Original article

Tissue α-tocopherol concentrations following supplementation with various forms of vitamin E in sheep

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Summary — Tissue α-tocopherol concentrations were determined in 40 lambs following oral supplementation of various forms of vitamin E. Lambs were allocated to 8 dietary groups of 5 animals each and supplemented with or without equimolar amounts (300 mg equivalence) of different vitamin E compounds daily for 60 d as follows: 1) control, no supplemental vitamin E; 2) D-α-tocopheryl acetate; 3) DL-α-tocopheryl acetate; 4) DL-α-tocopheryl nicotinate; 5) D-α-tocopheryl polyethylene glycol 1 000 succinate (TPGS); 6) DL-α-tocopheryl nicotinate + TPGS; 7) D-α-tocopheryl acetate + TPGS; and 8) D-α-tocopheryl succinate. At the end of the 60 d the lambs were killed and portions of adrenal, fat, heart, kidney, liver, lung, skeletal (brachiocephalicus and gluteus) muscles, pancreas and spleen were removed. Daily supplementation with various vitamin E compounds (equivalence) in lambs resulted in significant differences in tissue α-tocopherol concentration in heart, liver, gluteus medius muscle, and spleen. Correlations between the plasma and tissue α-tocopherol levels were highly significant for all tissues except adrenal, fat, and pancreas.

vitamin E / tissue concentration / α-tocopherol / sheep

Résumé — *Concentrations d'α-tocophérol dans les tissus selon l'apport de différentes formes de vitamine E chez le mouton.* *Les concentrations d'α-tocophérol dans les tissus ont été déterminées sur 40 agneaux ayant reçu un apport de différentes formes de vitamine E, par voie orale. Les agneaux ont été répartis en 8 groupes de régime de 5 individus, et ont reçu — ou non — chaque jour des quantités équimolar (= 300 mg) de différents composés de vitamine E, pendant 60 j. Selon les groupes, le régime était le suivant : 1) groupe contrôle, sans apport de vitamine E ; 2) D-α-tocophéryl acétate ; 3) DL-α-tocophéryl acétate ; 4) DL-α-tocophéryl nicotinate ; 5) D-α-tocophéryl polyéthylène glycol 1000 succinate (TPGS) ; 6) DL-α-tocophéryl nicotinate + TPGS ; 7) D-α-tocophéryl acétate + TPGS ; 8) D-α-tocophéryl succinate. À l'issue des 60 j, les agneaux ont été tués, pour procéder à l'extraction de la glande surrénale, de la graisse, du cœur, du rein, du foie, des poumons, des muscles* brachiocephalicus *et* gluteus, *du pancréas et de la rate. L'apport journalier de différents composés de vitamine E chez l'agneau a donné des différences considérables de concentrations d'α-tocophérol dans les tissus du cœur, du*

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foie, du muscle gluteus medius *et de la rate. Les corrélations entre les taux d'α-tocophérol du plasma et des tissus étaient très significatives pour tous les tissus, sauf ceux de la glande surrénale, de la graisse, et du pancréas.*

vitamine E / concentrations dans les tissus / α-tocophérol / mouton

INTRODUCTION

Various vitamin E compounds have different biopotencies, since the natural and synthetic chemical forms of vitamin E are not used equally in biological systems. While the alcohol form of D-α-tocopherol has the highest biopotency (McDowell, 1989), it is easily oxidized. Therefore, more stable forms such as acetate, nicotinate, and succinate esters with reduced biopotencies (Machlin, 1984) have been used in the feed industry. When compared on an equimolar basis, supplementation with D-α-tocopheryl acetate resulted in higher ($P < 0.05$) plasma α-tocopherol levels than DL-α-tocopheryl acetate in beef cattle and calves (Hidiroglou *et al*, 1988a; Roquet *et al*, 1992) as well as in

Table I. Dry matter content of basal diet administered to lambs [a].

