting from 80% grass silage to 80% corn silage linearly increased proportions of *trans*-10-C18:1 and C18:2n-6 in milk fat, whereas the proportions of *trans*-11,*cis*-15-C18:2 and C18:3n-3 linearly decreased. Significant interactions between CL supplementation and F:C were found for proportions of *trans*-10-C18:1, *trans*-15-C18:1, *cis*-15-C18:1, *trans*-11,*cis*-15-C18:2, and C18:3n-3 in milk fat, with the highest levels achieved when the diet contained 5% CL and a 35:65 F:C ratio. The effect of supplementing CL on several milk FA proportions, including C18:2n-6 and C18:3n-3, depends significantly on the F:C ratio and forage type in the basal diet.
Key words: linseed, grass silage, forage to concentrate ratio, milk fatty acid

INTRODUCTION

Because of its relatively large proportion of saturated fatty acids (**FA**), dairy milk fat has been associated with human cardiovascular health problems (Bauman and Lock, 2010; Elwood et al., 2010). On the contrary, monounsaturated FA such as oleic acid (*cis*-9-C18:1), long-chain n-3 FA, and conjugated linoleic acid in milk fat have been associated with potential benefits for human health (Bauman and Lock, 2010). Because of these effects of milk FA profile on human health, the manipulation of milk FA profile has been the subject of extensive research in recent years. The FA profile of milk fat is largely dependent on FA intake and FA metabolism in the rumen (Jenkins et al., 2008), and on lipid mobilization and FA metabolism in the mammary gland (Chilliard et al., 2007). Dietary FA are extensively metabolized and hydrogenated in the rumen, resulting in a wide range of ruminal biohydrogenation intermediates (Chilliard et al., 2007). Ruminal biohydrogenation of *cis*-9,*cis*-12-C18:2 (C18:2n-6) and *cis*-9,*cis*-12,*cis*-15-C18:3 (C18:3n-3) results in the secretion of various *trans*-C18:1, *cis*-C18:1, and nonconjugated

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and conjugated C18:2 and C18:3 isomers in milk fat. Chilliard et al. (2007) reported that the main factor in the variation of biohydrogenation is the forage to concentrate ratio (**F:C**) in the diet. After adding linseed oil to a high concentrate diet, the major biohydrogenation intermediates secreted in milk fat were *trans*-11-C18:1, *trans*-13+14-C18:1, *cis*-9,*trans*-13-C18:2, and *trans*-11,*cis*-15-C18:2 (Loor et al., 2005), whereas *trans*-15-C18:1 and *cis*-15-C18:1 were increased in duodenal flow (Loor et al., 2004). Compared with grass silage, corn silage inclusion in a diet supplemented with fish oil and sunflower oil resulted in higher proportions of *trans*-C18:1 and lower proportions of C18:0 and *trans*-C18:2 in milk fat (Shingfield et al., 2005). Basal diet appears to have a profound effect on ruminal metabolism of FA from supplemental fat sources (Shingfield et al., 2005; Soita et al., 2005), which might be related to shifts in rumen pH and microbial populations. Feeding a high starch diet markedly affects the ratio of cellulolytic to propionogenic, lactogenic, and amylolytic bacteria, which in turn affects ruminal biohydrogenation (Latham et al., 1972; Loor et al., 2004). Thus, interactions between level of lipid supplementation and other dietary changes are likely to occur.

Few direct comparisons exist between the different characteristics of the basal diet, such as type of forage and F:C, and lipid supplements. In addition, a large diversity of diets exists and quantifying interactions is important. To our knowledge, the effects of adding crushed linseed (**CL**) to diets that vary in F:C and in the proportion of grass silage to corn silage and their interactions on milk FA profile within a single experiment have not been reported. Designing an experiment in which multiple factors are considered simultaneously allows quantification of the curvature in relationships as well as interactions among factors (St-Pierre and Weiss, 2009). The Box-Behnken design (Box and Behnken, 1960) is a multifactor experimental model specifically designed for the exploration of response surfaces and it involves a smaller number of experimental points compared with a full-factorial design. The objective of this study was therefore to evaluate the effects of CL supplementation, varying forage type and F:C, and their mutual interactions, on intake, production performance, and milk FA profile. The study was carried out by varying grass silage at the expense of corn silage, F:C ratio, and level of CL supplementation in a 3-factor multivariate Box-Behnken design.

MATERIALS AND METHODS

Experimental Design and Diets

The experimental design was a 3-factor Box-Behnken design with forage type (grass silage or corn silage), F:C, and proportion of CL supplementation as the main factors. Forage type included 20, 50, and 80% grass silage (DM basis), with the remainder being corn silage. Forage to concentrate ratio was 35:65, 50:50, and 65:35 (DM basis) and CL was supplied at 1, 3, and 5% of diet (DM basis). Thirteen treatment diets with varying levels of grass silage, corn silage, F:C, and CL were formulated according to the Box-Behnken design, including the center point treatment (50% grass silage, 50:50 F:C, and 3% CL). The experiment consisted of 4 experimental periods of 21 d each, with 4 treatments evaluated, including the center point treatment, during each period (Table 1). To formulate the treatment diets, 3 commercial concentrate mixtures were used and the treatment diets were balanced for CP content. Contents of starch and NDF were allowed to differ for the different treatment diets because of the varying forage type and F:C ratio. Starch and NDF content (DM basis) in the treatment diets ranged from 117 to 209 g/kg and 311 to 388 g/kg, respectively. The treatment diets met or exceeded the requirements for NE_L (Dutch NE_L system; Van Es, 1975) and intestinal digestible protein (**DVE**; Tamminga et al., 1994). All treatment diets were offered as TMR diets. The CL was obtained from Vegolia (Falkenberg, Sweden). The specified ingredient and chemical composition of the diets are shown in Tables 2 and 3, respectively. Increasing the grass silage percentage mainly decreased starch and C18:2n-6 contents, whereas C18:3n-3 content increased (Table 3). Increasing the forage proportion mainly increased NDF and forage NDF contents, whereas starch, NE_L, DVE, C12:0, C14:0, C16:0, *cis*-9-C18:1, C18:2n-6, and C18:3n-3 contents decreased. Increasing the CL proportion mainly increased C18:3n-3 content in the diets.

Animals and Housing

The experiment was approved and carried out under the Swedish Law on Animal Experimentation. Sixteen Holstein and 20 Swedish Red cows (620 ± 50 kg of BW; 2.1 ± 0.9 parity; 72 ± 17 DIM; 48.1 ± 5.3 kg/d milk; values expressed as means ± SD) were blocked according to breed, parity, and milk yield, and randomly assigned to 4 groups. Groups were fed the different treatment diets during the 4 experimental periods. Cows were housed in freestalls with slatted floors and boxes bedded daily with sawdust on top of rubber mattresses. Individual feed intake was continuously monitored using automated feed bins with weighing equipment (BioControl A/S, Rakkestad, Norway). Each group of 9 cows had ad libitum access to 5 automated feed bins. Cows were fitted with transponders to enable individual feed intake recording from the automated feed bins. Weight changes of the bins (accuracy 0.1 kg) were