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Effect of the intake of carotenoids and tocopherols on the deposition in the suckling lamb

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Abstract. Carotenoids, present in great quantities in fresh forages, have been proposed as tracers of the feeding system in growing lambs. The aim of this study was to assess the transfer of carotenoids and tocopherols from the ewes' milk to the tissues of the suckling lambs(slaughtered at 10-12 kg LW). The intake of carotenoids and tocopherols of the lambs was estimated from the weekly milk production and the concentration of the respective analytes in milk. The content of carotenoids and tocopherolswas determined in the subcutaneous and perirenal fat, muscle and liver of the lamb. The lambs were classified in 4 groups according to the intake of carotenoids, retinol and tocopherol from milk.The contents of lutein and retinol in the tissues increased with the intake of the respective analyte (P<0.05 to P<0.001). The intake of α-tocopherol was reflected in deposition in the liver (P<0.001), muscle, subcutaneous fat (P<0.01) and perirenal fat (P<0.05). However, only the contents of γ-tocopherol in the subcutaneous fat were affected by the intake of the analyte, although not clearly (P<0.05). The deposition of the analytes depended on the tissue.

Keywords. Lutein – Retinol– Milk – Muscle – Fat.

Effet de l'ingestion de caroténoïdes et de tocophérols sur la deposition chez l'agneau de lait

Résumé. *Les caroténoïdes présents en grande quantité dans les fourrages frais, ont été proposés comme traceurs du système d'alimentation des agneaux en croissance. L'objectif de cette étude était d'évaluer le transfert de caroténoïdes et de tocophérols du lait de brebis vers les tissus des agneaux allaités (abattus à 10-12 kg de poids vif). L'apport en caroténoïdes et en tocophérols des agneaux a été estimé à partir de la production hebdomadaire de lait et de la concentration des analytes respectifs dans le lait. La teneur en caroténoïdes et en tocophérols a été déterminée dans la graisse sous-cutanée et périrénale, le muscle et le foie de l'agneau. Les agneaux ont été classés en 4 groupes en fonction de la consommation de caroténoïdes, de rétinol et de tocophérol du lait. La teneur en lutéine et en rétinol dans les tissus augmentait avec la prise de l'analyte respectif (p <0,05 à p <0,001). L'apport d'α-tocophérol se reflétait dans les dépôts dans le foie (P <0,001), le muscle, la graisse sous-cutanée (P <0,01) et la graisse périrénale (P <0,05). Cependant, seulement les teneurs en γ-tocophérol dans le graisse sous-cutanée ont été affectés para la ingestion de γ-tocophérol, mais pas clairement (P <0,05). Le dépôt des analytes dépendait du tissu.*

Mots-clés. *Lutéine – Rétinol – Lait – Muscle – Graisse.*

I – Introduction

Reintroducing the grazing management in sheep farms increases farm self-resilience, profitability and sustainability(Ripoll-Bosch *et al*., 2014), besides provides the fulfilment of the demands of the consumers for animal products from grass-based systems, which are considered safe, natural and respectful of animal welfare (Prache*et al*., 2005). The consumers, however, demand guarantees of the feedstuffs given to the animals, thus it is necessary to look for tools that allow tracing the feeding system of animals.

Carotenoids and liposoluble vitamins in the animal tissues have been studied to trace forage-feeding in ovine (Prache*et al*., 2005; Alvarez *et al*., 2014) as they are present in important amounts in the green forage and can not be synthesized by animals. Carotenoids and tocopherolsin forages, which differ among species, preservation methods and phenological stages, are transferred through the milk to the lamb (Nozière *et al*., 2006). They are deposited in the lambs' tissues, but differently depending on the tissue studied (Kasapidou *et al*., 2009). The aims of this study were to studythe transfer of carotenoids and tocopherols from the ewes' milk to the tissues of the suckling lambs.

