4-P35

CHARACTERISTICS OF SARCOPLASMIC RETICULUM FROM MUSCLES OF PASTURE- AND GRAIN-FED CATTLE WITH OR WITHOUT VITAMIN E SUPPLEMENT

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Background:

The discolouration in retail meats during display conditions is a combined function of muscle pigment oxidation and lipid oxidation occurring in membrane phospholipids. Much research has been published on the effect of dietary vitamin E supplementation on improving the lipid and colour stability in meat and meat products (for reviews see Fautsman et al. 1989; Morrissey et al. 1998). Vitamin E is a membrane-associated lipid-soluble antioxidant and as such can quench free radicals capable of initiating and propagating lipid oxidation which in turn protects oxymyoblobin against oxidation. The requirement for vitamin E to be effective, however, is influenced by many factors including the amount and type of dietary fat and the presence of antioxidants. There is limited information on lipid stability in muscle membranes of pasture-fed cattle and the effect of supplementation with vitamin E on its efficacy in improving membrane stability.

Objective:

The objective of this study was to examine the effects of dietary vitamin E supplementation at 2000 i.u. per animal per day on some characteristics of the muscle sarcoplasmic reticulum (SR) membrane from grass- and grain-fed cattle.

Methods

Materials. Thirty-two Hereford steers of similar weight were randomly divided into four groups of eight. Two groups were kept on pasture and the other two on a standard feedlot ration. One of these two dietary groups was supplemented with 2000 i.u. vitamin E (α-tocopherol acetate) per day for 126 days until slaughter. After the carcase had been in the chiller overnight, muscles were removed and the portion for SR preparation was frozen immediately and kept at –20°C. SR was prepared using the procedure of Martinosi et al. (1968). The protein concentration of the SR preparation was determined immediately using the Bradford method (1976) before it was frozen in liquid nitrogen and stored at –67°C until use.

Chemical assays. α-Tocopherol content in SR was determined using a reversed phase high pressure liquid chromatography (HPLC) system containing a Waters 5μ Resolve C18 column and a fluorescence detector with excitation and emission wavelengths set at 295nm and 325nm respectively. The mobile phases were methanol : water (97:3) and methanol and the flow rate was 2 ml/min. Lipid was extracted from 1 ml of the SR using Bligh and Dyer method (1959) and then redissolved in 1 ml chloroform. Phospholipid content in SR was determined by phosphorus assay according to the procedure of Bartlett (1959) with the modification of using perchloric acid in the initial digestion. Phospholipid classes were determined using a modified method of Becart et al. (1990) with phospholipids being detected on an evaporative light scattering detector (Mark III, Alltech). Fatty acid profile was determined by methylating the fatty acids of the SR lipid in 1 ml 5% sulphuric acid in methanol overnight at 60°C, extracting the esters with petroleum ether and injecting a suitable amount onto gas-liquid chromatography with a capillary column.

Lipid oxidation studies. Lipid oxidation studies were performed in duplicate at 37°C on half of the SR preparations. The incubation mixture contained 40 mM Tris-maleate buffer, pH 7.4, 1 mM ascorbate, 0.1 M iron ($FeSO_4$), 0.1 M EDTA and 0.15 mg SR protein. Water was added to make up the final 1ml incubation volume. The incubations were terminated with 0.3 ml 30% TCA with 0.2% BHT and 0.6 ml 50 mM TBA (added together in 0.9 ml). Five mg BSA was added before the contents were centrifuged at 1000 *g* for 10 minutes. The supernatant was transferred into a screw-top tube, capped tightly and heated in boiling water for 10 minutes. The tubes were cooled to room temperature and the amount of coloured product was measured at 535nm. TBARS was quantified using an extinction value of 1.56 x 105 M^{-1} x cm^{-1} and the results were expressed as nmole MDA produced per mg protein per minute.

Statistical analysis. All data were analysed for analysis of variance using SAS (SAS, 1997)

Results and Discussion

The SR from unsupplemented grain-fed cattle had significantly lower α-tocopherol contents (per mg SR protein) than did SR from the three other treatment groups (Table 1), as was found for intact muscle (Lanari et al. 1999). For pasture-fed cattle, supplementation did not significantly increase the α-tocopherol content of SR; the control cattle had high amounts of this antioxidant already present in the pasture. No differences were observed in either protein or phospholipid contents nor in the distribution of the individual phospholipid classes in the SR preparations in any of the four treatments. Similarly, the proportion of neutral lipid to phospholipid did not change. The fatty acid profile in muscle SR preparations is presented in Table 2. There were no significant differences observed for any of the fatty acids between the four treatment groups. Membrane composition was however different between the pasture- and grain-fed cattle with membranes from the pasture-fed groups being more unsaturated overall. Although C18:2 was higher with grain-feeding, the content of fatty acids having 3 or more double bonds was markedly higher in the membranes of the pasture groups particularly C18:3, C20:5 and C22:6.

Fig. 1 shows that SR from control grain-fed cattle was significantly more prone to lipid oxidation than was SR from the other three treatment groups. This corresponds with the low α-tocopherol content in the SR. It appears that, when fatty acid composition and phospholipid content and class distribution were similar, α-tocopherol content is the deciding factor in determining the extent of lipid oxidation in SR membranes. Clearly, the α-tocopherol content was high enough to protect the highly unsaturated fatty acids present in membranes of pasture-fed cattle.