Ingredient	Percentage (DM basis)
Ground yellow corn	59.00
Cottonseed hulls	21.00
Soybean meal (44% CP)	12.00
Alfalfa meal (14% CP)	3.00
Corn oil	3.00
Trace minerals and salt [b]	1.00
Ground limestone	0.75
Vitamin A and D_3 [c]	0.25

[a] Diet contained 25 IU of vitamin E/kg of feed by chemical analysis. [b] Provided per kg of diet: 2.1 g of NaCl; 0.22 mg of I; 0.088 mg of Co; 9 mg of Fe; 0.725 mg of Cu; 2.625 mg of Mn; and 8.75 mg of Zn. [c] Provided per kg of diet: 2 000 IU of vitamin A and 550 IU of vitamin D_3.

sheep (Hidiroglou *et al*, 1992). Hidiroglou *et al* (1992) studied the bioavailability of several vitamin E compounds using blood plasma as an index of their biological activity. They reported that the bioavailability of D-α-tocopheryl acetate outranked all the other forms of vitamin E except those of D-α-tocopheryl acetate + D-α-tocopheryl polyethlene glycol succinate (TPGS). A slightly higher bioavailability index was observed for D-α-tocopheryl succinate than for DL-α-tocopheryl nicotinate. This work provides data on the tissue α-tocopherol levels following the supplementation of various vitamin E compounds and provides a more reliable index of bioavailability.

MATERIALS AND METHODS

Animals

Forty crossbred wether lambs averaging 35 kg were provided with water *ad libitum* and fed a diet (table I) adequate in protein, energy, vitamins and minerals. Lambs were housed in individual pens (1.4 m^2) for a 10-d adjustment period before beginning the 60-d experiment. The standard diet, which contained 25 IU/kg of vitamin E, was offered at 1 kg/d during the experiment. Supplementary forms of vitamin E were provided so as to be equivalent to 300 mg per lamb per day of DL-α-tocopheryl acetate. Other forms were administered to be equimolar to this amount. The lambs were randomly allotted to 8 treatments of 5 animals each as follows: 1) control, no supplemental vitamin E; 2) D-α-tocopheryl acetate; 3) DL-α-tocopheryl acetate; 4) DL-α-tocopheryl nicotinate; 5) D-α-tocopheryl polyethylene glycol 1 000 succinate (TPGS); 6) DL-α-tocopheryl nicotinate (150 mg) + 150 mg TPGS (3:1 molar ratio α-tocopheryl basis); 7) D-α-tocopheryl acetate (150 mg) + 150

mg TPGS; and 8) D-α-tocopheryl succinate, to be mixed with the diet.

At the end of the 60 d, the lambs were killed by exsanguination. Portions of adrenal, fat, heart, kidney, liver, lung, gluteus medius and brachiocephalius muscles, pancreas, and spleen were removed. All tissues were rinsed in water and their surfaces were dried with filter paper. During the removal of tissues, care was taken to dissect away superfluous adipose tissue. Tissues were stored at –70°C until they were analyzed for α-tocopherol concentration.

Analytical methods

Quantification of α-tocopherol in tissues was performed by HPLC using a fluorescence detector (Hidiroglou et al, 1992). Tissue samples were prepared for α-tocopherol determination according to the method of Ingold et al (1987).

Statistics

One-way ANOVA were performed on the logarithms of the tissue values and the tissue-to-plasma ratios. Logarithms were taken to stabilize the variances, which increased with increasing concentrations (Snedecor and Cochran, 1980). The statistical model used was as follows:

$$Y_{ij} = \mu + g_i + e_{ij}$$

where g_i is the effect of the ith treatment, and e_{ij} is the residual error term.

Treatment means were calculated by the least-squares method (SAS, 1985). ANOVA as well as Duncan's multiple range test were used to compare tissue α-tocopherol concentration among treatments. No adjustment for the plasma levels was made for the treatment comparison.

RESULTS

Tissue α-tocopherol concentrations following supplementation of various tocopherol sources are presented in figure 1. There were large variations in the tissue α-toco-

pherol concentrations as indicated by the large standard errors. Logarithmic transformation was used to stabilize the variances, which increased with increasing concentrations. ANOVA of the logarithms of tissue α-tocopherol values showed significant ($P < 0.05$) effect of treatments for heart, liver, hip muscle, and spleen. In the heart, liver, gluteus medius muscle, and spleen lambs supplemented with the combination of D-α-tocopheryl acetate + TPGS outranked the TPGS and control group ($P < 0.05$). In the liver and spleen, the control group had lower α-tocopherol levels than in the other treatments. In the gluteus medius muscle, the control group had significantly ($P < 0.05$) lower α-tocopherol levels than the D-α-tocopheryl acetate, DL-α-tocopheryl nicotinate and DL-α-tocopheryl acetate. There were tendencies for higher α-tocopherol levels in adrenal, fat, brachiocephalicus muscle, and pancreas following D-α-tocopheryl acetate + TPGS supplementation. Across all the supplemented treatments, the TPGS group contained the lowest tissue α-tocopherol concentration for all tissues, except adrenal, fat and lung.

