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Original article

Serum total cholesterol, high-density lipoprotein–cholesterol and triglyceride concentrations in lambs following supplementation with various forms of tocopherol

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Summary — A 61-d study involving 40 crossbred lambs evaluated the effect of various forms of tocopherol provided daily in equimolar amounts on total cholesterol, high-density lipoprotein–cholesterol and triglyceride concentrations in the serum of lambs. Thirty-five lambs were allotted to 7 treatment groups of 5 animals each, supplemented with 300 mg tocopherol either as: 1) DL-α-tocopheryl acetate; 2) D-α-tocopheryl acetate; 3) D-α-tocopheryl succinate; 4) D-α-tocopheryl polyethylene glycol 1 000 succinate (TPGS); 5) DL-α-tocopheryl nicotinate; 6) DL-α-tocopheryl nicotinate (150 mg) + 150 mg TPGS; and 7-D-α-tocopheryl acetate (150 mg) + 150 mg TPGS mixed with the commercial flock diet. In addition, another group of 5 lambs were used as control (no vitamin E supplementation). Dietary supplementation of various vitamin E sources resulted in no overall treatment effects for total cholesterol, triglycerides or high density lipoprotein–cholesterol. A significant variation was noticed among animals. The levels of all measured serum components varied throughout the experimental period ($P < 0.0001$). The day x treatment interaction was not significant ($P > 0.05$) for any serum measured component. The present data strongly suggest that short-term treatment (< 2 mo) with pharmacological oral doses of various forms of vitamin E did not influence serum lipid metabolism of lambs. The data also showed that the bioavailability of α-tocopherol is dependent on the form administered. D-α-tocopherol acetate is a highly available form, the bioavailability of which is further increased when combined with D-α-tocopheryl polyethylene glycol succinate.

vitamin E / tocopherol / cholesterol / triglyceride / lamb

Résumé — Concentrations sériques en cholestérol total, HDL cholestérol et triglycérides chez des agneaux recevant un supplément de diverses formes de tocophérol. *Dans une expérience de 61 j portant sur 40 agneaux, l'influence de diverses formes de tocophérol, fournies quoti-*

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diennement en quantités équimolaires, sur le cholestérol total, le cholestérol HDL et les triglycérides sériques, a été étudiée. Trente-cinq agneaux ont été répartis en 7 groupes de 5, supplémentés avec 300 mg de DL-α-tocophéryl-acétate (1), ou de D-α-tocophéryl-acétate (2), ou de D-α-tocophéryl succinate (3), ou de D-α-tocophéryl polyéthylène glycol 1000 succinate (TPGS) (4), ou de DL-α-tocophéryl nicotinate (5), ou de DL-α-tocophéryl nicotinate (150 mg) + 150 mg TPGS (6) ou de D-α-tocophéryl acétate (150 mg) + 150 mg TPGS (7), mélangés dans l'aliment commercial. En outre, un autre groupe de 5 agneaux était utilisé comme témoin (sans vitamine E ajoutée). Aucun effet des traitements avec les différentes formes de vitamine E n'a été observé sur le cholestérol total, les triglycérides et le HDL-cholestérol. Des variations significatives entre animaux ont été constatées. Les taux de tous les composants sériques étudiés ont évolué au cours de l'expérience ($P < 0,0001$). L'interaction jour x traitement n'était pas significative ($P > 0,05$), pour aucun des composants. Les résultats obtenus suggèrent fortement qu'un traitement à court terme (moins de 2 mois) avec des doses orales pharmacologiques de diverses formes de vitamine E n'a pas d'influence sur les lipides sériques chez l'agneau. Ils montrent aussi que la biodisponibilité de l'α-tocophérol dépend de sa forme d'apport et que la D-α-tocophéryl acétate est une forme très disponible, dont la biodisponibilité augmente encore quand elle est associée au D-α-tocophéryl polyéthylène glycol succinate.

vitamine E / tocophérol / cholestérol / triglycéride / agneau

INTRODUCTION

Currently health professionals are recommending diets low in cholesterol and indicate that cholesterol in a greater proportion as high density lipoprotein (HDL) reduces the risk of chronic atherosclerosis. The relationship between dietary vitamin E and cholesterol metabolism has been investigated in studies with laboratory animals and human subjects; conflicting results have been observed. In studies with rats, supplementation with various dietary levels of vitamin E decreased cholesterol levels in serum and liver (Harrill and Gifford, 1966) while Kritchevsky *et al* (1980) reported no effect on serum cholesterol levels. Since the suggestion of a lower risk of coronary heart disease among persons with a relatively higher proportion of cholesterol present in their HDL fraction (Gluck *et al*, 1976), a series of clinical trials has been carried out on the redistribution of cholesterol among the lipoproteins in humans by the administration of megadoses of vitamin E. This paper reports data on lambs concerning the possible effect of various chemical forms of *per os*-administered tocopherol on different forms of cholesterol present in the blood plasma in lambs.

