Effect of grazing alfalfa on α-tocopherol content and FA composition in *Longissimus* and *Semitendinosus* muscles of light lambs

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Abstract. Grazing can increase α-tocopherol content in the lamb muscle without additional expenses compared with tocopheryl acetate supplementation and modify the fatty acid (FA) profile. The aim of the study was to compare the effect of alfalfa grazing on α-tocopherol and FA composition of *Longissimus thoracis* (LT) and *Semitendinosus* (ST) muscles. Twenty single reared male lambs of Rasa Aragonesa breed were used. A group of weaned lambs was indoors and fed concentrate and a group of suckling lambs was grazing alfalfa continuously with their dams. The LT and ST muscles were extracted to determine α-tocopherol content and FA composition. In both muscles, grazing lambs had more than 2-fold higher α-tocopherol content, C18:2n-6tt, C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3 and total n-3 FA contents (P<0.001) but lower n-6:n-3 ratio (P<0.001) than concentrate-fed lambs. In both muscles, grazing lambs had lower Monounsaturated FA than concentrate-fed lambs. Generally, grazing had a greater impact on n-3 FA and α-tocopherol content in ST than in LT muscle. Grazing improved the n-6:n-3 ratio and could increase the meat shelf-life. The α-tocopherol content and individual n-3 FA could be used to authenticate forage intake.

Keywords. Fatty acids – α-tocopherol – Meat – Light lamb.

Effet du pâturage de luzerne sur le contenu α-tocophérol et la composition en acides gras dans les muscles* Longissimus *et* Semitendinosus *d`agneaux légers

Résumé. *Le pâturage peut augmenter la teneur en α-tocophérol dans le muscle sans dépenses supplémentaires comparée avec la supplementation avec acétate de tocopherol et améliorer la composition des acides gras (AG). L'objectif de l'étude était de comparer l'effet du pâturage sur le teneur en α-tocophérol et la composition en AG des muscles Longissimus thoracis (LT) et Semitendinosus (ST). Vingt agneaux simples de la race Rasa aragonaise ont été utilisés. Un groupe d´agneaux sevrés a été nourri exclusivement au concentré à la bergerie et un autre groupe a eu accès continuellement à une parcelle de luzerne avec leurs mères. Dans les deux muscles, les agneaux au pâturage avaient plus de 2 fois plus d´α-tocophérol, C18:2n-6tt, C18:3n-3, C20:5n-3, C22:5n-3, C22:6n-3 et AG n-3 totaux (P<0,001), mais un rapport n- 6:n- 3 inférieur (P<0,001) que les agneaux nourris au concentré seul. Dans les deux muscles, les agneaux de pâturage avaient des teneurs en AG mono-insaturés inférieures à celles des agneaux nourris avec le concentré seul. Le pâturage a un impact plus important sur le teneur des AG n-3 et la teneur en tocophérol dans le ST que dans le muscle LT. Le pâturage de luzerne amélioré le rapport n-6:n-3 ce qui pourrait augmenter la conservation de la viande. La teneur en α-tocophérol pourrait être utilisée pour authentifier l'ingestion de fourrage avec les AG n-3 individuels.*

Mots-clés. *Acides gras – α-tocopherol – Viande – Agneau léger.*

I – Introduction

The α-tocopherol is a lipid-soluble antioxidant often fed to ruminants in the concentrate (in the form of tocopheryl acetate) due to its powerful antioxidant activity. It is an effective method to reduce the oxidative processes in meat and to increase meat shelf life (Liu *et al*., 1995). However the high cost of α-tocopherol requires accurate feeding and quantity of α-tocopherol must be ad-

justed to reduce the cost of the diet. Grazing increases the intake of α-tocopherol cheaply. Lambs finished on pasture had higher α-tocopherol content in meat than those fed high concentrate diets (Turner *et al.*, 2002). Besides, grazing also can improve of the fatty acid (FA) composition from a human health point of view (Wood *et al.*, 2004). But, the effect of grazing might be muscle-dependent. The objective of the study was to compare the effect of grazing on α-tocopherol and FA profile of *Longissimus* (LT) and *Semitendinosus* (ST) muscles.

