Effect of diet on the metabolism of labelled tocopherol in sheep*

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1. Eighteen crossbred wethers were allotted at random (six per treatment) to each of the following diets: (1) maize-silage; (2) grass-silage; and (3) chopped hay. After 6 months a single oral dose of D-α-[5-*Me*-^{3}H]tocopherol was given to each sheep on these three treatments.

2. Blood plasma, rumen liquor and urine radioactivity were measured for 4 d and, at the end of this period, the animals were killed and tissue distribution of ^{3}H was determined.

3. Maize-silage generally contained less α-tocopherol than grass-silage or hay. Tissue uptake of ^{3}H was greater on maize-silage than other diets.

4. In muscle, spleen and liver, tocopherol concentrations were lower in the maize-silage than the grass-silage fed animals.

5. A tendency to higher uptake of radioactivity was recorded at all times in the plasma and its lipid extract of sheep fed on maize-silage than those fed on grass-silage or hay.

6. Urine clearance of radioactivity tended to be higher in animals fed on the maize-silage than those fed on grass-silage or hay. This difference of magnitude in urinary excretion was probably related to the rate of metabolism of the ingested radiotocopherol.

Evaluation of vitamin E nutrition in ruminants has recently received considerable attention (Lannek, 1973; Sharman, 1973; Oksanen, 1973). Their vitamin E metabolism is distinguished from that of simple-stomached animals by the fact that the ruminant is fed on roughages containing different tocopherols, whereas diets for simple-stomached animals contain mostly α-tocopherol. The relative amounts of the various types of tocopherols in cattle feed are important, because of the differences in their biological potencies. Therefore, knowledge of the vitamin E content of the feed at the time of consumption is of great importance. Robowsky & Knabe (1972) reported a processing loss of vitamin E activity of about 25% for grass-silage, and 50–70% for hay. The role of vitamin E in preventing nutritional muscular dystrophy (NMD) in young cattle was studied by Hidiroglou, Jenkins, Wauthy & Proulx (1972) and Hidiroglou, Wauthy & Proulx (1976), who reported that giving first crop grass-silage rich in tocopherol (90 mg/kg dry matter (DM)) but low in selenium (33 ng/g DM), resulted in no clinical, biochemical or post-mortem evidence of NMD in calves, despite their very low plasma Se levels.

In the present experiment comparisons were made for tissue uptake, metabolism and the elimination of tritiated α-tocopherol from sheep fed different roughages.

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EXPERIMENTAL

Animals and diets

Eighteen Suffolk–Shropshire crossbred wethers were allotted at random to three dietary groups. The diets were maize-silage, grass-silage and chopped grass-legume hay. Urea and mineral mix were added to the maize-silage (420 g DM/kg) at the rate of 8 g of each/kg wet weight. The mineral mix supplied (g/100 g): $Ca_2H(PO_4)_2$ 23·5, $CaCO_3$ 51·8, NaCl 18, S 5, $ZnSO_4.7H_2O$ 1·19, $MnSO_4.7H_2O$ 0·4, $CuSO_4.5H_2O$ 0·1, and $CoSO_4.7H_2O$ 0·01. In the grass-silage and hay groups, a mixture of steamed bone meal and sodium chloride (40:60, w/w) was available at all times to the sheep.

After 6 months of experimental feeding, one sheep from each treatment, chosen on an equal weight basis, was placed in a metabolism cage and given, by stomach tube into the rumen, a dose of 20 μCi/kg body weight of D-α-[5-*Me*-^{3}H]tocopherol in 10 ml aqueous-ethanolic (50%) solution. Faeces and urine were collected separately during the metabolism trial.

Sampling procedures

Rumen and blood samples were taken at 1, 3, 5, 7, 24, 48, 56, 72, 80 and 96 h after administration of the radioisotope. The reliability of rumen sampling by stomach tube was confirmed by the uniformity of the radioisotope distribution in samples taken from various locations in the rumen after the sheep were killed. Rumen samples were strained through four layers of cheesecloth. Bacterial and cell-free fractions of the rumen liquor were prepared according to the procedure of Wright & Hungate (1967). All animals were killed 96 h after the radioisotope treatment and samples of tissues were collected and stored at $-20°$ until analysed. The tissue samples were saponified within 2–3 d and analysed within 2–3 weeks. The above procedure was repeated until all the sheep had been treated.

