Studies on the requirement of the young chick for vitamin E

The effects of different sources and levels of dietary starch on gain in weight and body vitamin E storage

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(*Received* 19 *March* 1959—*Revised* 8 *February* 1960)

The exact requirement of poultry for vitamin E under practical feeding conditions has not been established. It has been suggested by Blaxter & Brown (1952) that it is unwise to state absolute dietary requirements, since the needs at any time depend on the combination of current stress factors, dietary, physiological and environmental.

Investigations into the vitamin E requirement of young chicks have centred round the study of nutritional encephalomalacia and exudative diathesis. However, it was first pointed out by Dam (1944) and later confirmed by Zacharias, Goldhaber & Kinsey (1950) and Singsen, Matterson, Kozeff, Bunnell & Jungherr (1954) that exudative diathesis and encephalomalacia could be induced or suppressed by dietary changes unrelated to the tocopherol content of the diet. The introduction of so-called dietary stress factors has generally involved the fat and fatty-acid components of the diet. Little attention has been paid to the diet's carbohydrate fraction as such.

In a recent review, Norris (1958) has stated that the increasing field incidence of encephalomalacia in the United States was thought to be due in part to the development of high-energy rations containing large amounts of maize. I report here the results of a series of experiments designed to compare the effects on young chicks of different levels of α-tocopherol when given in a purified diet containing wheat or maize starch as a major constituent. Each type of starch was given at two levels, roughly corresponding to those in a normal and a high-energy diet and in conjunction with four dietary levels of α-tocopheryl acetate.

EXPERIMENTAL

Treatment of chicks

The birds used in all the experiments were of a commercial broiler strain (New Hampshire–Light Sussex × White Rock) and were obtained from a hatchery as day-old male chicks. They were weighed and wing-banded on arrival and distributed at random between subtreatments. Thereafter they were weighed at weekly intervals until 6 weeks of age. The birds were caged and fed in units corresponding to subtreatments. Housing was in wire-floored cages heated electrically. Food and water were offered *ad lib.* and the weight of food eaten was recorded.

Plan of the experiments

The layout of the experiments is shown below. The four basal diets differed only in their non-protein, non-fat energy sources. Vitamin E was added as synthetic DL-α-tocopheryl acetate 10% premix (Roche Products Ltd, Welwyn Garden City, Herts), in amounts which were calculated to be equivalent to 2, 4 and 8 mg D-α-tocopheryl acetate/100 g diet on the assumption that the activity of the D form is 1·36 times that of the DL form (Ames, 1956). Dietary levels are quoted below in terms of D-α-tocopheryl acetate, and represent the sum of the D form present in the natural oil and the added DL form, corrected for its lower activity.

Expt no.	Main treatment, starch: Source	Main treatment, starch: Percentage in diet	Subtreatment, DL-α-tocopheryl acetate (mg/100 g)	No. of birds per subtreatment
1	Wheat	65	0, 2·72, 5·44, 10·88	10
2	Maize	65	0, 2·72, 5·44, 10·88	10
3	Wheat	55	0, 2·72, 5·44, 10·88	15
4	Maize	55	0, 2·72, 5·44, 10·88	15

Diets

The basal diets were designed to contain adequate amounts of all known nutrients except α-tocopherol. The level of all other vitamins was approximately twice that recommended by the (U.S.A.) National Research Council: Committee on Animal Nutrition (1954). It was found on calculation that diets containing casein and blood albumin required supplementation with glycine and arginine to bring up the levels of essential amino acids to those recommended by the National Research Council. The mineral mixture used was a modified form of the Phillips–Hart salt mixture (Bliss & György, 1951). Ground Whatman filter-paper was used as a source of roughage. The detailed compositions of the basal diets are given in Table 1.

