Meat Science 83 (2009) 499–505

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat

Eliana Jerónimo [a,c], Susana P. Alves [a,b], José A.M. Prates [c], José Santos-Silva [a], Rui J.B. Bessa [a,c,*]

[a] Unidade de Investigação em Produção Animal, INRB, Fonte Boa, 2005-048 Vale de Santarém, Portugal
[b] REQUIMTE, ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Campus Agrário de Vairão, 4485-661 Vairão VC, Portugal
[c] CIISA, Centro de Investigação Interdisciplinar em Saúde Animal, Faculdade de Medicina Veterinária, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 9 January 2009
Received in revised form 26 May 2009
Accepted 22 June 2009

Keywords:
Conjugated linoleic acid
Lamb meat
Linseed oil
Meat
Polyunsaturated fatty acids
Sunflower oil

ABSTRACT

The effect of stepwise replacement of dietary sunflower oil (SO) with linseed oil (LO) on carcass composition, meat colour and fatty acid (FA) composition of intramuscular lipids of lamb meat was investigated. Thirty-six lambs were fed one of four diets consisting of pellets of lucerne with oil (60 g/kg): the diet varied in the composition of oil added and were: 100% SO; 66.6% SO plus 33.3% LO; 33.3% SO plus 66.6% LO and 100% LO. The experimental period was 7 weeks. Live slaughter weight, hot carcass weight and intermuscular fat percentage of chump and shoulder increased linearly with replacement of SO by LO.

Total FA content of *longissimus dorsi* muscle and polar and neutral lipids were not affected by the treatments. Replacement of SO with LO increased the content of 18:3$n-3$ and total $n-3$ long chain ($\geqslant C_{20}$) PUFA (LC-PUFA) and decreased the 18:2$n-6$, total $n-6$ LC-PUFA and 18:2 *cis*-9, *trans*-11 in meat lipids. Maximum CLA concentration (42.9 mg/100 g fresh muscle) was observed with 100% of SO, decreasing linearly by SO with LO replacement. Maximum $n-3$ LC-PUFA was predicted to be 27 mg/100 g of fresh muscle at 78% of SO with LO replacement. Considering both CLA and $n-3$ LC-PUFA, the maximum levels were estimated to be reached at 52% of replacement of SO with LO. The utilization of blends of SO and LO is a good approach for obtaining lamb meat enriched with both CLA and $n-3$ LC-PUFA.

1. Introduction

Ruminant meats have been associated with an increase in the risk of cardiovascular diseases, due to their high content of saturated fatty acids (SFA) (Givens, 2005). However, ruminant meats may also be a good dietary source of some nutrients with health benefits including some fatty acids (FA) such as long chain ($\geqslant C_{20}$) polyunsaturated fatty acids (LC-PUFA) and conjugated linoleic acid isomers (CLA). The decrease of SFA and the increase of health-beneficial FA has been a main topic of ruminant meat research. The beneficial effects of eicosapentaenoic (20:5$n-3$, EPA) and docosahexaenoic (22:6$n-3$, DHA) acids are well documented and include anti-atherogenic, antithrombotic and anti-inflammatory actions (Givens, Kliem, & Gibbs, 2006). It is important to increase the intake of EPA and DHA in the human diet because the synthesis of these FA from dietary α-linolenic acid (18:3$n-3$) is very limited (Burdge & Calder, 2005). Although, foods of marine origin are the richest sources of EPA and DHA, its contribution to human diet in most Western countries is low (Givens & Gibbs, 2008). Thus, the enrichment of foods consumed in relatively high quantities with EPA and DHA is one option to increase the intake of these $n-3$ PUFA. There are opportunities to increase the concentration of very long chain $n-3$ FA ($n-3$ LC-PUFA) in ruminant meats (Givens et al., 2006). The inclusion of 18:3$n-3$ source in lamb diets, such as forages (Bessa, Portugal, Mendes, & Santos-Silva, 2005), pastures (Santos-Silva, Bessa, & Santos-Silva, 2002), linseed (Demirel et al., 2004; Wachira et al., 2002) or linseed oil (Bessa et al., 2007; Cooper et al., 2004) increases the concentration of $n-3$ LC-PUFA in meat.