Analytical methods

Tocopherol determination in body tissues. Tissue samples from three sheep in each treatment group were saponified (Cunningham & Morton, 1959), the non-saponified fraction was placed on a 70 × 40 mm Florisil column in benzene and the tocopherol fraction was eluted from the column with 25 ml benzene. The various components in the benzene eluate were separated by two-dimensional thin-layer chromatography (TLC), using Eastman (6060) silica gel sheets (Eastman Kodak, Rochester, New York, USA) and Skellysolve–B-diethyl ether–isopropyl ether–acetone–acetic acid (127:1·5:16:6:1·5, by vol.) or benzene as the developing solvents (Hidiroglou, Jenkins, Lessard & Borowsky, 1970). The appropriate areas were located under ultraviolet light (wavelength 254 nm), eluted with ethanol, and the tocopherol content of the alcoholic extracts was determined by the procedure of Emmerie & Engel (1939). A non-UV-absorbing area of the chromatogram comparable in area to that of the UV-absorbing area was washed with ethanol and used as a blank.

Tocopherol determination in diets. Solvent extraction of tocopherols from the roughages was carried out according to the technique of Livingston, Nelson & Kohler

(1968). This consisted of soaking overnight approximately 5 g roughage in 250 ml of a hexane–acetone mixture (7:3, v/v) plus 2 ml 8·7 M-acetic acid. An inert atmosphere was maintained in the flask during this extraction. The filtrates were concentrated almost to dryness on a rotary vacuum evaporator. The concentrate was saponified by the method of Threlfall, Griffiths & Goodwin (1967), and the non-saponified fraction subjected to chromatography on a Florisil column. The tocopherol fraction was eluted from the column, separated by TLC and the various fractions eluted and analysed as described above. A standard curve was prepared for each of the three tocopherols (α, γ, δ), because of the differences in response to the Emmerie–Engel reagent.

Materials

All reagents were analytical grade and all solvents were redistilled. Standard D-α, D-γ, and D-δ-tocopherols were purchased from Eastman Kodak (Rochester, New York, USA) and D-α-[5-*Me*-^{3}H]tocopherol with a specific activity of 11 Ci/mmol or 2·6 mCi/mg was purchased from the Radiochemical Centre, Amersham, Bucks.

Analysis of radioactivity

Plasma radioactivity. Triplicate samples (0·5 ml portions) of plasma were assayed for total radioactivity in 10 ml phase-combined system (PCS)-solubilizer scintillation fluid (Amersham-Searle, England). The radioactivity present in hexane extracts of plasma was determined by transferring the extract to counting vials, evaporating to dryness and dissolving the residue in 10 ml toluene containing 0·4% 2,5-diphenyloxazole (PPO) and 0·01% *p*-bis-[2-(5-phenyloxazolyl)]benzene (POPOP). Saponified plasma samples were extracted with benzene. The extracts were transferred quantitatively to chromatoplates (silica gel G containing fluorescent salt) and developed in hexane–ether–acetic acid (80:20:1, by vol.). Areas absorbing UV light were scraped off and eluted with ethanol and the eluates were transferred quantitatively to counting vials.

All samples were counted in a Beckman LS-250 liquid scintillation spectrometer (Beckman Instruments Ltd, Fullerton, Calif., USA) using an automatic external standard. The counting efficiency for ^{3}H was 40 ± 3% (SD) and the counts were corrected for background and quenching effects.

Urine radioactivity. Triplicate portions (0·05 ml) of urine were assayed for total radioactivity using the PCS-solubilizer scintillation fluid. In addition, portions of acidified urine (pH 1) were extracted with diethyl ether and the residue remaining after evaporation of the extract to dryness was dissolved in benzene and subjected to TLC and analysis of radioactivity as described for plasma.

Tissue radioactivity. Fresh samples (50–100 mg) of tissue were placed on ashless cellulose pellets (400 mg) and burned in a Packard Model 306 Sample Oxidizer (Packard Instrument Co., Downers Grove, Ill., USA). The tritiated water formed was dissolved in Monophase-40 (Packard scintillation fluid) added automatically by the sample oxidizer. The recovery of ^{3}H by this procedure was found to be 99%.

Table 1. *Tocopherol content of the roughages* fed to sheep*

(Mean values for three analyses/sampling date)

Sampling date	Group 1, maize-silage: Total tocopherols (mg/kg DM)	Group 1, maize-silage: Tocopherols (% total) α-tocopherol	γ-tocopherol	δ-tocopherol	Group 2, grass-silage Total tocopherol† (mg/kg DM)	Group 3, hay Total tocopherol† (mg/kg DM)
May	63	48	24	28	107	84
June	74	52	25	23	93	70
July	65	55	20	25	99	58
August	48	40	30	30	76	68
September	39	51	26	23	46	70
October	42	53	22	25	68	39

* For details of groups and diets, see p. 216.
† Only α-tocopherol was detected.
DM, dry matter.