Table 1. *Percentage composition of basal diets*

	W 65	M 65	W 55	M 55
Wheat starch	65·0	—	55·0	—
Maize starch	—	65·0	—	55·0
Casein (fat and vitamin free)*	15·0	15·0	15·0	15·0
Albumin (blood)	10·0	10·0	10·0	10·0
Arachis oil B.P.	3·0	3·0	3·0	3·0
Mineral mixture†	5·0	5·0	5·0	5·0
Ground filter-paper	2·0	2·0	12·0	12·0

To 1 kg of basal diet were added 10000 i.u. vitamin A, 1000 i.u. cholecalciferol, 2·0 mg menaphthone, 4·0 mg thiamine hydrochloride, 8·0 mg riboflavin, 50·0 mg nicotinic acid, 8·0 mg pyridoxine, 30·0 mg calcium-D-pantothenate, 0·5 mg biotin, 1·5 mg folic acid, 1 g inositol, 50 mg *p*-aminobenzoic acid, 0·04 mg cyanocobalamin, 2 g choline chloride, 0·5 g ascorbic acid, 0·125 g Nicarbazin (4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine complex, May and Baker Ltd, Dagenham), 0·02 g chlortetracycline hydrochloride, 5·2 g glycine, 0·9 g arginine hydrochloride.

* British Drug Houses Ltd, Poole, Dorset.

† Modified form of Phillips–Hart salt mixture (Bliss & György, 1951) with percentage composition: K_2HPO_4, 32·0; $CaCO_3$, 30·0; NaCl, 17·0; $MgSO_4.7H_2O$, 10·0; $CaHPO_4.2H_2O$, 7·5; $FeC_6H_3O_7.5H_2O$, 2·8; $MnSO_4.4H_2O$, 0·54; KIO_3, 0·10; $CuSO_4.5H_2O$, 0·03; $ZnCl_2$, 0·025; $CoCl_2$, 0·025; SeO_2, 0·002.

Fresh diets were prepared at frequent intervals to avoid rancidity. They were given in a crumb form to improve palatability and reduce wastage. The crumbing technique consisted of mixing with approximately 40% of water, rubbing the mixture through a sieve and drying in a hot-air cabinet at 40°.

Histological and biochemical methods

Collection of material. All surviving birds were killed at 6 weeks old and examined *post mortem*. Livers were removed, and samples of blood were taken for chemical analysis. Material not immediately required was stored at −20°. Samples of droppings were collected at random during the experimental period and dried.

Histological examination. The whole cerebellum was excised from the brain after death and divided longitudinally, one half being used for sections. The tissue was fixed in formol saline; longitudinal sections were cut from paraffin blocks and stained with haematoxylin and eosin.

Tocopherols in serum. Total tocopherols in 5 ml blood serum were determined by a modified form of the method of Blaxter & Brown (1953). The serum was extracted with light petroleum after saponification. Vitamin A, carotenoids and sterols were adsorbed on activated Floridin earth XS (British Drug Houses Ltd) and total tocopherols, i.e. total reducing substances, were measured by the reaction of Emmerie & Engel (1938). Individual tocopherols were separated in pooled samples of blood serum from each of several subtreatments by the paper-chromatographic method of Green, Marcinkiewicz & Watt (1955) after preliminary removal of lipids, sterols, carotenoids and vitamin A as outlined above. The papers were impregnated with sodium fluorescein and tocopherol 'spots' were identified under ultraviolet light.

Tocopherols in liver. Macerated livers were extracted by the method of Bloor (1914) with a 4:1 mixture of peroxide-free ethanol and diethyl ether for 10 h in the Soxhlet apparatus of Swick & Baumann (1952). Total tocopherols were determined as stated above after removal of lipids by saponification and of most of the sterols by freezing in methanol at −15° (Eggitt & Ward, 1953). Individual tocopherols were separated by the two-dimensional paper-chromatographic procedure of Green *et al.* (1955).

Fatty acids in liver. Since whole livers were required for tocopherol determinations, total fatty acids were determined after saponification. The aqueous phase after ether extraction was acidified, and the liberated acids were taken up in diethyl ether. The ethereal solution was evaporated to dryness and the residue was taken up in acetone and titrated with 0·1 N-NaOH. The results were expressed as oleic acid.