The CLA acronym refers to a group of positional and geometric isomers of linoleic acid (18:2$n-6$), in which the double bonds are conjugated, and many studies suggest that CLA exhibits anti-carcinogenic, anti-adipogenic, anti-diabetogenic, anti-atherogenic and anti-inflammatory effects (Wahle, Heys, & Rotondo, 2004). Ruminant fats are among the richest natural sources of CLA isomers, particularly of rumenic acid (18:2 *cis*-9, *trans*-11), and are the main sources of these isomers in the human diet (Chin, Liu, Storkson, Ha, & Pariza, 1992). The 18:2 *cis*-9, *trans*-11 is formed during the ruminal biohydrogenation of 18:2$n-6$ to stearic acid (Harfoot & Hazelwood, 1997) and by endogenous conversion of 18:1 *trans*-11 by Δ^9-desaturase in tissues (Griinari et al., 2000). Feeding lipid sources rich in 18:2$n-6$ and 18:3$n-3$ will increase the 18:2 *cis*-9, *trans*-11 content of ruminants meat (Bessa et al.,

* Corresponding author. Address: Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Pólo Universitário do Alto da Ajuda, 1300-477 Lisboa, Portugal. Tel.: +351 213652871; fax: +351 213652889.
E-mail address: rjbbessa@fmv.utl.pt (R.J.B. Bessa).

0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.06.033

2005, 2007; De La Torre et al., 2006; Santos-Silva, Mendes, Portugal, & Bessa, 2004). However, feeding linseed oil (rich in 18:3$n-3$) seems to be less effective in the increase of 18:2 *cis*-9, *trans*-11 in muscle than sunflower oil (rich in 18:2$n-6$) (Bessa et al., 2007; Noci, French, Monahan, & Moloney, 2007). Bessa et al. (2007) observed that a blend of sunflower and linseed oils may be a good approach to obtain simultaneously an enrichment in $n-3$ PUFA and CLA in lamb meat. Thus, in this work we intended to further explore this approach, studying more levels of stepwise substitution of sunflower oil (SO) with linseed oil (LO) in order to determine the best blend, as well as to extend the FA analyses to neutral and polar muscle lipid fractions.

2. Materials and methods

2.1. Animals management and sampling procedures

Animal handling followed the EU directive 86/609/EEC, concerning animal care. Thirty-six Merino Branco ram lambs were used in a trial carried out in the Centro de Experimentação do Centro Alentejo (Reguengos de Monsaraz, Portugal). The lambs were born in September 2005, and were reared on pasture with their dams until weaning, that occurred at about 90 days of age. The average initial weight of lambs was 22.9 ± 2.78 kg (mean ± s.d.). Animals were randomly assigned to four groups of nine lambs each. The four experimental diets were as follows: pelleted dehydrated lucerne with sunflower oil (S); pelleted dehydrated lucerne with a blend of 66.6% of sunflower oil and 33.3% of linseed oil (2SL); pelleted dehydrated lucerne with a blend of 33.3% of sunflower oil and 66.6% of linseed oil (S2L); pelleted dehydrated lucerne with of linseed oil (L). The target for oil inclusion was 60 g/kg on a dry matter basis, resulting in pellets with ether extract range between 70 and 76 g/kg of dry matter. The diets were prepared in an industrial unit and oil was sprayed over the pelleted dehydrated lucerne. The chemical composition of the diets is presented in Table 1.

After an adaptation period of 7 days to the experimental conditions, lambs stayed on trial for 7 weeks. Feed was offered daily in the morning at a rate of 110% of *ad libitum* intake calculated by weighing-back refusals daily. The animals were weighed weekly just before feeding. At the end of trial, lambs were transported to the experimental abattoir of the Estação Zootécnica Nacional (Vale de Santarém, Portugal). After weighing, to obtain the live slaughter weight, lambs were stunned and slaughtered by exsanguination. Carcasses were immediately weighed to obtain the hot carcass weight, which was used to assess the dressing percentage. The carcasses were kept at 10 °C for 24 h, and graded according to weight using the EU scales for the classification of lamb carcasses. Then, the carcasses were chilled at 2 °C until the third day after slaughter. The kidney knob channel fat (KKCF) and the kidneys were removed and the carcasses were split along the spine. The left sides of the carcasses were separated into eight joints (Santos-Silva, Mendes, & Bessa, 2002), and the chumps and the shoulders were dissected into muscle, subcutaneous and intermuscular fats and bone. The colour of *longissimus muscle* was measured at the level of the 13th thoracic vertebra, using a Minolta CR-300 chromometer (Konica Minolta, Portugal) in the L^*, a^* and b^* system after 1 h of exposure to air to allow blooming. After removing the epimysium, the *longissimus dorsi* muscle was minced, vacuum packed, freeze-dried and stored at −80 °C until lipid analysis.

