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Replacing cereals with dehydrated citrus pulp in a soybean oil supplemented diet increases vaccenic and rumenic acids in ewe milk

José Santos-Silva,*† Maria T. Dentinho,* Alexandra Francisco,*† Ana P. Portugal,* Ana T. Belo,* António P. L. Martins,‡ Susana P. Alves,†§ and Rui J. B. Bessa†§[1]
*Unidade Estratégica de Investigação e Serviços em Produção e Saúde Animal–Instituto Nacional de Investigação Agrária e Veterinária (UEISPSA-INIAV), Vale de Santarém, Portugal
†CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Avenida da Universidade Técnica 1300-477 Lisboa, Portugal
‡Unidade Estratégica de Investigação e Serviços em Tecnologia e Segurança Alimentar–Instituto Nacional de Investigação Agrária e Veterinária (UEISPA-INIAV), Lisboa, Portugal
§Faculdade de Medicina Veterinária, ULisboa, Avenida da Universidade Técnica 1300-477 Lisboa, Portugal

ABSTRACT

This study evaluates the effect of the replacement of cereals by dried citrus pulp (DCP) in diets supplemented with 5% of soybean oil, on ewe milk yield and composition, including milk fatty acid (FA). Four Serra da Estrela multiparous ewes in the second month of lactation were used in a double 2 × 2 Latin square design. Ewes were individually penned and milked twice a day with an 8-h interval. Each experimental period included 14 d of diet adaptation followed by 5 d of measurements and sampling. The 2 diets included on dry matter basis 450 g/kg of corn silage and 550 g/kg of either a soybean oil-supplemented concentrate meal containing barley and maize (cereal) or dried citrus pulp (DCP; citrus). Feed was offered ad libitum, considering 10% of orts, and intake was measured daily. Milk yield was higher and dry matter intake tended to be higher with the citrus diet. Milk composition and technological properties for cheese production were not affected by treatments, except for lactose, which was lower with the citrus diet. Replacement of cereals by DCP resulted in a 3-percentage-point decrease of both 18:0 and *cis*-9–18:1 that were mostly compensated by the 4.19- and 1.68-percentage-point increases of *trans*-11–18:1 and *cis*-9,*trans*-11–18:2, respectively. The intake of C18 FA tended to increase with the citrus diet compared with the cereal diet, but the apparent transfer of 18:2n-6 and of 18:3n-3 did not differ between diets. The milk output of C18 FA increased with the citrus compared with the cereal diet, mostly due to the increase of *trans*-11–18:1 and *cis*-9,*trans*-11–18:2 because the daily milk output of 18:0, *trans*-10–18:1, *cis*-9–18:1, 18:2n-6 and 18:3n-3 did not differ between diets. Replacing cereals with DCP in an oil-supplemented diet resulted in a selective increase of *trans*-11–18:1 and *cis*-9,*trans*-11–18:2 in milk, with no major effect on other biohydrogenation intermediates.

Key words: dairy ewe, dried citrus pulp, milk fatty acid, biohydrogenation intermediates, soybean oil

INTRODUCTION

Rumen lipid metabolism, particularly the biohydrogenation of PUFA, is a major determinant of the fatty acid (**FA**) profile of ruminant milk. Conjugated linoleic acid increase, particularly rumenic acid (*cis*-9,*trans*-11–18:2) and its precursor vaccenic acid (*trans*-11–18:1), is one of the main objectives for improving the nutritional value of milk fat (Pintus et al., 2013; Shingfield et al., 2013; Nudda et al., 2014). This goal can be achieved by supplementing ruminant diets with vegetable oils rich in PUFA (Shingfield et al., 2013) among other strategies. However, the nature of the carbohydrates present in these diets influence the rumen biohydrogenation pathways and thus the content of *trans*-11–18:1 and *cis*-9,*trans*-11–18:2 in products. Diets rich in starch and PUFA, particularly 18:2n-6, are frequently associated with changes in the dominant biohydrogenation pathways, markedly increasing the concentration of *trans*-10–18:1 in fat from ruminant products (*trans*-10 shift; Aldai et al., 2013; Bessa et al., 2015). In dairy cows, large increases in *trans*-10–18:1 are often accompanied by a decrease in *trans*-11–18:1 and *cis*-9,*trans*-11–18:2 and reduction of milk fat concentration (Shingfield and Griinari, 2007). Dairy ewes seem less susceptible to the *trans*-10 shift, although the general pattern is also recognizable (Toral et al., 2010; Gómez-Cortés et al., 2011a,b).