Counting efficiency for ^3H as measured by internal or external standards varied from 25 to 33%.

Rumen liquor radioactivity. Triplicate portions (0·5 ml) of rumen liquor and its cell-free fractions were assayed directly for radioactivity using 2 ml Bio-Solv BBS-3 (Beckman Instruments, Fullerton, Calif., USA) as solubilizer and 15 ml of a scintillation fluid containing 0·4% PPO and 0·01% POPOP in toluene. The bacterial fraction was burned in the Packard Sample Oxidizer and the tritiated water counted as described previously. Aliquots of rumen liquor were saponified and extracted with diethyl ether. The diethyl ether extract was evaporated to dryness, the residue was dissolved in benzene and transferred to a Florisil column and the tocopherol fraction was eluted with benzene. Separation of the tocopherol fraction and measurement of the radioactivity were carried out as described for plasma.

Faecal radioactivity. Faecal samples (each of approximately 5 g) were homogenized at 10000 rev./min for 10 min and extracted overnight with 250 ml chloroform–methanol (2:1, v/v). Carrier DL-α-tocopherol (2 mg) was added to each aliquot before extraction, to act as an antioxidant. The chloroform layer was evaporated to dryness, and the residue was dissolved in hexane and subjected to TLC and analysis of radioactivity as described for plasma.

Statistical analysis

The analysis of variance of a completely random design was used to test ration effects for the tissue data. The rest of the data were analysed as a block design with rations as a main effect and time as a block effect.

RESULTS AND DISCUSSION

Tocopherol content of roughages

The average tocopherol contents of roughage at the time of consumption by sheep are presented in Table 1. The tocopherol content was influenced by the method of preservation of the forage. Grass-silage (except in September) contained more toco-

Table 2. *Radioactivity (disintegrations/min) in rumen liquor (RL) and its cell-free and bacterial fractions in wethers after a single oral dose of 20 μCi* D-α[5-*Me*-^{3}H]*tocopherol/kg body-weight*

(Mean values for six wethers/group)

	RL (/ml)	Cell-free (/ml RL)	Bacterial (/ml RL)
Diet† mean	28811	6463	3464
Maize-silage	29961	7772	3321
Grass-silage	29167	6281	2231
Hay	27304	5336	5040
Sampling time interval (h)			
1	84726	13083	6765
3	55876	12299	6069
5	50517	11026	7114
7	41733	9202	5553
24	23829	5958	3112
32	17249	4755	2959
48	13200	3956	2178
56	10206	3218	1696
72	8269	2811	1292
80	6192	2563	877
96	6275	2320	558

	Statistical significance		
Variable	Diets (D)	Times (T)	D × T
Rumen liquor	NS	*	NS
Cell-free fraction	NS	*	NS
Bacterial fraction	NS	*	NS

* $P < 0.05$. NS, not significant. † For details of diets, see p. 216.

pherol than hay and maize-silage. These values also indicated a trend towards a decrease in the tocopherol level of forage during storage which, in general, was greater in hay than in grass-silage.

It should also be noted that there were great differences in the tocopherol compositions of the three fodders. Only α-tocopherol was found in hay and grass-silage. In maize-silage α-tocopherol was on the average about half the total tocopherol, that is, equal to the sum of β-, γ- and δ-tocopherols. The β- and γ-tocopherols in maize-silage could not be separated by TLC and are reported as γ-tocopherol.

The values for grass-silage were generally lower than those reported by Brown (1953) but those for maize-silage were similar to the values of Burrows (1968) for maize-silage at the full-growth stage. The tocopherol values for hay were similar to those reported by Robowsky & Knabe (1972) but lower than the values reported by Krukovsky, Trimberger, Turk, Loosli & Henderson (1954). Although there are many reports in the literature of the tocopherol content of feeding-stuffs, they are in most instances of limited value. Most of them are derived from isolated analyses of tocopherol, whereas those presented here were obtained at several times during an experimental feeding period and enabled us to relate the vitamin E content of fodder to the vitamin E status of the animal.