Tocopherol determinations in diet constituents and droppings. Initial extraction of lipids was carried out with peroxide-free diethyl ether for dried droppings and with peroxide-free ethanol for wheat and maize starch. Tocopherols were subsequently determined by the methods outlined for liver.

RESULTS

Rate of gain in weight and efficiency of food conversion

Summarized results for the live-weight gains and food-conversion efficiencies of each group of birds at 6 weeks are given in Table 2. The addition of α-tocopherol had no significant effect on live weight. Although comparisons between the two sources and levels of starch were confounded with other differences between experiments, consistently better live-weight gains were obtained with wheat starch than with maize starch, but the food-conversion efficiencies were consistently better on maize starch. The addition of α-tocopherol at all levels gave better food utilization only on the diets containing 65 % of starch.

A further feature was that, although the rate of feathering was slower than with normal diets, it was rather better on maize starch than on wheat starch.

Table 2. *Mean weight increases (g) and food-conversion efficiencies (g food/g weight increase) of the chicks at 6 weeks of age*

Diet	Variable	DL-α-tocopheryl acetate added (mg/100 g diet) None	2·72	5·44	10·88	No. of birds/sub-treatment
W 65	Live-weight gain	573 (9)	600 (8)	607 (9)	584 (7)	10
	Food-conversion efficiency	2·78	2·40	2·50	2·68	
M 65	Live-weight gain	446 (8)	477 (9)	492 (9)	477 (8)	10
	Food-conversion efficiency	2·67	2·27	2·50	2·36	
W 55	Live-weight gain	561 (10)	546 (13)	529 (14)	507 (12)	15
	Food-conversion efficiency	2·82	2·90	2·78	2·91	
M 55	Live-weight gain	452 (13)	475 (12)	468 (12)	444 (14)	15
	Food-conversion efficiency	2·35	2·35	2·43	2·45	
	Mean difference in live-weight gain (g)					
	Wheat-starch diet − maize-starch diet, 65 % level	127	123	115	107	—
	Wheat-starch diet − maize-starch diet, 55 % level	109	71	61	63	—

Figures in parentheses show the number of surviving birds.

Mortality

A fairly constant mortality was encountered with all treatments during each experiment (Table 3); with occasional exceptions deaths occurred during the first 2 weeks of life. By far the most frequent cause of death was exudative diathesis, sometimes associated with an accumulation of fluid in the pericardium. Except once, the condition was not accompanied by the type of haemorrhage reported by Dam & Glavind (1938) and by Ames (1956), and death generally occurred during the 2nd week of life. It was not prevented by α-tocopherol or selenium, since the incidence was unrelated to treatment. Further, the basal diet contained 0·7 p.p.m. selenium, whereas 0·1 p.p.m. selenium has been stated to reverse signs of exudative diathesis in chicks (Reid, Rahman, Creech & Couch, 1958).

Post-mortem findings

The carcasses of the birds at 6 weeks old appeared rather pale, owing to the absence of pigments from the diet, but otherwise the signs found represented minor lesions, and none was specifically related to the level of α-tocopherol in the diet. A few distended proventriculi were found in birds fed on the diets containing 12% filter-paper. The most noticeable feature at post-mortem was the heavy fat deposition in the intestinal cavity of some birds. Again the incidence bore no relation to tocopherol levels, but was related to the live weight of the bird and the energy content of the diet.

Table 3. *Mortality of chicks in all experiments*

	DL-α-tocopheryl acetate added (mg/100 g diet)			
	None	2·72	5·44	10·88
Total no. of chicks	50	50	50	50
Mortality (%)	20	16	12	18
Exudative diathesis (no. of deaths)	7	8	5	7
Feather pecking (no. of deaths)	1	—	—	—
Unabsorbed yolk sac (no. of deaths)	—	—	—	1
Cause unknown (no. of deaths)	2	—	—	1
Perosis (no. of deaths)	—	—	1*	—

* Killed.

Histological findings

No macroscopic lesions were found in any of the specimens of brain that were examined. Specimens from groups of chicks on two subtreatments, i.e. basal and basal with 2·72 mg DL-α-tocopheryl acetate/100 g, were examined for microscopic lesions attributable to vitamin E deficiency. All of the sections examined appeared normal.

