The relative biological potencies of retinyl palmitate and β-carotene for the milk-fed lamb

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1. Thirty-four cross-bred newborn lambs were used. A high-protein diet (25 % protein calories) was supplemented daily with retinyl palmitate (range, 13·75–440 μg/kg live weight 24 h), or with β-carotene (range, 68·5–2200 μg/kg live weight 24 h) for 21 days.

2. Live-weight gain was not increased by supplementing the basal diet with either retinyl palmitate or β-carotene.

3. The relative weights of β-carotene and retinyl palmitate (expressed as retinol) that were required to produce equivalent concentrations of retinol in the serum varied from 5:1 to 25:1, and for the liver (retinol + retinyl esters, expressed as retinol) the ratio varied from 3:1 to 9:1.

The biological potency of β-carotene, relative to preformed vitamin A, is not a single standard value but is dependent on the species of animal used, the level of supplementation, the previous nutritional history of the animal and the criteria used to determine the relative potencies.

The experiment now described compares the relative biological potencies of retinyl palmitate and β-carotene for the milk-fed lamb, using as criteria the live-weight gain of the lamb and the ability to raise the blood and liver concentrations of retinol from the low levels at birth. Vitamin A activity in the serum and liver is expressed as retinol in all tables.

EXPERIMENTAL

Animals and their management

Thirty-four cross-bred newborn lambs (Border Leicester ♂ × Merino ♀) × Dorset Horn ♂, were used. Pregnant ewes were confined in pens for some days before parturition and fed *ad lib.* on a diet of 50:50 (w/w) wheaten and lucerne chaff. The newborn lambs were taken away from the ewes before they had suckled, dried and housed in individual metabolism cages. The birth weights ranged from 2·0 to 6·4 kg. The mean daily maximum and minimum temperatures in the animal house were 28° and 15° respectively.

The newborn lambs were given their first feed within 6 h of birth and, after two feeds of cow's separated colostrum (Faruque & Walker, 1970), were allotted to their dietary treatments. Since the lambs were born over a period of several weeks, it was not always possible to balance the groups exactly as regards initial live weight and sex.

Experimental design

All lambs (fifteen males and nineteen females) were given a diet containing 25 % protein calories for an experimental period of 21 days. Three lambs (group A) were

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given the diet unsupplemented with either retinyl palmitate or β-carotene. The remaining thirty-one lambs were allotted at random to eight groups. Groups B–G contained two male and two female lambs. Group H contained one male and two females and group I one male and three female lambs. The lambs in groups B–E were given 13·75, 55·0, 220 and 440 μg retinyl palmitate/kg live weight 24 h respectively. The lambs in groups F–I were given 68·5, 275, 1100 and 2200 μg β-carotene/kg live weight 24 h respectively.

The supplements of water-miscible retinyl palmitate and all-*trans*-β-carotene were given once daily in the evening feed, dissolved in the milk. The vitamin supplements and the total milk intakes were adjusted daily to allow for the increase in live weight of each lamb. The level of intake of gross energy was 210 kcal/kg$^{0.73}$ 24 h.

The significance of the differences in the liver retinol reserves between groups was estimated by an analysis of variance.

Blood samples for the estimation of serum retinol were taken from the jugular vein soon after birth and before the first feed, then at weekly intervals approximately 3 h after the morning feed and some 16 h after supplementation with retinyl palmitate or β-carotene.

At slaughter, the liver was weighed and samples were taken for the estimation of retinol. The lambs were weighed daily 4 h after the morning feed and the mean daily live-weight gain was estimated by a regression analysis of the daily weights.

Diets

The composition and preparation of the experimental diet, and the supplements of vitamins and trace elements were the same as those for diet no. 2, used in the experiment of Faruque & Walker (1970).

All lambs were dosed with 1 ml of a groundnut-oil solution of 10000 i.u. ergocalciferol on the 2nd day of life. Aureomycin soluble (0·45 g; Cyanamid of Great Britain Ltd), which contained chlortetracycline hydrochloride (25 mg), was given daily, dissolved in the milk, to each lamb. The lambs were bottle-fed three times daily at 07.00, 14.00 and 22.00 h for the first 3 days, and twice daily at 07.00 and 18.00 h thereafter.

Water-miscible retinyl palmitate and water-miscible all-*trans*-β-carotene (F. Hoffmann-La Roche and Co. Ltd) were used as the supplements.

Analytical methods

Blood and liver. Serum retinol concentrations were determined on 1 ml samples stored at $-20°$ under nitrogen by a modification of the method of Bessey, Lowry, Brock & Lopez (1946). Liver samples were stored at $-20°$ under nitrogen and retinol was extracted by the method of Röse–Gottlieb and estimated by the Carr–Price reaction as described by Hausheer, Moor, Nobile, Mueller & Wagner (1964).

