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Effect of milk-replacer *vs* ewe milk rearing on vitamin E content of suckling lamb meat

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SUMMARY – Ten carcasses from suckling lambs of the Churra breed hand reared with milk substitute and ten carcasses from lambs reared with ewe milk were studied in order to evaluate the effect of the type of rearing on the content of vitamin E in the m. *longissimus dorsi* of suckling lamb. For analysis of vitamin E, the fat from m. *longissimus dorsi* was extracted and HPLC analyses were carried out. *α*-, *γ*- and *δ*- tocopherols were detected. Substantial differences in vitamin E levels in intramuscular fat from lambs of the two treatments were observed. Fat of lambs reared exclusively with milk substitute showed a higher content of α- and γ-tocopherols ($P<0.05$ and $P<0.001$, respectively) than the fat from breast-reared lambs. This study showed that vitamin E content in the meat of suckling lambs is influenced by the type of feeding.

Keywords: Milk substitute, suckling lamb meat, tocopherols, vitamins.

RESUME – *"Effet d'un substitut du lait par rapport à l'élevage au lait de brebis sur la teneur en vitamine E de la viande d'agneaux allaitants". On a étudié dix carcasses d'agneaux allaitants de race Churra nourris manuellement avec un substitut de lait, et dix carcasses d'agneaux élevés au lait de brebis, afin d'évaluer l'effet du type d'élevage sur la teneur en vitamine E dans le muscle* longissimus dorsi *des agneaux allaitants. Pour analyser la vitamine E, le gras du* longissimus dorsi *a été extrait et des analyses HPLC ont été réalisées. Des tocophérols α-, γ- et δ- ont été détectés. On a observé des différences substantielles concernant les niveaux de vitamine E dans le gras intramusculaire des agneaux des deux traitements. Le gras des agneaux nourris exclusivement avec le substitut de lait montrait une teneur plus élevée en α- et γ-tocophérols (P<0,05 et P<0,001, respectivement) par rapport au gras des agneaux allaitants. Cette étude montre que la teneur en vitamine E dans la viande d'agneaux allaitants est influencée par le type d'alimentation.*

Mots-clés : *substitut du lait, viande d'agneaux allaitants, tocophérols, vitamines.*

Introduction

"Castilla y León" is the Spanish region with the largest sheep stock (c.a. 6 millions of animals) from which approximately 2.5 millions are slaughtered annually for human consumption. Most of these are suckling lambs aged between 25 and 45 days and with a carcass weight lower than 7 kg. Two different suckling lamb rearing practises coexist: weaning the lambs at three days of age and then feeding them on milk replacer, in order to favour ewe milk processing, or rearing the lambs only with ewe milk (unweaned lambs). Rearing suckling lambs with milk substitute is increasingly used in this region, especially with lambs of foreign milk breeds, i.e. Assaf or Awassi.

Vitamin E is a highly effective antioxidant that protects cellular membranes against oxidative damage (Morrissey *et al.*, 1994) and it is considered to be the main physiological fat-soluble antioxidant (Rodas Mendoza *et al.*, 2003). Vitamin E is composed of a number of tocopherol analogues (*α*-, *β*-, *γ*- and *δ*-tocopherols and *α*-, *β*-, *γ*- and *δ*-tocotrienols). Some authors have determined the effects of dietary vitamin E supplementation on tissue tocopherols level of lamb muscles (Guidera *et al*., 1997; Salvatori *et al*., 2004). It has been shown that a large fraction of animal body tocopherol (almost 90% of the body pool) is concentrated in adipose tissue (Casal *et al*., 2001), and that its concentration in adipose tissue may be a better indicator of its abundance than plasma levels over a relatively long time (Rupérez *et al*., 1998).

α-tocopherol has been recognised as one of the most effective tocopherols for preventing oxidative changes in meat (Jensen *et al*., 1998), whereas the antioxidant activity of *β*-, γ- and *δ*-tocopherols are also important (Hewavitharana *et al.*, 2004). Some studies have demonstrated that oxidative stability

of fresh meat during storage was affected by tocopherol levels (Arnold *et al*., 1993; Guidera *et al*., 1997; Lynch *et al*., 1999; Lo Fiego *et al*., 2004).

Tocopherols supplementation in milk substitutes normally amounts to 30-80 mg/kg (according to the milk substitute producer) while ewe milk is reported to contain lower tocopherols levels, c.a. 1-1.6 mg/kg (Debier *et al*., 2005). Thus, differences in tocopherols content in suckling lamb meat are to be expected depending on milk source.