Biochemical findings

Serum tocopherols. Serum tocopherols were determined in blood samples from all birds. Preliminary examination of the results showed that the spread tended to increase with tocopherol intake, and therefore the values were transposed into logarithmic form. Mean values for serum tocopherols from all treatments have been plotted in Fig. 1 and from these values the regression of serum tocopherols on dietary level has been calculated. The linear regression was found to be highly significant. The regression line has been plotted in Fig. 1.

The method of qualitative separation applied to the tocopherol in pooled samples of serum from several subtreatments showed that only α-tocopherol was present.

Tocopherols in liver. Because of the relatively small amounts of tocopherol present in the livers of birds on the basal diets, it was necessary to bulk up to four individual livers into one sample for tocopherol assay. Again only α-tocopherol was identified. Mean results for both total tocopherols and α-tocopherol in fresh liver are given in

Table 4. The effects of the different diets on the degree of liver storage of α-tocopherol have also been examined, and the results are shown in Fig. 2.

Liver fatty acids. The relationship between the dietary level of vitamin E and liver fatty acids has also been investigated. The mean values for fatty acids have been compared in Fig. 3 with the calculated regression line.

Tocopherols in diet constituents and dried droppings. Preliminary investigations were

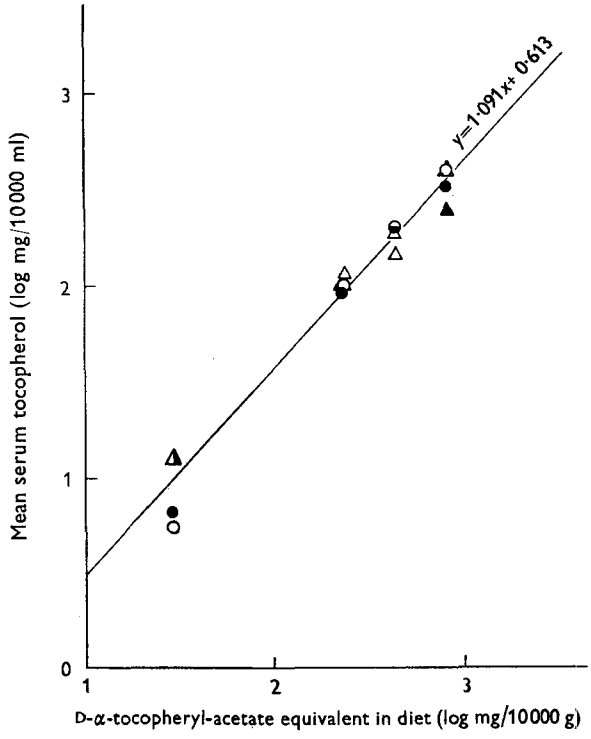

Fig. 1. Relationship between mean serum-tocopherol levels and tocopherol intake of young chicks in all experiments. ○, diet W 65; ●, diet M 65; △, diet W 55; ▲, diet M 55.

Table 4. *Mean values for storage of tocopherol in the liver (μg/g fresh liver) by chicks on the different diets*

		DL-tocopheryl acetate added (mg/100 g diet)			
Diet	Tocopherol	None	2·72	5·44	10·88
W 65	Total	2·0	4·3	8·6	20·6
	α	0·47	3·3	6·5	19·9
M 65	Total	2·38	6·9	9·6	28·5
	α	0·84	4·41	9·5	26·2
W 55	Total	2·80	7·0	9·3	14·0
	α	0·34	3·98	6·62	11·9
M 55	Total	2·0	8·2	11·8	21·4
	α	0·50	6·0	10·2	18·2