II – Materials and methods

The experimental and slaughter procedures were approved by the Animal Ethics Committee of the Research Centre and met the guidelines of Directive 2010/63/EU (European Union, 2010) on the protection of animals used for experimental and other scientific purposes.

The experiment was conducted in La Garcipollera Research Station located in the mountain area of the Spanish Pyrenees (North-East Spain, 42º 37' N, 0º 30' O, 945 m a.s.l.).Thirty-nine pairs of dams-lambs were used.Two groups of 10 suckling lambs grazed with their dams in adjacent permanent pastures. The remaining 19 suckling lambs were stalled indoors with their dams in 2 pens, where pasture hay was offered to the ewes *ad libitum*. All the ewes received 300 g ewe^{-1} d^{-1} of concentrate (10.3 MJ Metabolisable energy kg^{-1} 14% crude protein as fed basis). The experiment lasted from birth until the lambs reached 10-12 kg, when they were slaughtered in the experimental abattoir of CITA research station.

Milk intake (Lobón *et al*., 2017) and the concentration of carotenoids, retinol and tocopherols in the milk (Bertolin *et al*., 2018) were used to calculate the intake of carotenoids and tocopherols of the lambs and grouped into 4 groups with increasing intakes (Class 1 to 4, in increasing order, Table 1). Samples of the subcutaneous and perirenal fat, longissimus thoracis muscle and the liver were obtained from the carcasses that had been cooled at 4 ºC for 24 h in the dark. Samples were immediately vacuum-packed and frozen at -80 ºC. Muscle and liver samples were lyophilized. The analytes were determined by liquid chromatography as described in Bertolin *et al*. (2018). Carotenoids and retinol were detected by absorbance at 450 nm and 325 nm, respectively; and tocopherols by fluorescence emission at λ_{exc} = 295 and λ_{emi} = 330 nm. β-carotene, lutein, retinol and tocopherols were identified by comparison of their retention times and spectral analyses and were quantified by external calibration with those pure standards.

Table 1. Intake of lutein, retinol and tocopherols according to the category

	1	2	3	4	s.e.	P-value
n	10	10	10	9		
Lutein, ng	16^{d}	118^{c}	344^{b}	615^{a}	14.8	0.001
Retinol, mg	2^{d}	3^{c}	5^{b}	9^{a}	0.19	0.001
α-tocopherol, mg	9^{d}	17^{c}	24^{b}	36^{a}	0.7	0.001
γ-tocopherol, mg	0.6^{d}	0.9^{c}	1.2^{b}	1.9^{a}	0.04	0.001

Within a line, means with different letter differ at P<0.05.

Data were analyzed using the SAS statistical software (SAS V.9.3). Normality of the residues of the contents was verified except for of α-tocopherol in the muscle and the contents of γ-tocopherol in the liver and perirenal fat. For these analytes, means were compared with the Kruskal–Wallis non-parametric test of the NPAR1-WAY procedure.The contents with normal distribution of the residues were tested by ANOVA using the GLM procedure of SAS with the group of intake as fixed effect. Least square means and their associated standard errors were estimated. For all tests, the level of significance was set at 0.05. Trends were discussed when P-values were < 0.10.

III – Results and discussion

The intake of lutein, retinol and α– and γ–tocopherol of the lambs differed among the groups (Table 1). Lutein content in the liver and muscle increased according to the intake (P<0.001) (Fig. 1). It can be hypothesized that the lambs in the current experiment were too young to deposit detectable amounts of lutein in the fat as detected in fattened lambs (Prache *et al*., 2003). Lambs grouped in Class 1 and 2, with low intake, presented similar contents in the liver and muscle but lower than the lambsin Class 3 and 4 (P<0.05). Similarly, the content of retinol in the liver (P<0.05), muscle and both fat tissues (P<0.01) increased with retinol intake. However, the contents only differed between Class 1 and Class 4 (P<0.05).