Oral supplementation of vitamin E resulted in accumulation of different levels of α-tocopherol in different organs. This implies different rates of uptake, breakdown, or turnover of α-tocopherol by different organs. Some tissues, such as adrenal, liver, and pancreas, appeared to accumulate large amounts of α-tocopherol following oral supplementation of different forms of vitamin E. Treatment effect was not significant ($P > 0.05$) for the ratio of the tissue to plasma α-tocopherol levels for the different tissues. This suggests that the form in which vitamin E was supplemented orally had no effect on the ratio of tissue to plasma α-tocopherol levels. However, following logarithmic transformation, the correlation coefficient between the plasma α-tocopherol and various tissues α-tocopherol levels were significant ($P < 0.05$) for all tissues except fat, adrenal and pancreas (table II).
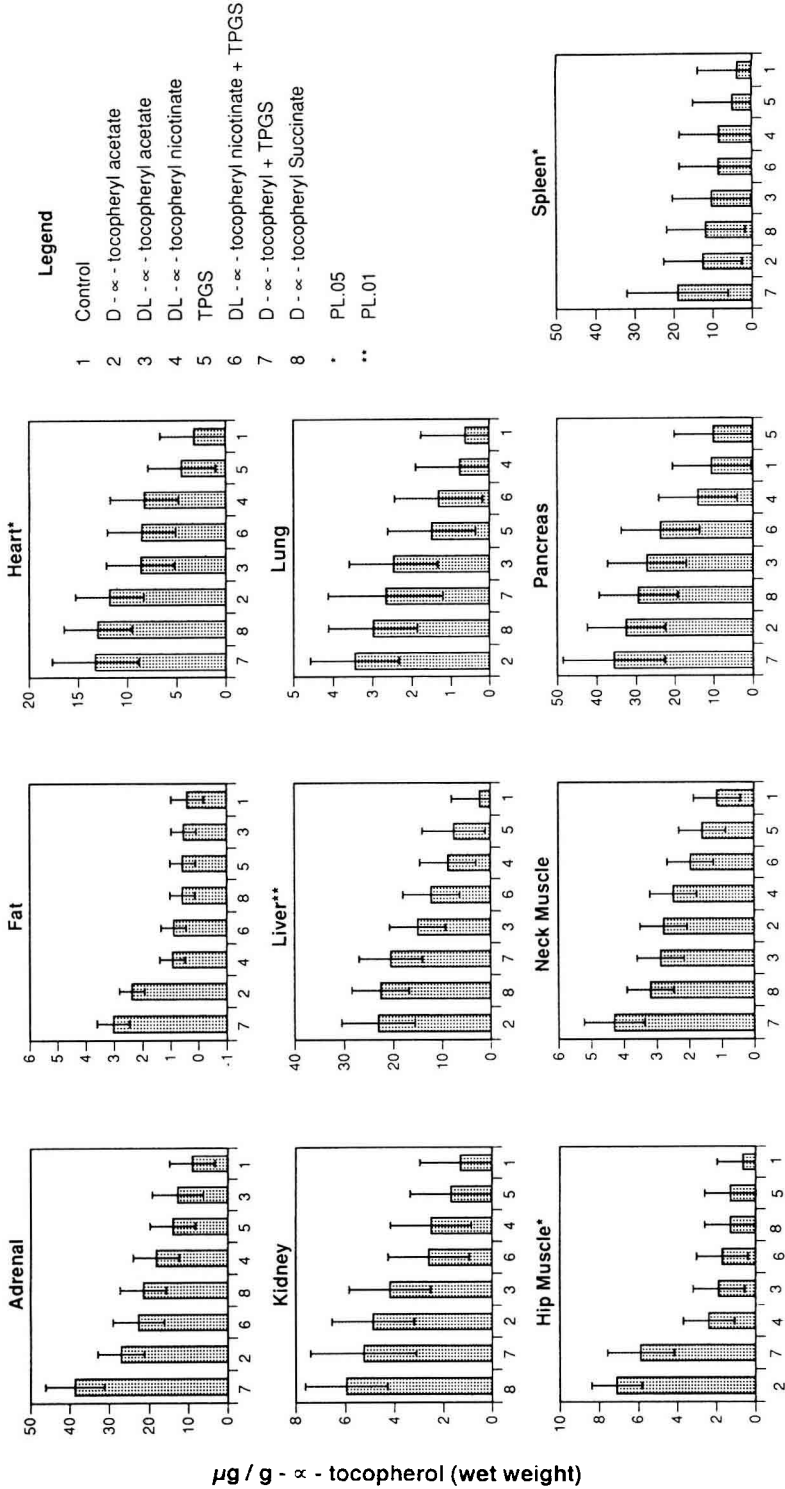
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Fig 1. Tissue level of α-tocopherol as affected by tocopherol sources. (* *P* < 0.05; ** *P* < 0.01).