MATERIALS AND METHODS

Animals

A total of 40 crossbred wether lambs averaging 35 kg were fed a standard commercial diet consisting primarily of corn, cottonseed hulls and soybean meal that was calculated to be adequate in protein, energy, vitamins and minerals for this class of animal. Sheep were placed in individual pens (1.4 m^2) for a 10-d adjustment period before beginning the 61-d experiment. The standard diet, which contained 25 IU/kg vitamin E, was offered at 1 kg/d during the experiment.

Thirty-five lambs were allotted to 7 treatment groups of 5 animals each. Supplemental forms of vitamin E were provided to be equivalent to 300 mg/lamb/d of DL-α-tocopheryl acetate. Other forms were administered to be equimolar to this amount. The treatments were as follows: 1) DL-

α-tocopheryl acetate; 2) D-α-tocopheryl acetate; 3) D-α-tocopheryl succinate; 4) D-α-tocopheryl polyethylene glycol 1 000 succinate (TPGS); 5) DL-α-tocopheryl nicotinate; 6) DL-α-tocopheryl nicotinate (150 mg) + 150 mg TPGS; and 7) D-α-tocopheryl acetate (150 mg) + 150 mg TPGS mixed with the commercial diet. In addition, 5 lambs were used as control (no vitamin E supplementation). All vitamin E compounds in this experiment were provided by Eastman Chemical Company, Kingsport, TN.

Blood samples were taken at various intervals by jugular venipuncture on d −1, 0, 1, 2, 8 and biweekly thereafter for 8 wk. Blood was centrifuged immediately at 4°C and serum removed and stored at −70°C until analyzed for α-tocopheryl, triglycerides (TG), total cholesterol and HDL-cholesterol.

Analytical procedures

Serum samples were prepared for TG, total cholesterol and HDL–cholesterol determinations via an appropriate enzymatic reaction kit (Kodak Ektachem DT slide kit) and quantified by a Kodak Ektachem DT60 Analyzer. High performance liquid chromatography (HPLC) with a fluorescent detector was used for analysis of vitamin E in serum (McMurray and Blanchflower, 1979).

Statistical procedures

A split-plot in time analysis was used to examine the data; the between-animal variation was used to evaluate treatment differences. The statistical model included the corresponding reading at d 0 as a covariate. The day effect was partitioned to allow examination of the low-order polynomials.

RESULTS

Data on serum α-tocopherol concentrations are summarized in table I. On the basis of serum α-tocopherol concentrations, the various tocopherol preparations given orally to lambs were not absorbed with equal effectiveness despite equimolar dosing. The greatest area under serum curve (AUC) was observed in the D-α-tocopheryl

Table I. Pharmacokinetic values for various vitamin E compounds after their supplementation for 2 months to sheep.

Treatment	*Serum α-tocopherol (μg/ml)*		
	C terminal -initial (C_t–C_i)	*C maximum -initial* (C_{max}–C_i)	*Area under serum curve* ($\mu g/ml^{-1} \cdot h$)
Control	0.74^{e}	1.19^{d}	62.92^{b}
DL-α-Tocopheryl acetate	3.04^{c}	4.20^{ab}	175.30^{c}
D-α-Tocopheryl acetate	4.02^{b}	5.11^{a}	231.94^{b}
D-α-Tocopheryl succinate	3.19^{bc}	3.84^{bc}	167.43^{cd}
D-α-Tocopheryl polyethylene Glycol 1000 succinate (TPGS)	2.06^{d}	2.91^{c}	115.11^{e}
DL-α-Tocopheryl nicotinate	2.78^{cd}	4.12^{b}	152.90^{cde}
DL-α-Tocopheryl nicotinate + TPGS	3.04^{c}	3.54^{abc}	150.00^{cde}
D-α-Tocopheryl acetate + TPGS	5.34^{a}	5.55^{a}	282.23^{a}
SE	0.30	0.40	14.76

a, b, c, d, e Values in a column not sharing a common superscript are significantly different by analysis of variance for C_t–C_i, C_{max}–C_i, and area under the serum curve at $P < 0.05$.

acetate + TPGS treatment, which outranked ($P < 0.05$) all the other treatments. The AUC in the D-α-tocopheryl acetate was second highest ($P < 0.05$) and the lowest AUC was observed for the control animals. The AUC in the TPGS treatment was lower ($P < 0.05$) than in the DL-α-tocopheryl acetate treatment.

There were no overall treatment effects ($P < 0.05$) (tables II, III) for total cholesterol, TG, or HDL-cholesterol; in each case, the covariate (appropriate d 0 reading) was

Table II. Overall ANOVA from split-plot in time.

	Total cholesterol		*Triglycerides*		*High density lipoprotein-cholesterol*	
	df	*ms*	*df*	*ms*	*df*	*ms*
Treatment	7	1 049.87	7	282.83	7	374.49
Covariate [a]	1	29 521.67****	1	653.25*	1	6 462.43****
Between animals (error a)	28	1 014.42****	28	147.19****	28	223.73****
Day	13	2 882.12****	13	200.42****	13	1 070.88****
Linear	1	31 396.06****	1	31.01	1	12 407.61****
Quad	1	1 159.45***	1	226.80*	1	202.34**
Rest	11	446.56****	11	213.42****	11	119.22****
Day x treatment	91	82.02	91	45.34	91	28.68
Residual (error b)	370	96.55	361	35.76	369	23.78

*, **, ***, **** Significant at 5, 1, 0.1 and 0.01%, respectively; [a] covariate is corresponding value at d 0.