To our knowledge, no studies have investigated the effects of type of rearing (ewe milk *vs* milk substitute) or of milk tocopherol levels on the deposition of tocopherols in suckling lamb meat. The purpose of this work was to evaluate the effect of the type of rearing on the content of vitamin E in the m. *longissimus dorsi* of suckling lamb of an autochthonous Spanish breed (Churra).

Materials and methods

Samples

Twenty suckling lambs of Churra breed were used in this study. Ten animals were reared with local milk substitutes while the other ten lambs were suckling ewe milk. After slaughtering at age comprised between 25 to 35 d, samples of m. *longissimus dorsi* were excised from the carcasses and kept frozen at -40ºC prior to chemical analysis.

Tocopherol extraction

For analysis of vitamin E, fat from 30-40 g of *longissimus dorsi* muscle was extracted according to the chloroform and methanol method described by Bligh and Dyer (1959).

Vitamin E was extracted from the fat modifying the method described by Yang *et al*. (1992) as follow: duplicate lipid extracts of 0.25 g were hydrolysed by using 1 mL of 20% KOH in methanol at 68ºC for 45 min. Tocopherols were extracted twice with 3 mL of diethyl ether and the extracts were washed three times with 3 mL of distilled water to remove the KOH. Extracts were evaporated under nitrogen at room temperature and filtrated through a 0.45 *μ*m pore size nylon filter before injection into HPLC.

Cromatographic analysis

Separation by HPLC was carried out using a Waters 2690 Separation Module with a Waters 996 Photodiode Array detector. The column was an OmniSpher 5 C_{18}, 250 x 3.0 mm i.d., reversed phase. Cromatographic conditions were as described by Rodas Mendoza *et al*. (2003). The mobile phase was 100% methanol and the elution was performed at a flow-rate of 1 mL/min. The column was kept at 50ºC during analysis. The total run time required was 30 min. The working standard solutions were analysed together with the samples. Detection of *α*-, *γ*- and *δ*-tocopherols was performed by UV spectrophotometer at the wavelength of 292 nm.

Chromatographic peaks were identified by comparing retention times of samples with those of standard compounds (Sigma-Aldrich) and calibration curves were constructed from the standards for quantification.

Statistical analysis

Tocopherol contents were analysed by one way ANOVA including rearing system in the model.

Results and discussion

The effect of rearing system on *α*-, *γ*- and *δ*-tocopherols levels (*μ*g/100g) in the intramuscular fat extracted from *longissimus dorsi* muscle is shown in Table 1. Miguélez (2004) reported in m.

longissimus dorsi from Churra suckling lambs 1.98±0.41% of intramuscular fat. The mean *α*- and *γ*-tocopherols concentrations in suckling lamb muscle were significantly influenced by diets. α-tocopherol content of fat from animals reared with milk substitute was double compared to meat from lambs reared with ewe milk (P<0.05). Animals reared with milk replacer had a much higher content of *γ*-tocopherols compared with those reared with ewe milk (P<0.01). No significant differences were observed in the content of *δ*-tocopherol in the m. *longissimus dorsi* between lambs reared with milk substitute or with ewe milk.

Table 1. *α*-tocopherol, *γ*-tocopherol and *δ*-tocopherol content (*μ*g/100g) in fat extracted from *longissimus dorsi* muscle of suckling lambs

	Treatments		
	Ewe milk* (n=10)	Milk replacer* (n=10)	P value
α-tocopherol	100.3 ± 21.4	192.2 ± 107.8	P<0.05
γ-tocopherol	3.4 ± 3.2	125.2 ± 94.3	P<0.001
δ-tocopherol	7.3 ± 3.1	5.1 ± 2.7	NS

*The values given are: means ± s.d.

Differences in the accumulation of α- and γ-tocopherols in suckling lamb meat can be attributed to the supplementation of tocopherols into the milk replacers. According to Guidera *et al*. (1997), vitamin E supplementation in lamb diet increased the α-tocopherol levels in animal tissues. Wulf *et al*. (1997) showed that vitamin E supplementation elevated α-tocopherol concentrations in the *longissimus lumborum* muscle by 65%. Arnold *et al*. (1993) observed in beef that tissue vitamin E concentrations increased by increment of vitamin E supplementation.

Conclusions

In this study substantial differences on the accumulation of *α*- and γ-tocopherols in *longissimus dorsi* muscle from suckling lambs reared with ewe milk or reared with milk substitute were observed. Fat from lambs reared exclusively with milk substitute showed a higher content of *α*- and γ-tocopherols and slightly lower content of *δ*-tocopherols than fat from ewe reared lambs. This fact is expected to have influence in meat quality. In this regard, further studies are required.

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