Table 3. *Radioactivity (disintegrations/min per g fresh tissue) of various tissues of wethers 96 h after administration of an oral dose of* D-α-[5-*Me*-^3H]*tocopherol*

(Mean values with their standard errors for six wethers/group)

Tissue	Diets† Group 1 Maize-silage	Group 2 Grass-silage	Group 3 Hay	SE	Statistical significances: maize-silage *v.* grass-silage and hay
Oesophagus	4299	1541	1759	690	*
Rumen	5069	1140	1561	870	*
Reticulum	6642	1436	2067	1407	*
Omasum	7074	1527	2667	744	*
Abomasum	9982	4315	3855	1844	*
Duodenum	6915	3187	3926	1075	*
Jejunum	13434	7378	7706	2584	NS
Ileum	10114	4301	3854	1859	*
Caecum	10384	3867	4388	1437	*
Colon	5291	1814	1517	1300	NS
Liver	13584	6558	6996	2031	*
Pancreas	9927	4463	5104	1921	NS
Spleen	23387	9523	9492	4666	NS
Adrenal gland	40604	17630	23379	3407	**
Kidney cortex	10683	4872	3906	1666	*
Depot fat	2757	1384	1706	1949	NS
Muscle (vastus medialis)	2704	1070	873	424	*
Heart	10141	3702	4589	1799	*
Lung	14582	5654	6446	2636	*
Bone marrow	2835	884	1166	459	*

* $P < 0.05$; ** $P < 0.01$. NS, not significant.
† For details of groups and diets, see p. 216.

Rumen liquor radioactivity

The results (Table 2) were analysed statistically in order to examine the effects of diet and times of sampling on radioactivity levels in rumen liquor, in the cell-free fractions and in the bacteria. No differences ($P > 0.05$) in radioactivity levels were noted between diets nor was the interaction significant for diet and the time after administration of the tritiated tocopherol. This does not appear to confirm the suggestion of Alderson, Mitchell, Little, Warner & Tucker (1971) that the microbial fermentation of vitamin E is enhanced in the presence of high levels of readily-soluble carbohydrate. There was, however, a significant ($P < 0.05$) decrease in radioactivity levels in the whole rumen liquor as well as its fractions as a function of time, and radioactivity was variable among animals on the same diet. The amounts of radioactivity in the rumen liquor fractions showed that, whereas there was appreciable tocopherol in the cell-free fraction, the bacterial fraction contained less.

TLC treatment of the rumen liquor lipid extracts showed one radioactive spot, identified as α-tocopherol, as reported by Draper (cited by Dawson & Kemp, 1970).

Tissue radioactivities

Twenty tissues, in addition to the blood, of sheep slaughtered 96 h after dosing with tritiated tocopherol were analysed for radioactivity content (Table 3). Labelled tocopherol was widely distributed throughout the body. As reported previously (Hidiroglou, Jenkins, Lessard & Carson, 1970), high levels of radioactivity were recorded in some tissues such as adrenal, spleen, liver and jejunum, which finding might be related to the physiological role of the vitamin E at these sites. The high concentration of vitamin E (tritiated tocopherol) in liver is not surprising since this organ plays a very active role in the metabolism of all lipid fractions. Indeed the high levels of tocopherol in this organ confirmed it as the main storage area for vitamin E, as well as having a vital role in the regulation of the pool size of vitamin E. The heart and kidney, which are sites of rapid metabolism, contained appreciable amounts of radioactivity. The small intestine also contained appreciable amounts of radioactivity, perhaps because this tissue is the site of vitamin E absorption or because high amounts of radioactivity were excreted through the bile. Skeletal muscle was among the tissues with a lower level of radioactivity.

The higher tissue uptake of the labelled vitamin E for the sheep of group 1 (maize-silage) compared with that for groups 2 (grass-silage) and 3 (hay) may be due to the fact that maize-silage contained appreciable amounts of γ- and δ-tocopherols whereas the grass-silage and hay contained only α-tocopherol. It is well known that the number of methyl groups in the tocopherol molecule affects its biological activity, which depends upon the rate of tissue retention. According to Peake & Bieri (1971), γ-tocopherol disappears from the body faster than α-tocopherol resulting in a rapid decrease in tissue level of this tocopherol.