II – Materials and methods

Twenty single reared Rasa Aragonesa male lambs were used. Twelve weaned lambs (49±0.2 days old) were indoors fed a commercial concentrate (C) (175 g crude protein (CP)/kg dry matter (DM), 180 g neutral detergent fibre (NDF)/kg DM, 45 g acid detergent fiber (ADF)/kg DM, 13.2 MJ metabolisable energy (ME)/kg DM, 27 mg dl-α-tocopheryl acetate/kg). A group of 8 unweaned lambs (29.5 ± 0.95 days old) grazed continuously on alfalfa pasture (154 g CP/kg DM; 326 g NDF/kg DM and 204 g ADF/kg DM, 154 mg α-tocopherol/kg DM) with access to their dam's milk and the commercial concentrate (Gr). When the lambs reached 22-24 kg of live weight (75 and 66 days old, C and Gr lambs respectively), lambs were slaughtered. After chilling for 24 h at 4 ºC, the LT and ST muscles were extracted to determine α-tocopherol content and FA composition.

The content of α-tocopherol in muscle was determined following the procedure of Molino *et al.* (2012). The extraction was performed in duplicate. One hundred mg of lyophilized meat were placed in a tube with 0.4 ml ethyl alcohol and vortexed. Afterwards, 1 ml of hexane was added to the tube, which was vortexed for 15 min and centrifuged (3.500 rpm for 5 min). The upper layer was collected and evaporated under nitrogen. The dry residue was dissolved in 0.5 ml of acetonitrile–dichloromethane–methanol (75–10–15). HPLC was run on a HPLC 1100 Agilent equipped with a DAD. The α-tocopherol was detected at 295 nm. FA was determined according to Bligh and Dyer (1959) method, with the following modifications: 2.5 g of lyophilized muscle were mixed with chloroform, methanol and KCl 0.88%. The lower phase (FA and chloroform) was extracted, dry evaporated and stored at -20 ºC. Meat FA composition was determined by GC-FID (Bruker 436-GC) of the fatty acid methyl esters (FAMEs) (Joy *et al.*, 2012). These FAMEs were prepared by base-catalysed methanolysis of the glycerides with KOH according to the UNE-EN ISO 5509:2000 methods.

III – Results and discussion

The α-tocopherol content was affected by the interaction between the muscle and diet (P=0.007). Grazing increased 3.0 and 3.7-fold α-tocopherol content in LT and ST muscles in comparison with indoors concentrate-fed lambs (C) (Fig. 1). In studies with weaned lambs, grazing increased 4 times α-tocopherol content in LT muscle (Santé-Lhoutellier *et al.,* 2008; Turner *et al.*, 2002). The α-tocopherol contents in the abovementioned studies were greater probably because the feeding periods were longer than in the current experiment and α-tocopherol deposition in muscle increased with feeding length (Turner *et al.*, 2002), In relation to that, Liu *et al.* (1995) concluded that age of lambs affects the α-tocopherol deposition, which can explain the differences among studies. In the current experiment Gr lambs were still lactating thus α-tocopherol intake could be different than in weaned grazing lambs. Regarding the muscle effect, In C lambs treatment, both muscles, LT and ST, presented similar α-tocopherol content, whereas in Gr lambs, ST muscle presented greater content than LT muscle (P<0.05) as reported in beef cattle (Lynch *et al.*, 2000).The different proportion and size of fibres of muscles affects the membrane volume, which might affect the potential retention of α-tocopherol (Liu *et al.*, 1995).