Table III. Average treatment effects on serum total cholesterol, triglycerides and high density lipoprotein (HDL)-cholesterol distribution.

Treatment	*Total cholesterol (mg/100 ml serum)*		*Triglycerides (mg/100 ml serum)*		*HDL–cholesterol (mg/100 ml serum)*	
	x [a]	*SE* [b]	x	*SE*	x	*SE*
DL-α-Tocopheryl acetate	70	4.29	22	1.73	39	2.01
D-α-Tocopheryl acetate	78	3.83	26	1.46	42	1.81
D-α-Tocopheryl succinate	68	4.23	24	1.50	40	1.88
D-α-Tocopheryl polyethylene glycol succinate (TGPS)	77	3.93	27	1.48	43	1.84
DL-α-Tocopheryl nicotinate	73	3.85	28	1.53	41	1.84
DL-α-Tocopheryl nicotinate + TPGS	76	3.87	24	1.54	40	1.82
D-α-Tocopheryl acetate + TPGS	78	5.22	27	2.01	47	2.57
Control	80	3.98	23	1.47	44	1.88

[a] Least squares mean; [b] SE of *x*.

significant ($P < 0.05$). The data also showed a significant variation between animals ($P < 0.0001$). There was a highly significant day effect for each of the response parameters (tables II, IV) with the pattern remaining the same for all treatments (table II). The day effect cannot exclusively be attributed to the linear component, although it predominates for total cholesterol and HDL–cholesterol, but not TG (table II).

DISCUSSION

Results of this study showed that ingestion of high doses of vitamin E administered to lambs daily for 2 mo had no appreciable effect on their lipid profile; these findings seem to contradict some previous work with other species. Hermann *et al* (1979) reported that in humans vitamin E administration in the form of synthetic all-rac-α-tocopherol acetate contributed in the increase of the HDL–cholesterol plasma concentration, with decreases in the very low-density lipoprotein (VLDL) fraction of cholesterol and total triglycerides (TG). However, several workers (Hatam and Kayden, 1981; Jacques *et al,* 1988) reported that in humans serum cholesterol or HDL–cholesterol is unrelated to vitamin E supplementation; this is in keeping with the results reported herein. In rabbits, Kritchevesky *et al* (1980) observed that pharmacological doses of vitamin E had no effect on serum cholesterol levels. However, according to Komaratat *et al* (1985), administration of vitamin E to rabbits after 9 wk on a basal diet supplemented with stripped corn oil and varying amounts of vitamin E depressed plasma cholesterol levels, with the effect being proportional to the amount of vitamin E supplemented. The hypocholesterolemic effect was accompanied by a

Table IV. Serum total cholesterol, triglycerides and high density lipoprotein (HDL)-cholesterol in lambs during a 61-d period.

Day	*Total cholesterol (mg/100 ml serum)*		*Triglycerides (mg/100 ml serum)*		*HDL–cholesterol (mg/100 ml serum)*	
	x [a]	*SE* [b]	*x*	*SE*	*x*	*SE*
1	61	1.62	25	1.04	35	0.84
2	59	1.60	24	0.98	35	0.83
8	69	1.60	25	1.00	36	0.83
15	73	1.62	26	1.00	38	0.85
19	72	1.60	25	0.98	40	0.83
22	75	1.60	26	0.98	42	0.83
26	75	1.65	27	1.01	41	0.85
29	71	1.60	26	1.00	42	0.83
33	82	1.60	30	0.98	45	0.83
36	76	1.69	26	1.06	45	0.87
40	90	1.60	28	0.98	53	0.83
43	81	1.60	20	0.98	45	0.83
50	85	1.60	21	1.03	47	0.83
61	87	1.60	26	1.00	50	0.83

[a] Least squares mean. [b] Standard error of *x*.

redistribution of plasma lipoprotein cholesterol. According to Judson *et al* (1991) sheep provided with different doses of vitamin E for 3 mo displayed no effect ($P < 0.001$) on plasma concentrations of cholesterol, TG, and total lipids.

The controversy of the influence of vitamin E supplementation on plasma lipid profile may be related to the length of feeding time of the antioxidant (Stone *et al,* 1986); according to the latter workers, alteration of the lipoprotein serum profile may occur after 3 mo of feeding the antioxidant nutrient. In the present experiment we have observed predominantly linear and highly significant time effects for cholesterol and HDL-cholesterol. While the hypocholesterolemic activity of tocopherol is under debate (Gey, 1993; Luc and Fruchart (1993) have established that vitamin E modifies lipoprotein metabolism by providing the various fractions increased protection against peroxidation phenomena. It would have been of interest to test this in ruminants.

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