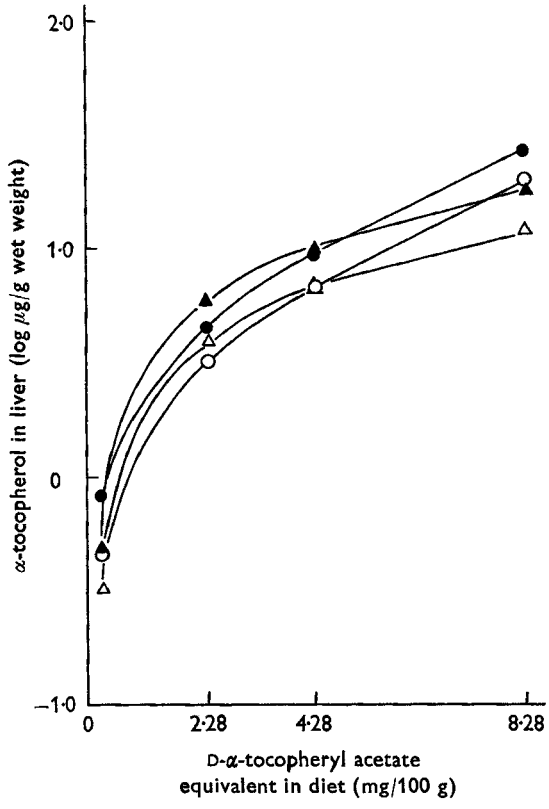

Fig. 2. Relationship between dietary level and storage of α-tocopherol in the liver by young chicks on diets W 65 (○), M 65 (●), W 55 (△) and M 55 (▲) (showing the effects of source and percentage of starch in the diet).

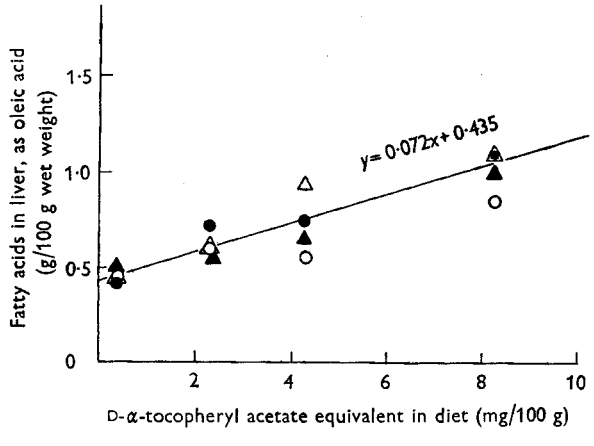

Fig. 3. Effect of increasing tocopherol intake by young chicks on concentration of fatty acids in the liver in all experiments. ○, diet W 65; ●, diet M 65; △, diet W 55; ▲, diet M 55.

carried out into the excretion of tocopherols in the droppings of the birds on some subtreatments. The values found (mg α-tocopherol/100 g dried droppings) were:

	Added DL-α-tocopheryl acetate/100 g diet		
	2·72 mg	5·44 mg	10·88 mg
Diet W 55	1·30	1·95	6·80
Diet M 55	0·81	1·00	2·56

No other tocopherols were identified in the droppings.

A sample of arachis oil assayed for vitamin E activity by the method of Eggitt & Ward (1953), as modified by Green *et al.* (1955), contained:

α-tocopherol	0·095 mg/g
γ-tocopherol	0·118 mg/g

γ-Tocopherol appears to be inactive for the chick (Harris & Quaife, 1952) and its contribution has been disregarded.

No vitamin E activity could be found in either the wheat or the maize starch used, although they contained 0·76 and 0·83 % total lipids, respectively.

DISCUSSION

The figures in Table 2 for the live-weight increases do not show any regular pattern, apart from an indication that slightly better gain in weight and food utilization resulted from the addition of vitamin E to the diets higher in energy, and the consistently better rates of weight increase on the wheat-starch diets. These were not expected but, as has already been pointed out, each source and level of starch was examined in a separate experiment, and some other differences between the experiments may have been in part responsible for the differences between the weight increases on wheat and maize diets. The highest overall weight increases and food conversion efficiencies were obtained with the addition of 2·72 mg DL-α-tocopheryl acetate/100 g diet, which agrees with the findings of Singsen, Bunnell, Matterson, Kozeff & Jungherr (1955) that between 1·6 and 2·4 mg vitamin E/100 g diet is required by the chick under conditions of stress. However, no addition of tocopherol resulted in a significantly greater increase in weight. Singsen *et al.* (1955) consider the requirements in the absence of stress to be less than 0·7 mg/100 g.