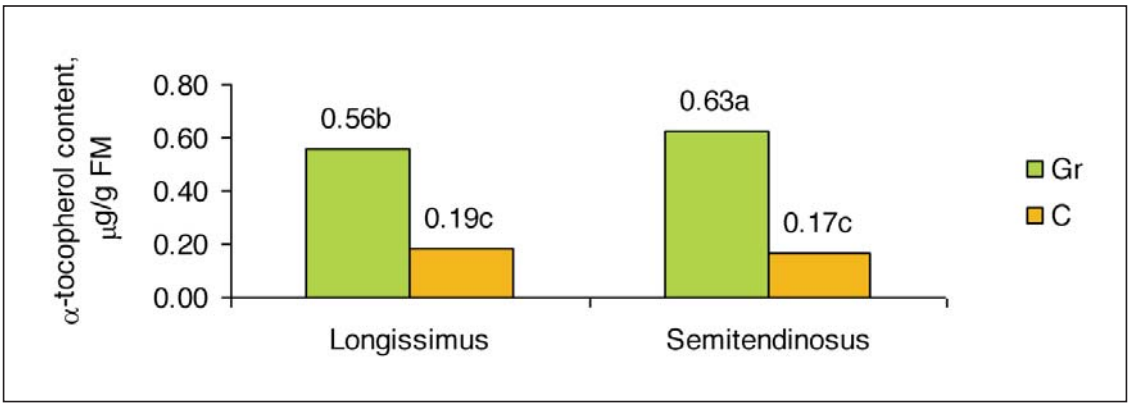

Fig. 1. The α-tocopherol content in *Longissimus* and *Semitendinosus* muscles of grazing (Gr) and concentrate (C) lambs. Different letters mean differences at P<0.05.

Table 1. Effect of diet (D) and muscle (M) on fatty acids profile *(g/ 100g FAME)*

	diet		muscle		P-value		
	Gr	C	LT	ST	D	M	DxM
C10:0	0.24	0.18	0.21	0.21	***		
C12:0	0.51	0.40	0.44	0.47	†	†	
C14:0	5.12	4.08	4.53	4.68	**		
C16:0	23.02	24.80	23.76	24.06	**		
C16:1	1.77	1.85	1.80	1.82			
C17:0	1.16	1.02	1.04	1.14	*	†	
C17:1	0.82	0.77	0.78	0.81			
C18:0	11.93	12.01	12.13	11.82		***	
C18:1n9	34.89	39.12	37.50	36.52	***	***	
C18:1n7	1.11	1.15	1.10	1.16			
C18:2n6tt	0.59	0.16	0.37	0.38	***		
C18:2n6ct	6.86	6.92	6.70	7.08		*	
C18:3n6	0.07	0.07	0.07	0.07			
C20:0	0.12	0.08	0.10	0.10	***	***	
C18:3n3	2.57	0.37	1.45	1.49	***		
CLA	0.83	0.48	0.67	0.64	***	*	
C20:1	0.08	0.09	0.09	0.08		***	
C22:0	0.20	0.19	0.18	0.21		**	
C20:4n6	2.11	2.71	2.25	2.57	†	**	
C20:5n3	1.11	0.17	0.55	0.73	***	***	**
C24:0	0.10	0.26	0.18	0.18	***		
C22:5n3	1.19	0.55	0.80	0.94	***	***	
C22:6n3	0.63	0.23	0.38	0.48	***	***	*
SFA	42.43	43.03	42.60	42.87			
MUFA	38.68	42.98	41.27	40.39	***	***	
PUFA	15.99	11.66	13.26	14.39	***	***	
n-6	9.63	9.86	9.39	10.10		**	
n-3	5.54	1.32	3.18	3.68	***	***	*
n-6:n-3	1.75	7.81	5.03	4.52	***	***	***
PUFA:SFA	0.38	0.27	0.31	0.34	***	**	

† P < 0.10 *P<0.05, **P<0.01, ***P<0.001.

