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Effects of linseed oil and natural or synthetic vitamin E supplementation in lactating ewes' diets on meat fatty acid profile and lipid oxidation from their milk fed lambs

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ABSTRACT

Forty-eight Churra ewes with their new-born lambs were separated into four dietary treatments: Control (without added fat), LO (with 3% linseed oil), LO-Syn E (LO plus 400 mg/kg TMR of synthetic vitamin E) and LO-Nat E (LO plus 400 g/kg TMR of natural vitamin E). Linseed oil caused an increase in *trans*-11 C18:1 (VA), *trans*-10 C18:1, *cis*-9, *trans*-11 C18:2 (RA), *trans*-10, cis-12 C18:2 and C18:3 n-3 (ALA) in milk fat compared to the Control. The addition of vitamin E to the LO diets did not influence significantly the majority of milk fatty acids compared with the LO diet alone. *Trans*-10 C18:1, VA, RA, *trans*-10, *cis*-12 C18:2 and LA levels were higher in intramuscular lamb fat from treatments with linseed oil. No statistically significant differences were observed in these FA due to vitamin E supplementation or the type of vitamin E (synthetic vs. natural). Vitamin supplementation resulted in lipid oxidation levels below the threshold values for detection of rancidity in lamb meat.

1. Introduction

In recent years, there has been a growing interest in identifying strategies to enhance the concentration of healthy fatty acids in ruminant foods (meat and milk), such as conjugated linoleic acid (CLA) and n-3 polyunsaturated fatty acids (PUFAs). Till now the dietary inclusion of PUFA-rich lipids has been the most commonly investigated nutritional strategy (Raes, De Smet, & Demeyer, 2004; Wood et al., 2008).

Current research in European Mediterranean regions has been focused on improving the fatty acid profile of suckling lamb meat, owing to its importance as a traditionally consumed food in that area. Suckling lambs, covered by a protected geographical indication (PGI), are reared with their dams, fed exclusively on maternal milk and slaughtered after a suckling period of 30–35 days. As suckling lambs are considered to be functional nonruminants, maternal milk enrichment with health-promoting FAs by supplementing ewe diets with fat from appropriate sources could be a good strategy for naturally enhancing the levels of these FA in suckling lamb meat (Manso, Bodas, Vieira, Mantecon, & Castro, 2011). In this regard, vegetable oil supplementation has been used in order to increase rumenic acid (RA) and PUFA n-3. However, increases in dietary PUFA intake appear to affect the rumen environment and thus, the biohydrogenation pathways of linoleic and linolenic acid (ALA). This shift in intermediate FAs is characterized by an increased formation of *trans*-10, *cis*-12 C18:2 and *trans*-10 C18:1 instead of *cis*-9, *trans*-11 C18:2 and *trans*-11 C18:1 (Shingfield, Bernard, Leroux, & Chilliard, 2010). *Trans*-10, *cis*-12 CLA has possible unfavorable effects on human cholesterol level (Tricon et al., 2004) and has been shown to decrease *de novo* synthesis of FAs in the mammary gland and induce milk fat depression (Toral et al., 2010b). In contrast, *cis*-9, *trans*-11 CLA is more desirable because of its anticarcinogenic, antiatherosclerosis and other health-promoting properties (Lock, Kraft, Rice, & Bauman, 2009).

Some studies have indicated a possible role for high doses of vitamin E in preventing shifts in PUFA biohydrogenation pathways (Juárez et al., 2011; Pottier et al., 2006), thus minimizing any negative effect of plant oil on milk production, milk fat yield and/or milk fatty acid composition. Vitamin E could act either as an inhibitor of bacteria producing *trans*-10 C18:1 or as an electron acceptor for *Butyrivibrio fibrisolvens* (Pottier et al., 2006). Hou, Wang, Wang, and Liu (2013) have reported that vitamin E could affect CLA content and the accumulation of biohydrogenation intermediates in rumen fluid.

It is well known that increasing the content of unsaturated fatty acids in muscle cell membranes increases their susceptibility to oxidation (Wood et al., 2004). Therefore, the addition of antioxidants to animal diets has emerged as a strategy for increasing the commercial value of meat, and one of the most widely used antioxidants in this regard is vitamin E. Vitamin E supplementation of lamb and ewe diets

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(Capper et al., 2005; Kasapidou et al., 2012; Ripoll, Joy, & Muñoz, 2011) is usually carried out by using a synthetic source of α-tocopherol (all-rac-α-tocopheryl-acetate), due to its stability and lower cost in animal feeds (Vagni, Saccone, Pinotti, & Baldi, 2011). However, the use of natural solutions to minimize oxidative rancidity and increase meat shelf-life has a growing interest due to consumer demand for natural products and their willingness to pay a price premium for natural foods. In view of the foregoing, another vitamin E source to consider is natural vitamin E (RRR-α-tochopheryl-acetate) which is derived from vegetable oils and exhibits higher biological activity than synthetic vitamin E (Lauridsen, Engel, Craig, & Traber, 2002). Recent studies in dairy cows have estimated that the relative bioavailability of vitamin E from natural sources is 1.36 times greater than that of synthetic vitamin E (Weiss, Hogan, & Wyatt, 2009).

The aim of this work was to determine the effects of including linseed oil and vitamin E (natural or synthetic) in early lactating ewe diets on the meat quality of their suckling lambs, with particular reference to muscle fatty acid composition, vitamin E content and its subsequent effect on color and lipid oxidation. This work is a part of a series examining the relationship between ewe diet and milk fatty acid composition on suckling lamb fatty acid composition and *trans* fatty acid content.