RESULTS AND DISCUSSION

Mean values for the intakes of energy, live weights and live-weight gains, liver weights and liver retinol reserves are given in Table 1. All lambs gained in weight throughout the experimental period. No refusals of the diet occurred and all lambs were active and keen to drink. There were no significant differences between the live-weight gains of the groups.

Table 1. *Mean values with their standard errors for the live weight, energy intake and live-weight gain per 24 h, liver weight and liver retinol content of lambs given different supplements of retinyl palmitate or β-carotene*

Group	No. of lambs	Supplement (μg/kg)	Live weight (kg)	Energy intake (kcal)	Live-weight gain (g)	Liver weight (g)	Retinol per liver (μg)
A	3	0	5·3	700	128	96	15±3
				Retinyl palmitate			
B	4	13·75	4·9	660	111	91	63±32
C	4	55·0	5·1	670	109	101	487±51
D	4	220	5·4	700	116	105	4700±286
E	4	440	5·8	750	118	115	10800±1080
				β-Carotene			
F	4	68·5	4·7	630	119	91	152±54
G	4	275	4·6	610	100	94	934±162
H	3	1100	5·5	670	111	95	5840±112
I	4	2200	4·8	650	96	95	8980±505
SE of group mean	—	—	±0·3	±27	±5	±4	—

Liver. Liver retinol reserves were closely related to the intake of retinyl palmitate and the differences between groups were highly significant ($P < 0.01$). The regression equations ($n = 16$), with the correlation coefficients (r), residual standard deviations (RSD), and the RSD expressed as a percentage of the mean of the dependent variable were

$$L = 0.225 I - 4.87 \tag{1}$$
$$\text{RSD} = \pm 4.5 \ (12.5\%) \quad (r = +0.99),$$

$$\text{or} \quad I = 4.391 L + 23.59 \tag{1a}$$
$$\text{RSD} = \pm 19.9 \ (10.9\%) \quad (r = +0.99),$$

$$L_T = 0.210 I_T - 302 \tag{2}$$
$$\text{RSD} = \pm 293 \ (8.7\%) \quad (r = +0.99),$$

$$\text{or} \quad I_T = 4.742 L_T + 1507 \tag{2a}$$
$$\text{RSD} = \pm 1359 \ (7.7\%) \quad (r = 0.99),$$

where $I = $ μg retinyl palmitate intake/kg live weight 24 h, $I_T = $ total intake of retinyl palmitate (μg), $L = $ μg retinol/g liver and $L_T = $ total retinol per liver (μg). The regressions were highly significant ($P < 0.01$).

There were no measurable amounts of β-carotene in the livers of any of the lambs. There was a close relationship between the intake of β-carotene and the liver retinol reserves. The differences between the groups were highly significant ($P < 0.01$). The regression equations ($n = 15$), were

$$L = 0.044 I_C + 0.90 \tag{3}$$
$$\text{RSD} = \pm 9.3 \ (22.7\%) \quad (r = +0.98),$$

$$L_T = 0.042 I_{CT} + 101 \tag{4}$$
$$\text{RSD} = \pm 840 \ (21.8\%) \quad (r = +0.98),$$

where $I_C = \mu g$ β-carotene intake/kg live weight 24 h, $I_{CT} = $ total intake of β-carotene (μg), $L = \mu g$ retinol/g liver and $L_T = $ total retinol per liver (μg).

Table 2. *Mean values with their standard errors for the retinol content of the blood serum ($\mu g/100$ ml) of lambs given different supplements of retinyl palmitate or β-carotene*

Group	No. of lambs	Supplement (μg/kg 24 h)	Birth	1	2	3	SE of group mean
A	3	0	10·8	7·5	7·8	6·0	±0·4
			Retinyl palmitate				
B	4	13·75	9·0	10·2	10·2	10·2	±0·4
C	4	55·0	10·2	20·4	20·7	23·4	±1·0
D	4	220	9·0	19·5	29·7	26·7	±1·0
E	4	440	10·4	26·7	32·4	35·1	±1·1
			β-Carotene				
F	4	68·5	11·4	11·4	10·8	10·8	±0·1
G	4	275	9·0	13·2	14·1	16·8	±0·3
H	3	1100	9·3	17·1	17·4	25·5	±1·2
I	4	2200	8·4	18·6	20·1	29·1	±0·8

(Time of sampling (weeks) spans the Birth, 1, 2, 3 columns.)