The non-saponifiable material was subjected to chromatography on a Florisil column. The percentages of the total radioactivity recovered in the non-saponifiable fraction for various tissues were as follows (mean $\pm$ SE): liver $71 \pm 3{\cdot}5$, adrenal $73 \pm 2{\cdot}9$, spleen $79 \pm 3{\cdot}4$, muscle $74 \pm 3{\cdot}6$, pancreas $78 \pm 4{\cdot}6$. Diet had no effect on percentage recovery. The radioactivity which remained on the column was eluted with ethanol, indicating the polar nature of this fraction. Aliquots of liver tocopherol fractions, eluted from the Florisil column with benzene, were chromatographed on cellulose containing a fluorescent indicator (Eastman Chromagram Sheet 6065; Eastman Kodak). More than 90% of the radioactivity applied was recovered in a single spot which was shown to be α-tocopherol by means of repeated co-chromatography with the pure compound in various solvent systems.

Tissue tocopherol concentrations

The tocopherol levels in spleen and muscle were higher ($P < 0{\cdot}05$) in sheep fed on grass-silage than in sheep fed on the other two diets, but lower ($P < 0{\cdot}05$) in the liver of sheep fed on maize-silage than in the liver of the grass-silage-fed sheep (Table 4). The liver values for sheep given the grass-silage or hay agreed with those found by Bruggemen & Niesar (1954) for calf livers.

Buchanan-Smith (1969) reported 21 μg α-tocopherol/g fresh liver in a ewe given a

Table 4. *Concentrations of α-tocopherol (μg/g fresh tissue) in tissues of wethers fed for 6 months on maize-silage, grass-silage or hay*

(Mean values with their standard errors for three wethers/group)

	Diet†					
	Group 1 Maize-silage		Group 2 Grass-silage		Group 3 Hay	
Tissue	Mean	SE	Mean	SE	Mean	SE
Pancreas	19·1[a]*	2·81	21·1[a]	2·47	22·6[a]	5·32
Muscle (vastus medialis)	6·2[c]	0·46	12·2[a]	1·00	8·6[b]	0·14
Spleen	8·8[b]	0·34	16·0[a]	0·37	9·8[b]	0·99
Liver	9·1[b]	0·38	18·8[a]	2·23	16·7[ab]	3·17
Adrenal gland	7·2[a]	2·55	17·0[a]	3·63	11·6[a]	3·62

* Means in the same line without a common superscript letter are significantly different ($P < 0.05$) (*t*-test).

† For details of groups and diets, see p. 216.

purified, vitamin E-deficient diet; this high value, according to the author, was partly due to the analytical technique. Caravaggi & Wright (1969) reported a value of 6 μg tocopherol/g (wet weight basis) liver of sheep, which could be increased greatly by tocopherol administration. Tocopherol values for sheep liver, according to Dicks (1965) ranged from 15 to 40 μg/g and for edible meat up to 8 μg/g.

It is difficult, however, to compare our observed tocopherol concentrations with those reported in the literature because of the wide variation observed between individual sheep and the few values cited in the literature for sheep fed on different diets.

Plasma radioactivities

The tritium decay values for whole plasma and plasma lipid extracts, after a single oral dose of labelled tocopherol, are given in Table 5. Radioactivity was present in plasma as early as 1 h after administration and increased to reach a peak at 5–7 h and thereafter declined continuously. Plasma tritiated vitamin E varied considerably between individual sheep. There was a tendency for a higher uptake of radioactivity in the plasma and its lipid extract in sheep fed on maize-silage compared with those fed on grass-silage or hay, but the differences were not significant. After precipitation of 7 and 24 h plasma samples with ethanol (Quaife & Harris, 1944), TLC was carried out on the light petroleum extracts of plasma. An average of 85% (range 77–92%) was present in the region of the standard α-tocopherol, the remainder of the radioactivity being located almost entirely at the origin.

Urinary excretion of radioactivity

Excretion of radioactivity in the urine was maximal during the first 24 h after treatment (Table 6). According to Tikriti (1969), after administration of ^{11}C-labelled tocopheryl acetate to cows, total elimination of radioactivity from the urine occurred within 72 h. In the present experiment the major portion (75–86%) of the total

Table 5. *Radioactivity present in blood plasma and plasma lipid fractions of wethers after administration of an oral dose of* D-α-[5-*Me*-^3H]*tocopherol*