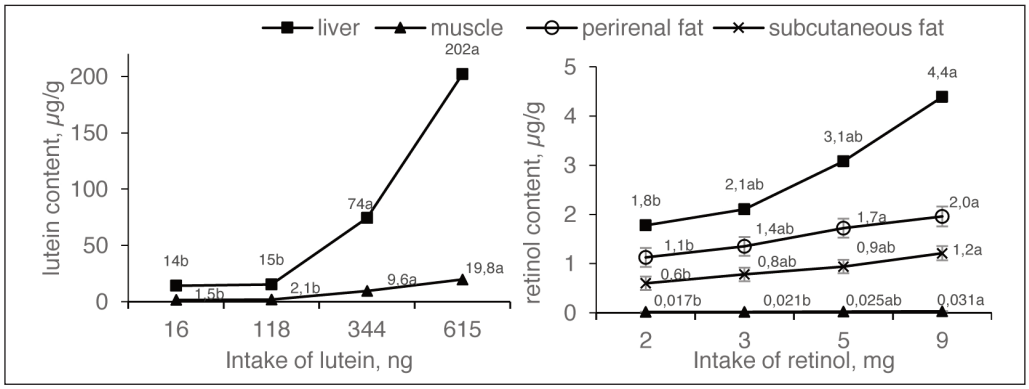

Fig. 1. Effect of the intake on the deposition of the analyte in tissues of the suckling lamb. Within a tissue, means with different letter differ at P<0.05.

The increase of α-tocopherol intakewas reflected in the liver (P<0.001), muscle (P<0.01), perirenal fat (P<0.05) and subcutaneous fat (P<0.01) (Fig. 2). Similarly, vitamin E supplementation of different forms in the diet increased α-tocopherol content in adipose tissue (Kasapidou *et al*., 2009). However, the increase in the intake of γ-tocopherol was onlyreflected in subcutaneous fat (P<0.05). The absence of a clear effect of the intake on the contents of γ-tocopherol could be related to a loss of γ-tocopherol apparently due to discrimination by the liver, although the absorption of α– and γ-tocopherol is similar (Debier and Larondelle, 2005). Then, it is more difficult to modify the content of γ-tocopherol than that of α-tocopherol through the diet.

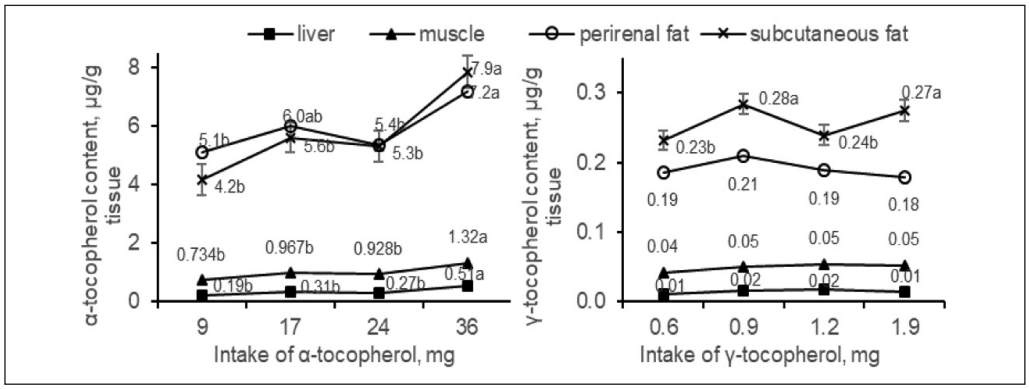

Fig. 2. Effect of α– and γ-tocopherol intake on the deposition of the analyte in tissues of the suckling lamb. Within a tissue, means with different letter differ at P<0.05.

IV – Conclusions

In conclusion, the contents of retinol, lutein and α-tocopherol in the tissues of the suckling lamb respond differently to the intake of the respective analytes. The contents of γ-tocopherol are difficult to modify with low intakes of the analyte.

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