Serum. The mean value for the serum retinol concentration before suckling was $9.8 \pm 0.4 \ \mu g/100$ ml ($n = 34$, range 6·9–13·8). The mean values for the serum retinol concentrations at each of the weekly sampling periods are given in Table 2.

The levels of retinyl palmitate supplementation given to lambs in groups C, D and E resulted in an increase in the serum retinol concentrations, whilst the level of supplementation given to group B was sufficient only to maintain the serum concentration at its initial value. In the unsupplemented group (group A) the serum concentration decreased slightly during the experiment.

There was a significant correlation ($P < 0.01$) between the retinol concentration in the serum samples taken just before slaughter and the logarithm of the retinyl palmitate intake. The regression equation ($n = 16$), was

$$S = 14.9823 \log I - 5.61 \tag{5}$$
$$\text{RSD} = \pm 6.4 \quad (r = +0.82),$$

$$\text{or} \quad \log I = 0.0452 S + 0.89 \tag{5a}$$
$$\text{RSD} = \pm 0.35 \quad (r = +0.82),$$

where $I = \mu g$ retinyl palmitate intake/kg live weight 24 h and $S = \mu g$ retinol/100 ml serum. The regression was highly significant ($P < 0.01$).

There was no measurable amount of β-carotene in the serum of the lambs in any group at any sampling period. The amounts of the β-carotene supplement given to groups G–I resulted in an increase in the serum retinol concentration, whilst the amount given to group F was sufficient only to maintain the initial concentration.

There was a significant correlation ($P < 0.01$) between the retinol concentration in the serum samples taken just before slaughter and the logarithm of the β-carotene intake. The regression equation ($n = 15$), was

$$S = 12.4212 \log I_C - 12.49 \tag{6}$$
$$\text{RSD} = \pm 5.8 \quad (r = +0.80),$$

where $I_C = \mu$g β-carotene intake/kg live weight 24 h and $S = \mu$g retinol/100 ml serum. The regression was highly significant ($P < 0.01$). The regression equations

Table 3. *The relative biological potencies of β-carotene and retinyl palmitate (expressed as retinol) in maintaining equivalent concentrations of retinol in the blood and liver of the milk-fed lamb as compared with published values for ruminant lambs and young of other species*

Amount of β-carotene given (μg/kg 24 h)	Ratio of β-carotene to retinol			
	Plasma*	Liver	Plasma	Liver
	Calves†		Pigs‡	
88·2	4·0	5·3	5·6	3·7
176	6·4	7·8	7·0	5·1
353	9·6	11·7	8·6	6·9
705	14·6	17·4	10·7	9·4
	Milk-fed lamb		Ruminant lamb‡	
68·5	5·4	3·1	—	—
88·2	(6·2)	(3·6)	6·6	5·5
176	(8·4)	(5·2)	8·2	7·4
275	10·2	6·2	—	—
353	(11·4)	(6·8)	10·4	10·0
705	(15·4)	(7·9)	13·1	13·4
1100	18·7	8·4	—	—
2200	25·3	8·9	—	—

Values in parentheses were calculated from published reports (see p. 28).
* Serum was used in the milk-fed lamb.
† Rousseau *et al.* (1956).
‡ Myers *et al.* (1959).

relating the retinol concentration of the serum samples taken just before slaughter to the logarithm of the retinol concentration in the liver for lambs given either retinyl palmitate ($n = 16$), or β-carotene ($n = 15$), were

Retinyl palmitate $\quad S = 9.4928 \log L + 14.28 \tag{7}$
$$\text{RSD} = \pm 6.1 \quad (r = +0.87),$$

β-carotene $\quad S = 8.1577 \log L + 11.05 \tag{8}$
$$\text{RSD} = \pm 5.6 \quad (r = +0.85),$$

where $L = \mu$g retinol/g liver and $S = \mu$g retinol/100 ml serum. The regressions were highly significant ($P < 0.01$).

Biological potency. The relative potencies of β-carotene and retinol for the milk-fed lamb are given in Table 3, together with comparable values for the biological potency of β-carotene as determined for calves by Rousseau, Eaton, Teichman, Helmboldt, Jungherr, Bacon & Beall (1956), and by Myers, Eaton & Rousseau (1959) for young pigs and lambs. The lambs used by Myers *et al.* (1959) weighed between 16 and 32 kg and were ruminating.

The relative biological potencies of β-carotene and retinol were calculated from the regression equations (1 *a*), (3) and (5 *a*), (6).

The ratios given in Table 3 suggest that β-carotene is less efficient, relative to retinol, in raising the retinol concentration in the serum than it is in raising the liver retinol reserves.

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