Feeds rich in pectic substances and sugars, such as dried citrus pulp (**DCP**), can be an alternative to cereals favoring a higher acetic/propionic ratio in rumen,

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[1]Corresponding author: rjbbessa@fmv.ulisboa.pt

and a higher rumen pH with a small or null effect on productivity (Bampidis and Robinson, 2006). Reports on the effect of dietary replacement of starch by DCP on biohydrogenation-derived FA in milk are limited to dairy cows (Solomon et al., 2000; Elfert et al., 2006; Cabrita et al., 2007). Studies on dairy ewes involving the dietary replacement of starch sources by DCP or other citrus products are scarce (Fegeros et al., 1995; Castrillo et al., 2004; Jaramillo et al., 2009; Shdaifat et al., 2013) and do not report a detailed milk FA analysis. Furthermore, no reports are available about the effects of replacing cereals by DCP in oil-supplemented diets for dairy ewes.

We hypothesized that replacement of cereal by DCP, in a diet providing a large supply of 18:2n-6, would favor the ruminal *trans*-11 biohydrogenation pathway to the detriment of the *trans*-10 shifted pathway, resulting in higher *cis*-9,*trans*-11–18:2 and *trans*-11–18:1 content in milk. Therefore, we conducted a trial to evaluate the effect of the replacement of cereals by DCP in diets supplemented with soybean oil, on the FA composition of ewe milk, particularly on *trans* octadecenoates and *cis*-9,*trans*-11–18:2.

Table 1. Composition of the experimental diets

Item	Diet	
	Cereal	Citrus
Ingredient, g/kg of DM		
Maize silage	450	450
Dried citrus pulp	0	240
Barley	190	0
Maize	70	0
Soybean meal, 48%	215	235
Soybean oil	50	50
Sodium bicarbonate	5	5
Calcium carbonate	13	13
Salt	4	4
Minerals and vitamins	3	3
Chemical composition, g/kg of DM		
DM	505	497
Ash	60	71
CP	156	167
Ether extract	76	76
NDF	278	300
ADF	154	184
Sugar	42	190
Starch	377	170
Fatty acid, g/100 g of fatty acids		
16:0	14.7	13.7
18:0	4.0	4.1
cis-9–18:1	23.8	23.6
18:2n-6	51.2	51.6
18:3n-3	6.2	7.0

MATERIALS AND METHODS

Animals, Diets, and Sampling

Four multiparous Serra da Estrela ewes from the flock of the Polo de Investigação of Fonte Boa, with an average parity of 4.8 ± 1.26 (±SD) and in the second month of lactation (49 ± 7.6 d after lambing), were used. After weaning at 35 d in milk, ewes were machine milked twice daily at 0900 and 1600 h. The trial occurred between March and April (2014). The ewes were housed in 3.1-m^2 individual pens, with permanent access to water. The experimental design consisted of a double 2 × 2 Latin square, with 2 diets and 2 sequential experimental periods of 19 d (14 d of adaptation and 5 d of data and sample collection). The animal handling followed European Union Directive 2010/63/UE concerning animal care.

The diets contained 450 g/kg of corn silage on a DM basis, supplemented with 550 g/kg of either a concentrate meal containing barley and maize or DCP as the main energy source. Both experimental concentrates included 50 g/kg DM of soybean oil to supply plenty of 18:2n-6 as a substrate for rumen biohydrogenation pathways. The concentrate feeds were produced by the Feed Compounds for Animal Unit of Polo de Investigação of Fonte Boa. The ingredients and chemical composition of the diets are presented in Table 1. Dried citrus pulp pellets were prepared using orange juice residues, following a conventional industrial process that includes pressing with addition of calcium oxide, dehydration of extracted liquor, and its incorporation into DCP (Citricos del Andevalo, S.A., Huelva, Spain). The corn silage and the meal fractions of the diets were manually mixed every day, immediately before distribution to the ewes.

Ewes were weighed at the beginning and at the end of the experimental periods. Feed was offered individually ad libitum, considering 10% orts, and intake was controlled daily, weighing the amounts of feed offered and refused. Feed samples were collected on a daily basis for chemical analysis to obtain a composite sample of the diets in each period.

Milk production was registered at every milking. Milk samples were collected daily as 10% of the total volume, obtaining 2 composite samples corresponding to d 15, 16, and 17, and to d 18 and 19 of the experimental periods. Sodium azide solution (0.8%, wt/vol) was used as preservative (0.05 g/L), and milk samples were kept refrigerated before processing for composition, technological properties, and fatty acid analysis.

Chemical Analysis

Feeds were analyzed for DM content (ISO 6496; ISO, 1999), ashes (ISO 5984; ISO, 2002), CP (ISO 5983; ISO, 1997), and ether extract (ISO 6492; ISO, 1999). Neutral detergent fiber, assayed with sodium sulfite,