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Short communication

Milk fat response to calcium salts of palm or soybean in a normal or milk fat depression scenario in dairy ewes

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ABSTRACT

A limited number of studies in lactating sheep have compared milk fat responses to calcium salts (Ca-salts) differing in fatty acid (FA) composition and their interactions with different dietary conditions. The objective of this study was to evaluate the effects of Ca-salts of palm or soybean FA on milk fat under Normal and CLA-induced milk fat depression (MFD) scenarios in lactating dairy ewes. Thirty-eight Lacaune and thirty-seven East Friesian multiparous ewes were used in a 2 × 2 factorial design. The main factor consisted of two diets designed to create two distinct scenarios: Normal and CLA-induced MFD (CLA-MFD, 30 g/d of CLA 29.9% *trans*−10, *cis*−12 CLA as methyl ester). The subfactor was supplementation of Normal or CLA-MFD scenarios with 27 g/d of Ca-salts of palm FA, or 30 g/d of Ca-salts of soybean FA, resulting in four treatments: 1) Normal + Ca-Palm, 2) Normal + Ca-Soy, 3) CLA-MFD + Ca-Palm, and 4) CLA-MFD + Ca-Soy. Overall, the CLA-MFD scenario decreased milk fat concentration by 1.64% units and decreased milk fat yield by 17.3 g/d. Ewes that received Ca-Palm had overall 0.29% units higher milk fat concentration, and 13.4% greater milk fat concentration when fed the CLA-MFD diet. Ca-Soy increased *trans*−10 C18:1 (131.0%), *trans*-11 C18:1 (30.4%), and *cis*-9, *trans*-11 CLA (21.1%) in the CLA-MFD diet. In conclusion, supplementation with Ca-Palm resulted in overall greater milk fat concentration. In a MFD scenario, supplementation with Ca-Soy increased concentration of milk *trans* FA linked to altered rumen biohydrogenation and further aggravated MFD.

1. Introduction

In addition to supplying dietary energy to dairy ruminants, the fatty acids (FA) present in fat supplements can also modify milk fat composition (Chilliard et al., 2007). In dairy ewes, the effect of Ca-salts of FA on milk yield and composition has not been consistent across studies and included findings such as increased milk fat and decreased protein concentration (Gargouri et al., 2006; Castro et al., 2009). This variation is highly related to the FA composition of the Ca-salts, which in turn, affects its degree of protection against rumen metabolism as showed by Sukhija and Palmquist (1990) that at a given rumen pH, Ca-salts rich in polyunsaturated FA (PUFA) were extensively dissociated compared to Ca-salts rich in saturated FA.

The *trans*-10, *cis*−12 CLA is a potent inhibitor of milk fat synthesis in lactating dairy ewes when used in a rumen-protected (Lock et al., 2006) or unprotected (Oliveira et al., 2012) supplement causing milk fat depression (MFD) described as CLA-induced MFD.

Under grazing conditions, lactating ruminants are normally fed during milking or immediately after with some type of concentrate that may contain Ca-salts of FA. Feeding large amounts of concentrate or cereal grains separately from forages can increase variations in rumen reducing pH and supplying PUFA represents a risk for diet-induced MFD (Jenkins, 2011). Due to the great economic value of milk fat for the dairy industry, milk fat depression represents a major obstacle for the implementation of some feeding strategies that include supplemental fat (Rico and Harvatine, 2013).

Limited research has evaluated the milk fat response to Ca-salts differing in FA composition and their interaction with MFD, particularly in dairy ewes. Therefore, our objective was to evaluate the effects of Ca-salts of palm or soybean FA on milk fat under normal and MFD scenarios in grazing dairy ewes supplemented with concentrated after milking. Because Ca-salts of soybean FA are rich in PUFA and are not completely inert in the rumen, we hypothesized that a mild diet-induced MFD may occur under the normal scenario when Ca-salts of

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soybean are fed. Under a CLA induced-MFD scenario, we hypothesized that CA-salts of soybean FA would aggravate MFD. Because CLA-induced MFD is a well-established model, we believed that feeding grazing dairy ewes with a supplement containing *trans* − 10, *cis* − 12 CLA could be used to create the MFD scenario.

2. Material and methods

2.1. Animals, treatments and experimental design

Santa Catarina State University Research Committee approved all management and animal care procedures. Thirty-eight Lacaune and thirty-seven East Friesian multiparous ewes weighing 62 ± 1.3 kg, averaging 70 ± 7 DIM, and producing 1.4 ± 0.1 kg of milk were balanced according milk production and parity to a 2 × 2 factorial design. The main factor was two diets designed to create two distinct scenarios: Normal and CLA-induced MFD (CLA-MFD). The Normal diet was grazed pasture supplemented with a concentrate (basal diet), whereas the CLA-MFD was basal + 30 g/d of rumen-unprotected CLA (29.9% *trans* − 10, *cis* − 12 CLA as methyl ester, Luta-CLA 60, BASF AG, SP, Brazil). The subfactor was supplementation of Normal or CLA-MFD diets with 27 g/d of Ca-salts of palm FA (Ca-Palm, 46% C16:0, Agelac 84, Raupp Nutraceutic Feeds, Chapeco, SC, Brazil), or 30 g/d of Ca-salts of soybean FA (Ca-Soy, 35.3% *cis* − 9, *cis* − 12 C18:2, Megalac-E, Church & Dwight Co., Nova Ponte, MG, Brazil), resulting in four treatments: 1) Normal + 27 g/d Ca-Palm (n = 20), 2) Normal + 30 g/d Ca-Soy (n = 17), 3) CLA-MFD + 27 g/d Ca-Palm (n = 19), and 4) CLA-MFD + 30 g/d Ca-Soy (n = 19). The Ca-salts of FA were selected to represent a supplement rich in palmitic acid (Ca-Palm) and a supplement rich in PUFA (Ca-Soy), and fed at a rate that provided equal amount of total FA (Supplemental Table 1). The CLA oil and Ca-salts were mixed with the concentrate at the time of concentrate feeding.

