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Influence of dietary grape pomace combined with linseed oil on fatty acid profile and milk composition

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ABSTRACT

Grape pomace is a by-product resulting from the winery industry that is rich in phenolic compounds. It could play a role as an antioxidant and, owing to its high fiber concentration, it would be an alternative ingredient to partially replace forage in the diet of small ruminants. The objective of this study was to evaluate the effect of dietary supplementation of vitamin E or different doses of grape pomace associated with linseed oil on milk fatty acid profile, composition, and yield. Forty-eight Churra ewes were fed with experimental diets consisting of a total mixed ration (TMR) containing 2.7% [on a dry matter (DM) basis] of linseed oil, forage, and concentrate at a 40:60 ratio. Ewes were assigned to 1 of 4 treatments: control (without grape pomace), vitamin E (with 500 mg/kg of TMR of vitamin E), grape pomace-5 (5 g/100 g of TMR of DM of grape pomace), and grape pomace-10 (10 g/100 g of TMR of DM of grape pomace). Experimental diets did not affect DM intake and milk yield and composition. The vitamin E supplementation had only a moderate effect on milk concentration of fatty acids (increase in α-linolenic acid and 16:0 and decrease in *cis*-9 18:1). Grape pomace supplementation did not affect the percentages of total saturated, monounsaturated, and polyunsaturated fatty acids. Levels of α-linolenic acid reached about 1% of total fatty acids as a consequence of the presence of linseed oil in the diets, were not modified with vitamin E, and remained unaltered in grape pomace-5 and -10 treatments. Linoleic acid was increased by the highest dose of grape pomace, but this ingredient did not modify the *cis*-9,*trans*-11 18:2 milk fat content. The concentration of total odd- and branched-chain fatty acids did not diminish in grape pomace-5 and pomace-10 treatments. The presence of grape residue did not modified the *trans*-11 18:1 and *trans*-10 18:1 contents, which might indicate that, under the conditions assayed, this winery by-product would not alter the pathways of rumen conversion of dietary unsaturated fatty acids.

Key words: grape pomace, fatty acid, milk, linseed oil, ewe

INTRODUCTION

It is well established that dietary ingredients have a noticeable ability to modify the fatty acid profile of ruminant milk fat, especially supplementation with a lipid source (Shingfield et al., 2013; Nudda et al., 2014). Compared with results from a control diet, the inclusion of 3 different unsaturated plant oils (linseed, sunflower, and olive) results in the highest contents of MUFA, CLA, and PUFA, and the lowest proportion of SFA (Bodas et al., 2010). The best option of these 3 oils for improving milk fat fatty acid composition from a nutritional point of view was linseed oil, because it allows for the highest n-3 fatty acid content as well as the lowest atherogenicity index and n-6-to-n-3 PUFA ratio (Bodas et al., 2010; Martínez Marín et al., 2011).

Increasing the content of unsaturated fatty acids in milk could also increase susceptibility to oxidation. Therefore the addition of antioxidants to PUFA-supplemented diets to improve milk quality seems to be an advisable practice. Vitamin E is the major antioxidant used to supplement ruminant diets (Atwal et al., 1990; Zened et al., 2012; Casamassima et al., 2014). It does not modify milk yield, milk fat, or protein percentage, causes only moderate effects on milk fatty acid concentration (Kay et al., 2005; Ferlay et al., 2010), and can prevent milk fat depression in ruminants fed with high doses of PUFA-supplemented diets (Pottier et al., 2006). The antioxidant effect of synthetic vitamin E could be replaced by natural antioxidants, such as plant polyphenols from fruits, which might induce an effect on PUFA rumen biohydrogenation (**BH**) and, consequently, on the milk fatty acid profile.

Grape pomace is a by-product resulting from the winery industry that is costly to dispose of. Seeds and

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skins of crushed grapes are very rich in phenolic compounds (Yi et al., 2009) and therefore could play an important role as antioxidants. Furthermore, because of its low cost and high fiber concentration, grape residue could be an alternative feed ingredient to partially replace the forage portion in the diet of ruminants. Grape pomace has traditionally been used in the Mediterranean area as an alternative feed for sheep during periods of scarce feed supplies. Furthermore, Santos et al. (2014) reported that grape residue silage improved antioxidant activity in milk, Moate et al. (2014) found that ensiled grape marc reduced methane emissions by approximately 20%, and Nudda et al. (2015) suggested that grape seed might have an immunomodulatory effect on dairy ewes.