Table 1
Chemical composition of the experimental diets.

	SO replaced with LO (%)[a]			
	0	33.3	66.6	100
g/kg Dry matter				
Crude protein	153	153	153	152
Ether extract	70	71	72	76
NDF[b]	500	482	485	481
Fatty acids composition (% of total fatty acids)				
16:0	8.2	8.3	7.9	7.0
18:0	2.4	3.0	3.3	3.4
18:1 *cis*-9	20.4	19.5	17.7	15.6
18:2$n-6$	57.1	43.9	30.6	18.3
18:3$n-3$	6.3	20.4	33.6	48.0

[a] Zero percentage of sunflower oil (SO) by linseed oil (LO) replacement – diet S; 33.3% of SO with LO replacement – diet 2SL; 66.6% of SO with LO replacement – diet S2L; 100% of SO with LO replacement – diet L.
[b] Neutral detergent fibre.

2.2. Lipid analysis

Fatty acid methyl esters (FAME) of feed lipids were prepared by a one-step extraction transesterification, using toluene and heptadecanoic acid (17:0) as internal standard, according to the procedure reported by Sukhija and Palmquist (1988).

Intramuscular lipids were extracted by the method of Folch, Lees, and Stanley (1957), using dichloromethane and methanol (2:1 vol/vol), instead of chloroform and methanol (2:1 vol/vol), as described by Carlson (1985). The lipid extract was separated into neutral (NL) and polar (PL) lipids, using a solid-phase extraction procedure described by Juaneda and Rocquelin (1985) and silica gel cartridges (LiChrolut® Si, 40–63 μm, 500 mg/ml, Standard, Merck KGaA, Darmstadt, Germany). The NL fraction was eluted with dichloromethane and the PL fraction with methanol. The total lipids, NL and PL of muscle were transesterificated with sodium methoxide followed by hydrochloric acid in methanol (1:1 vol/vol) as described by Raes and Smet (2001). Quantification of muscle lipids FAME was done using nonadecanoic acid (19:0) as internal standard. The FAME were analyzed using a HP6890A chromatograph (Hewlett–Packard, Avondale, PA, USA), equipped with a flame-ionization detector (GC–FID) and fused silica capillary column (CP-Sil 88; 100 m × 0.25 mm id 0.20 μm of film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA). Helium was used as the carrier gas and the injector split ratio was 1:50. The initial column temperature of 100 °C was held for 15 min, increased to 150 °C at 10 °C/min and held for 5 min. Then, was increased to 158 °C at 1 °C/min, held 30 min, and finally increased to 200 °C at a rate of 1 °C/min, and maintained for 60 min. The injector and detector temperatures were 250 and 280 °C, respectively. Identification was accomplished by comparison of sample peak retention times with those of FAME standard mixtures (Sigma, St. Louis, MO, USA). For the resolution of 18:1 *cis*-9 from both 18:1 *trans*-13 and 18:1 *trans*-14 (that co-eluted in our GC–FID conditions) a second temperature program was used. The initial temperature column of 70 °C was held for 4 min, increased to 110 °C at 8 °C/min and then increased to 170 °C at 5 °C/min, held 10 min, and finally increased to 220 °C at a rate of 4 °C/min, and maintained for 25 min. Thus, the relative amounts of 18:1 *cis*-9 and 18:1 *trans*-13/14 were calculated from the second temperature program and applied to the area of the common peak identified in initial temperature program. Also, the FA 20:3$n-9$ co-eluted with the 18:3 *cis*-9, *trans*-11, *cis*-15 in our GC conditions, its quantification was conducted as described in Bessa et al. (2007).

CLA reported here is the GC–FID peak that included the predominant 18:2 *cis*-9, *trans*-11 isomer but also the minor 18:2 *trans*-7, *cis*-9 and the 18:2 *trans*-8, *cis*-10 isomers.

2.3. Statistical analysis

The effect of dietary replacement of SO with LO was analyzed using the GLM procedure of SAS (SAS Institute, Inc., Cary, NC,