2. Material and methods

2.1. Animal and experimental diets

The study was carried out with forty-eight pregnant Churra ewes (BW 63.6 ± 9.17 kg). The selected ewes were fed on the same basal diet before and after parturition. The basal diet was supplied for two weeks before lambing and afterwards, each ewe, on the basis of milk production, age, initial BW, prolificacy and parity in randomisation, was assigned to one of four dietary treatments (12 ewes per treatment).

The experimental diets consisted of a total mixed ration (TMR) that varied according to the inclusion of linseed oil (LO) and the type of vitamin E (synthetic or natural). The four dietary treatments were: Control (without linseed oil), LO (with 3% linseed oil), LO-Syn E (LO plus 400 mg/kg TMR of synthetic vitamin E) and LO-Nat E (LO plus 400 mg/kg TMR of natural vitamin E). The ingredients and chemical composition of the experimental diets are given in Table 1.

Ewes were individually fed during the whole experimental period and each intake was recorded. The experimental diets were fed ad libitum to each ewe and fresh drinking water was always available. Diets were supplied twice a day with forage and concentrate at a 45:55 ratio. The amount of diets offered and of refusals were weighed daily in each ewe and samples were collected for subsequent analyses.

The newborn lambs (12 lambs per treatment), covered by the protected geographical indication (PGI) "Lechazo de Castilla y León," were housed with their respective mothers all day long and were exclusively milk fed during the entire experimental period (27 ± 2.7 days). All animal handling practices followed the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes.

2.2. Milk sampling and composition

Twelve ewes per treatment were milked once a day during the entire experimental period in a 2 × 24 low-line Casse system milking parlor, with twelve milking units and two milkers. The milking machines (Alfa-Laval Iberia, S.A., Madrid, Spain) were set to provide 180 pulsations per minute with a 50:50 ratio at a vacuum level of 36 kPa.

Once a week, individual ewe milk production was recorded in second and third weeks of lactation and samples were taken in milk collection jars. For this, milk production was recorded by the oxytocine technique: in the morning before milking each ewe was injected with 0.35 cc of oxytocin (Oxiton®, Laboratorios Ovejeros, S.A., Spain) and then immediately milked. Ewes were returned to their paddock for six hours while the lambs were confined and after that milked again for milk sampling. One sub-sample of milk was kept at 4 °C until analyzed for fat and protein, according to the International Dairy Federation (IDF, 2000), using a MilkoScan-400 analyzer (Foss Electric, Hillerød, Denmark). Another two sub-samples were stored at −80 °C for subsequent analysis of fatty acid and α-tocopherol concentrations.

Table 1
\Ingredients and chemical composition of the experimental diets.

	Diets[a]			
	Control	LO	LO-Syn E	LO-Nat E
Ingredients, % as feed				
Dehydrated alfalfa	35.5	34.4	34.4	34.4
Cereal straw	9.07	9.07	9.07	9.07
Soybean meal	15.6	15.2	15.2	15.2
Corn grain	10.7	10.4	10.4	10.4
Oat grain	9.39	9.11	9.11	9.11
Barley grain	7.11	6.89	6.89	6.89
Beet pulp	7.11	6.89	6.89	6.89
Molasses	4.54	4.43	4.43	4.43
Linseed oil[b]		2.61	2.61	2.61
Vitamin mineral premix	1.00	1.00	1.00	1.00
Chemical composition, % DM				
DM	88.6	88.9	89.9	87.9
Ash	7.78	7.63	7.73	7.69
Crude protein	16.8	16.3	16.4	16.5
NDF	34.4	33.5	33.2	33.4
ADF	23.16	22.6	22.6	22.5
Ether extract	2.70	5.56	5.44	5.61
Fatty acid profile (%)				
C14:0	0.52	0.25	0.25	0.25
C16:0	19.11	10.95	10.95	10.95
C16:1	0.35	0.19	0.19	0.19
C18:0	2.37	3.98	3.98	3.98
C18:1	21.73	21.90	21.90	21.90
C18:2	41.43	24.61	24.61	24.61
C18:3	12.67	37.26	37.26	37.26
C > 20	1.82	0.86	0.86	0.86

[a] Diets supplemented without linseed oil and vitamin E (Control), with linseed oil (LO), with linseed oil and 400 mg/kg of synthetic vitamin E (LO-Syn E) and with linseed oil and 400 mg/kg of natural vitamin E (LO-Nat E).
[b] Fatty acid composition (%): C12:0, <0.01; C14:0, 0.10; C15:0, <0.01; C16:0, 6.20; C16:1, 0.10; C18:0, 4.90; C18:1, 21.90; C18:2, 14.80; C18:3, 51.30: C20:0, 0.20; C22:0, 0.10.

2.3. Slaughter procedure, carcass and meat measurements

Lambs were weighed twice a week until they reached the slaughter live weight (approximately 12 kg). Then lambs were taken to a commercial EU-licensed abattoir, stunned and slaughtered by section of the jugular vein in the neck. After exsanguination, dehiding and evisceration, carcasses were immediately weighed (hot carcass weight, HCW) and transferred to a cooler at 4 °C. After 24 hours, carcasses were weighed again (cold carcass weight, CCW), and chilling losses were calculated as the difference between HCW and CCW expressed as a proportion of the initial HCW. Dressing percentage was calculated as the ratio of CCW to slaughter live weight. Two samples of *m. Longissimus lumborum* (dissected between the 6th and the 13th rib) were stored at −80 °C, one for fatty acid composition analysis and the other for α-tocopherol level determination.

2.4. Feed and muscle chemical composition

The chemical composition of the TMR was determined using the procedures described by the AOAC (2003).

The chemical composition of meat was determined on *m. Longissimus lumborum* samples, which were analyzed for dry matter (AOAC official method 950.46), ash (AOAC official method 920.153) and crude protein (AOAC official method 981.10).