The diet had effect on most of the FA studied (Table 1). Grazing lambs had greater contents of C18:2n-6tt, C18:3n-3, CLA, C20:5n-3, C24:0, C22:5n-3, C22:6n-3 and lower C18:1n9 than C lambs in both muscles (P<0.05). Consequently Gr lambs had greater total PUFA, PUFA n-3, lower MUFA contents and n-6:n-3 ratio (P<0.05), being in agreement with Santé-Lhoutellier *et al*. (2008). Grass-based diets can improve the FA composition of ruminant fat depots by increasing their C18:3n-3, CLA, C18:1n-7, and PUFA n-3 contents (Wood *et al.*, 2004). However, no effect of grazing was observed on C18:1n-7 what can be due to the pH of rumen and to the FA metabolic pathway (Joy *et al*., 2014).

Regarding muscle effect, C18:0, C18:1n9, C18:2n6ct, C20:4n6, C20:5n3, C22:5n3, C22:6n3, MUFA and PUFA contents were different in LT and ST muscles (P<0.05). Grazing affected PUFA n-3 contents to a greater extent in ST (5.9 and 1.4 g/100 g in Gr and C lambs, respectively) than LT muscle (5.2 and 1.2 g/100 g in Gr and C lambs, respectively). The ratio n-6:n3 was also affected by the interaction between diet and muscle (P < 0.001). Unweaned grazing lambs had similar n-6:n-3 ratio in the LT and ST muscles (1.8 and 1.7, respectively) whereas concentrate-fed lambs had greater n-6:n-3 ratio in the LT than in the ST muscle (8.3 and 7.4, respectively; P<0.001). Differences in muscle fibre type between muscles are reflected in differences in FA composition (Wood *et al.*, 2004).

IV – Conclusions

In conclusion, grazing of unweaned lambs increased α-tocopherol content and improved the FA composition in muscle compared with concentrate-fed lambs. However, the magnitude of the effect of grazing in α-tocopherol and PUFA n-3 contents differed between muscles.

Acknowledgments

The authors gratefully acknowledge the staff of the CITA Research Centre for technical support. This study was funded by the INIA of Spain and the EU Regional Development funds: RTA2009-0091-C02-01 and INIA-RZP2010-00002.

References

Bligh E.G. and Dyer W.J., 1959. A rapid method of total lipid extraction and purification. In: *Canadian Journal of Biochemistry and Physiology,* 37, p. 911-917.

Joy M., Ripoll G., Molino F., Dervishi E. and Alvarez-Rodriguez J., 2012. Influence of the type of forage supplied to ewes in pre/postpartum periods on the meat FA suckling lambs. In: *Meat Science,* 90, p. 775-782.

Joy M., Ripoll-Bosch R., Sanz A., Molino F., Blasco I. and Alvarez-Rodriguez J., 2014. Effects of concentrate supplementation on forage intake, metabolic profile and milk fatty acid composition of unselected ewes raising lambs. In: *Animal Feed Science and Technology*, 187, p. 19-29.

Liu Q., Lanari M.C. and Schaefer D.M., 1995. A Review of Dietary Vitamin-E Supplementation for Improvement of Beef Quality. In: *Journal of Animal Science,* 73, p. 3131-3140.

Lynch A., Kerry J.P., O'Sullivan M.G., Lawlor J.B., Buckley D.J. and Morrissey P.A., 2000. Distribution of alpha-tocopherol in beef muscles following dietary alpha-tocopheryl acetate supplementation. In: *Meat Science*, 56, p. 211-214.

Molino F., Blanco M., Calvo J.H. and Joy M., 2012. Easy, fast and economic determination method of lutein, tocopherol isoforms, tocopherol acetate and b-carotene in meat. *XII Scientific Meeting of SECYTA*.

Santé-Lhoutellier V., Engel E. and Gatellier Ph., 2008. Assessment of influence of diet on lamb meat oxidation. In: *Food Chemistry*, 109, p. 573-579.

Turner K.E., McClure K.E., Weiss W.P., Borton R.J. and Foster J.G., 2002. α-tocopherol concentrations and case life of lamb muscle as influenced by concentrate or pasture finishing. In: *Journal of Animal Science*, 80, p. 2513-2521.

Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R. and Enser M., 2004. Effects of fatty acids on meat quality: A review. In: *Meat Science*, 66, p. 21-32.