Table II. Correlation coefficient between the log of tissue tocopherol and plasma tocopherol in sheep supplemented with vitamin E at 60 d.

Tissue	P value	r
Adrenal	0.11	0.26
Fat	0.09	0.28
Heart	0.0001	0.59
Kidney	0.0028	0.47
Liver	0.0001	0.74
Lung	0.0043	0.45
Hip muscle	0.0001	0.64
Neck muscle	0.0001	0.59
Pancreas	0.0100	0.39
Spleen	0.0001	0.59

DISCUSSION

The paucity of bioavailability data from various forms of tocopherol esters in sheep limits comparison with the present data. Elevation of tissue α-tocopherol levels in lambs has been used to estimate vitamin E response to the various vitamin E supplements. Various forms of vitamin E given orally are not absorbed with equal effectiveness. Solubility and chemical form affect utilization of vitamin E (Papas et al, 1991). According to Burton and Traber (1990), a major reason for the difference in bioavailability of various forms of vitamin E may be due to the discrimination in the liver. Indeed Traber et al (1990a) suggested the existence of a specific mechanism in human hepatocytes in assembling very low density lipoproteins preferentially with the natural stereoisomer (RRR-α-tocopherol) relative to SSR-α-tocopherol following a single oral equimolar dosing. Studies with monkeys fed equimolar doses of deuterated α-tocopheryl acetates (RRR and SRR) demonstrated that nascent very low density lipoprotein fraction isolated from liver perfusate were strongly enriched in RRR-α-tocopherol (Traber et al, 1990b).

Previous studies with sheep and cattle have indicated that D-α-tocopheryl acetate resulted in higher plasma α-tocopherol levels than DL-α-tocopheryl acetate form (Hidiroglou et al, 1988b). In the present experiment, the data show a higher tendency ($P > 0.05$) in α-tocopherol concentrations in all tissues except brachiocephalicus muscle, following supplementation with D-α-tocopheryl acetate than DL-α-tocopheryl acetate. This supports our previous report (Hidiroglou et al, 1992) for a higher bioavailability following D-α-tocopheryl acetate than DL-α-tocopheryl acetate supplementation as measured by serum α-tocopherol concentrations.

The present results show that among the various vitamin E compounds supplemented in the diet, the water-soluble vitamin E (TPGS) ranked the least efficient. This is in agreement with the results reported for turkey (Soto-Salanova et al, 1993), chicken (Combs, 1978), and pig (Ewan, 1990). Vitamin E concentration in the tissues were amongst the highest following the synergistic effect of DL-α-tocopheryl acetate and TPGS. These data confirm earlier reports of the synergistic effect of DL-α-tocopheryl acetate and TPGS in plasma and red blood cells to increase α-tocopherol concentrations (Hidiroglou et al, 1992; Roquet et al, 1992). Hidiroglou and Charmley (1991) reported a higher index of bioavailability ($P < 0.05$) in sheep given a single dose of DL-α-tocopheryl acetate than in those given DL-α-tocopheryl nicotinate. The present study revealed no difference ($P > 0.05$) in the tissue α-tocopherol level of lambs supplemented orally either with DL-α-tocopheryl acetate or DL-α-tocopheryl nicotinate.

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