The ruminal bacteria of dairy cows were altered by dietary supplementation with grape marc (Moate et al., 2014), and the rumen metabolism of lactating dairy ewes was influenced by dietary supplementation with grape seed, alone or mixed with linseed (Correddu et al., 2015). However, evaluation of the effect of grape residue as a feed ingredient for lactating ruminants on the detailed milk fatty acid profile, including fatty acids derived from the ruminal BH process, has received less attention (Ferlay et al., 2010). The aim of our study was to determine the effect of dietary inclusion of vitamin E or different doses of grape pomace mixed with linseed oil on dairy performance and milk fatty acid profile during the first month of lactation.

MATERIALS AND METHODS

Animals and Dietary Treatments

Forty-eight pregnant Churra ewes (mean ± SD; BW = 64.3 ± 0.92 kg) were selected 2 wk before lambing and were fed the same control diet they would receive during the experimental period without oil added (Table 1). The ewes, aged 3 to 5 yr (4.1 ± 0.61), whose parity ranged from 4 to 6 (4.9 ± 0.91), all gave birth 3 to 4 d before starting the experiment. According to a continuous experimental design, after lambing, each ewe was housed in an individual tiestall with its respective newborn lamb and randomly assigned to 1 of 4 treatments (12 ewes per treatment) based on their milk production, age, initial BW, and parity. The newborn lambs (12 per treatment) were housed with their respective mothers and were fed exclusively by suckling throughout the whole experimental period. The trial lasted for 4 wk and all animal handling practices followed the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for experimental and other scientific purposes. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Valladolid.

The experimental diets consisted of a TMR containing 2.7% (on a DM basis) of linseed oil and forage and concentrate at a 40:60 ratio. The 4 dietary treatments were control (**CTRL**; without grape pomace), vitamin E (**VIT-E**; with 500 mg/kg of TMR of vitamin E), 5 g of grape pomace (**GP-5**; 5 g/100 g of TMR of DM of grape pomace), and 10 g of grape pomace (**GP-10**; 10 g/100 g of TMR of DM of grape pomace). The grape pomace (*Vitis vinifera* sp.) was collected from 3 wineries of red wine belonging to the Ribera de Duero designation of origin (Valladolid, Spain) and vitamin E (α-tocopherol acetate) was obtained from Inatega S.L. (León, Spain). Diets were formulated to meet the energy and protein requirements of the dairy ewes using INRA (2007) and FEDNA (2010). The composition of the experimental diets and the ingredients are given in Tables 1 and 2, respectively. The chemical composition was determined by using the procedures described by the AOAC International (2003). Dried feed samples were analyzed for NDF, ADF, and ADL using filter bag equipment (Ankom Technology Corp., Fairport, NY). Total mixed ration was supplied twice a day and fresh water was always available. The ewes were fed individually during the whole experimental period and each intake was recorded. The amounts of diet offered and refusals were weighed daily for each ewe, and samples were collected for subsequent analyses. Milk yield and composition were recorded weekly during the first month of lactation.

Milk Sampling and Composition

During the suckling period, as is common for Churra sheep, ewes were machine-milked once a day at 0900 h in a 2 × 24 low-line Casse system milking parlor (Alfa-Laval Iberia, S.A., Madrid, Spain) with 12 milking units and 2 milkers during the entire experimental period. The milking machine (Alfa-Laval Iberia, S.A., Madrid, Spain) was set to provide 180 pulsations per minute in a 50:50 ratio with a vacuum level of 36 kPa.

Once a week, individual ewe milk production was recorded and samples were taken in milk collection jars. For this purpose, milk production was recorded by the oxytocin technique: in the morning, before milking, each ewe was injected with 0.35 mL of oxytocin (Oxiton, Laboratorios Ovejeros, S.A., Spain) and then immediately milked. Ewes were returned to their paddock for 6 h while the lambs were confined and were then milked again for milk sampling. One subsample of milk was kept at 4°C until analyzed for fat and pro-