All ewes grazed six paddocks (0.5 ha/each) of Aruana guinea grass (*Panicum maximum* Jacq. cv. Aruana), with an average DM forage mass of 2840 ± 108 kg/hectare and a grazing cycle of 3d of grazing and 17d of resting. The concentrate (56% corn, 40% soybean meal, 4% vitamin/mineral mix) was formulated using the Small Ruminant Nutrient System (Tedeschi et al., 2010) to add to the estimated nutrients provided by the pasture (Supplemental Table 2). The complete diet was expected to meet the nutritional requirements according to the NRC Small Ruminants (2007). The ewes were mechanically milked twice daily (0630 and 1630 h) and after each milking maintained in an enclosed barn and individually fed 500 g of a concentrate containing the above mentioned treatments. Consumption of the concentrate was always complete for all animals and the ewes returned to pasture after the concentrate feeding. The experiment lasted 8 weeks, with the first one serving as an adaptation and the following six weeks for evaluation. During the last week, all animals were switched to the Normal + Ca-Palm treatment to evaluate recovery from eventual MFD.

2.2. Samples and analysis

Samples of pasture and concentrate were composite weekly and analyzed according to AOAC (2000) for DM (method 934.01; 95–100 °C) and CP (method 988.05; Kjeldahl). Concentrations of NDF, ADF, and lignin were determined according to Van Soest et al. (1991), with NDF being corrected for ash content. Milk production was recorded daily and averaged by week, sampled with preservative (bronopol tablet; D & F Control Systems Inc., San Ramon, CA) at both milkings every 2d, composite based on yield at each milking and analyzed for fat, protein, lactose, and total solids (AOAC, 2000; method 972.160) and SCC by flow cytometry. Additional milk samples were collected on d 14, 28, and 42 of the treatment period and stored at −20 °C without preservative for analysis of FA. All procedures used for milk fat extraction and FA analysis in milk fat, Ca-salts and CLA were previously described by Baldin et al. (2013).

2.3. Statistical analysis

Data were analyzed as a 2 × 2 factorial design using PROC MIXED of SAS (SAS 2009). The effect of animal was considered random in all models. Difference between breeds was not of interest and the two breeds (Lacaune or East Friesian) were balanced among treatments. Milk production and milk components were analyzed as repeated measures. The initial model assessed the fixed effect of diet (control or CLA-MFD), Ca-salt (Ca-Palm or Ca-Soy), time (day 0, 7, 14, 21, 28, 35, 42, 49), and their two- and three-way interactions. No interactions between diet and Ca-salt and time were observed, therefore the 3-way interaction was removed from the model. The heterogeneous first-order autoregressive (ARH(1)) or autoregressive (AR(1)) variance-(co) variance matrix was used depending on model convergence. Measurements of milk yield and composition at the end of the adaptation period (day 0) were used as covariate in the model. Data points with studentized residuals outside of ± 3.0 were considered outliers and excluded from analysis. Milk FA were analyzed as single observation collected on d14. This model contained the fixed effect of diet (Normal or CLA-MFD), Ca-salt (Ca-Palm or Ca-Soy), and their interaction. Pre-planned contrasts tested the effect of Ca-salts (Ca-Palm or Ca-Soy), within scenario (Normal or CLA-MFD), on the overall mean or individual time points for repeated measures. Statistical significance was declared at $P < 0.05$ and tendency at $P < 0.10$.

3. Results

The interactions of time with diet and Ca-salts have not been presented in tables but instead they were listed and discussed whenever relevant. Laboratorial issues precluded the analysis of FA in milk samples collected on d28 and 42; and therefore, only results from samples collected on d14 are presented.

Table 1
Response of milk production and composition to calcium salts of palm (Ca-Palm) or soybean (Ca-Soy) during normal or conjugated linoleic acid induced milk fat depression (CLA).

	Normal		CLA-MFD			*P*-value		
	Ca-Palm	Ca-Soy	Ca-Palm	Ca-Soy	SEM[a]	Diet	Ca-salt	Diet*Ca-salt
Milk yield, kg/d	1.39	1.35	1.42	1.48	0.05	0.09	0.82	0.20
Fat, %	6.47	6.23	4.88	4.54†	0.11	< 0.001	0.01	0.67
Fat yield, g/d	84.4	83.1	64.8	68.0	2.19	< 0.001	0.68	0.30
Protein, %	5.05*	4.87	4.80	4.90	0.06	0.06	0.48	0.02
Protein yield, g/d	67.5	65.9	66.0†	70.2	1.72	0.42	0.44	0.09
Lactose, %	4.79	4.79	4.74	4.77	0.02	0.13	0.44	0.45
Lactose yield, g/d	66.2	64.3	66.6	68.9	2.16	0.25	0.92	0.32

* Contrast Ca-Palm vs Ca-Soy whithin Control or CLA groups differ at: * = $P < 0.05$, † = $P < 0.1$.
[a] Pooled standard error of the mean.