Table 1. Protein, α-tocopherol and phosphorus content (n=24) and phospholipid classes distribution in muscle SR membranes of cattle with or without vitamin E supplement (n=12)

	Grass		Grain	
	Control	Supp	Control	Supp
Protein (mg/g)	8.0	6.8	6.1	6.8
α-Tocopherol (μg/mg protein)	0.81a*	0.94a	0.39b	0.96a
Phosphorus (nmole/mg protein)	804b	950a	1015a	902ab
Phospholipid classes (%)				
Neutral lipid	11.7	11.8	11.9	11.9
Cardiolipin	5.0	4.4	4.2	4.6
Phosphatidyl ethanolamine	24.3	25.1	24.5	24.0
Phosphatidyl inositol	6.9	7.1	6.9	6.6
Phosphatidyl serine	2.3	2.4	2.3	2.2
Phosphatidyl choline	43.1	42.8	43.6	44.0
Sphingomyelin	6.7	6.5	6.7	6.8

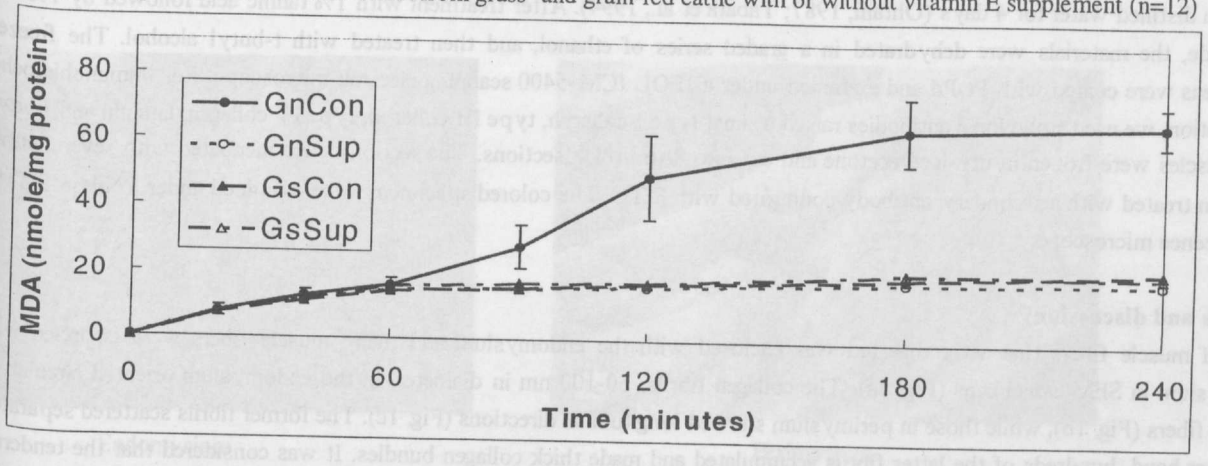
* Means within the same row with the same letter are not significantly different (P>0.05).

Table 2. Fatty acid profile in muscle SR preparations of cattle with or without vitamin E supplement (n=24)

	Grass		Grain	
	Control	Supp	Control	Supp
16:0	16.4	16.2	17.5	18.4
16:0 DMA[1]	5.2	6.1	6.2	6.0
16:1c	1.5	1.5	1.2	1.1
17:0	0.5	0.5	0.4	0.4
17:1	1.0a[2]	0.9a	0.4b	0.3b
18:0	14.1	13.6	14.1	15.3
18:0 DMA	3.1	3.5	4.0	4.0
18:1t11	1.1	1.0	1.2	2.0
18:1c9	23.9a	22.8a	20.1b	18.9b
18:1c11	1.7	1.8	1.8	1.6
18:2	10.0b	10.1b	14.0a	12.6a
18:3	2.6a	2.6a	0.7b	0.7b
22:0	0.9a	0.9a	0.6b	0.6b
22:1	0.9a	0.9a	0.6b	0.6b
20:4	5.8	5.9	6.8	6.6
20:5	2.4a	2.8a	1.4b	1.3b
Unknown 4	0.6b	0.6b	1.2a	1.7a
Unknown 6	3.1	3.7	2.4	2.3
22:6	0.5	0.7	0.4	0.4
Saturated (S)	31.9	31.1	32.7	34.6
Mono-unsaturated (M)	29.0a	27.6a	25.3b	24.4b
Poly-unsaturated (P)	21.3	22.1	23.3	21.5

[1] Dimethyl acetals formed from fatty aldehydes during methylation. [2] Means within the same row with the same letter are not significantly different (P>0.05).

Fig. 1. Lipid oxidation in muscle SR from grass- and grain-fed cattle with or without vitamin E supplement (n=12)



Conclusions

Vitamin E supplementation did not alter the α-tocopherol content in membranes of pasture-fed cattle but did with grain-fed cattle. For either nutritional group, there were no differences in fatty acid composition, phospholipid content and class distribution. α-Tocopherol content is critical in determining the lipid oxidation in SR membranes, even in those from pasture-fed cattle which contained more highly unsaturated fatty acid.

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