Sampling time interval (h)	Radioactivity (disintegrations/min per ml plasma) Group 1* Maize-silage	Group 2 Grass-silage	Group 3 Hay	Mean	Radioactivity (disintegrations/min per ml plasma lipid extract) Group 1 Maize-silage	Group 2 Grass-silage	Group 3 Hay	Mean
1	1257	1483	1082	1274	134	695	1029	619
3	13311	5582	4635	7842	9109	4862	2114	5362
5	25460	8202	9841	14501	20792	5882	4123	10267
7	29780	8969	7463	15404	21081	6971	3157	10403
24	18041	9011	7848	11660	14376	6341	5143	8620
32	16269	8439	7419	17709	13673	6705	5054	8477
48	13002	6854	5824	8560	9731	3717	4591	6013
56	11266	5876	5279	7474	9222	3989	2998	5043
72	9269	4549	4464	6094	5616	1823	2297	2346
80	8066	4971	4539	5858	5276	2654	1751	2335
96	6927	3962	3645	4844	6492	1900	1113	3169
Mean	13877	6180	5640	8566	10500	4140	3034	5891
	SE between rations (R)			2528	SE between rations (R)			2498
	SE between sampling time (T)			1340	SE between sampling time (T)			1259
	SE of interaction R × T			4378	SE of interaction R × T			4468

* For details of groups and diets, see p. 216.

Table 6. *Excretion of radioactivity in urine of wethers after administration of a single oral dose of* D-α-[5-*Me*-^3H]*tocopherol*

(Mean value for six wethers/group)

Sampling time interval (h)	Total urinary excretion (disintegrations/min × 10^{-4}) Group 1* Maize-silage	Group 2 Grass-silage	Group 3 Hay	Mean	Total urinary excretion of the ether soluble products after acid hydrolysis (disintegrations/min × 10^{-4}) Group 1 Maize-silage	Group 2 Grass-silage	Group 3 Hay	Mean
8	6553	2602	2877	4011	1803	2602	2520	2308
24	8689	4250	7409	6782	5106	859	2559	2841
32	2282	884	2366	1844	953	553	838	781
48	4273	1670	3872	3272	1151	515	1382	1016
56	1326	343	1453	1041	441	142	579	387
72	2935	783	2961	2226	723	307	925	652
80	1351	289	2567	1402	320	105	292	239
96	1643	630	1973	1416	593	215	756	521
Mean	3631	1431	3185	2749	1386	663	1231	1094
	SE between rations (R)			895	SE between rations (R)			333
	SE between sampling time (T)			548	SE between sampling time (T)			204
	SE of interaction between R × T			1551	SE of interaction R × T			577

* For details of groups and diets, see p. 216.

radioactivity measured during the 96 h experimental period was excreted in the urine during the first 48 h. There was a tendency for more radioactivity to be excreted early in the urine of sheep fed on maize-silage than in that of sheep fed on hay and grass-silage. This trend in the differences in urinary excretion appears to be related to the rate of metabolism of the ingested tocopherol.

Virtually all urinary radioactivity was water-soluble; less than 1 % could be extracted with chloroform. After evaporation of urine to dryness, all the radioactivity remained in the residue. After acid-hydrolysis of the urine, 17–57 % of the total urinary radioactivity could be extracted with diethyl ether. A scan of the TLC plate of the diethyl ether extract after acid-hydrolysis showed two radioactive spots in addition to that corresponding in mobility to α-tocopherol. Both of these areas were less polar than α-tocopherol and have not been identified. It is suggested that these two compounds, which are presumably metabolic products of the administered tritiated tocopherol, could be similar to those found in the urine of rats by Weber & Wiss (1963).

Faecal radioactivity

In all three treatment groups, the chloroform–methanol and light petroleum–diethyl ether soluble fractions accounted for over 85 % and 55 % of the radioactivity, respectively. Hydrolysis of the light petroleum–diethyl ether insoluble fraction converted approximately 50 % of the activity to a soluble form. TLC of the chloroform–methanol extract of faeces, processed as outlined in the Experimental section, indicated that over 70 % of the radioactivity was present in a single radioactive spot. This material was found to have a mobility similar to that of standard α-tocopherol after TLC. The remaining radioactivity was present either at the origin of the thin-layer chromatoplate or in an area of lower specific radioactivity than identified as α-tocopherol. This latter material has not been identified. Astrup, Mills, Cook & Scott (1974) reported that only one radioactive spot was found in extracts of goat faeces after administration of labelled tocopherol and showed by TLC that this was identical with the original α-tocopherol.

The results presented in this paper with respect to labelled tocopherol utilization indicate a greater uptake of radioactivity by sheep fed on maize-silage diets compared with those fed on hay or grass-silage. It is quite possible that the difference was due to different α-tocopherol intakes.

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