Briggs, Fox & Bieri (1956) found no difference in gain in weight between young chicks given a purified diet with or without a supplement of vitamin E. On the other hand, Patrick & Morgan (1943) had previously shown that chicks increased in weight almost twice as fast when DL-α-tocopherol was added at the rate of 0·3 mg/100 g to a basal diet freed of vitamin E by prolonged extraction with hexane, and that food utilization was also improved.

It must be remembered that the basal diets used by me contained small amounts of tocopherols from the arachis oil.

If the contribution of tocopherols other than α is ignored as of doubtful biological value (Ames, 1956) then the basal diet contained 0·28 mg D-α-tocopherol/100 g. This content is similar to that in the supplemented diet used by Patrick & Morgan (1943)

and probably also of the same order as that present in the basal diet of Briggs *et al.* (1956) since this diet was not extracted.

The low incidence of exudative diathesis has already been mentioned. The diathesis occurred earlier than has been previously reported (Dam & Glavind, 1938; Ames, 1956) and was unaccompanied by acute haemorrhage. It was not prevented by α-tocopherol or selenium (cf. Reid *et al.* 1958) and has been found in birds with high levels of vitamin E in liver and blood serum.

No encephalomalacia occurred, nor was there histological evidence to suggest any disturbance of brain tissue attributable to lack of vitamin E.

The serum-tocopherol levels of all birds on the basal diets were lower than the value of 500 μg/100 ml quoted by Zacharias *et al.* (1950) as that below which signs of encephalomalacia could occur in chicks fed on certain diets. In fact, in a few individuals no tocopherol at all could be detected in the blood serum. If the above-mentioned figure is accepted as a criterion of safety, then it is attained by a dietary level of 1·3 mg/100 g diet (see Fig. 1). Markson, Carnaghan & Parr (1957) have recently shown that there was no significant difference between liver or serum-tocopherol values of normal and diseased chicks and that other factors were concerned in precipitating the disease.

The relationship in poultry between the logarithms of the values for serum tocopherols and for dietary tocopherol levels appears to be linear in these experiments. Rousseau, Dicks, Teichman, Helmboldt, Bacon, Prouty, Dolge, Eaton, Jungherr & Beall (1957) working on calves, lambs and pigs concluded that the relation between dietary and plasma tocopherols could be expressed as a linear regression of log (plasma tocopherol) on log-log (tocopherol intake), although for pigs the fit of log (plasma tocopherol) on log (tocopherol intake) was slightly superior.

In previous work (Bunnell, Matterson, Singsen & Eaton, 1956; Bunnell, 1957; Markson *et al.* 1957) the storage of vitamin E in liver has been reported in terms of total tocopherols, i.e. total reducing substances, rather than as α-tocopherol itself, but for purposes of comparison both values are presented in Table 4. It is considered that true liver storage is represented by α-tocopherol only. Total tocopherols give a reliable indication of status only when relatively large amounts of α-tocopherol are present. Since only α-tocopherol was identified by paper chromatography the differences between the two sets of values represent other reducing substances not completely removed during early stages of the assay procedure.

Rousseau *et al.* (1957) and Bunnell (1957) have previously shown that a linear relationship exists between dietary intake and total tocopherol storage in liver for several species. In my experiments the relationship between dietary intake and α-tocopherol storage in the liver appeared to be linear only on the diets with 55% starch.

Examination of the relationship between dietary intake and the logarithm of the value for α-tocopherol storage in the liver (see Fig. 2) reveals that at all levels of dietary α-tocopherol examined the storage on maize was greater than on wheat, and by a constant proportion that appears to depend on the level of starch in the diet.

Greater weight gains by chicks on wheat-starch diets have not to my knowledge

been reported by other workers. The differences in the rates of liver storage might be in part a reflection of the differences in live weight and food consumption. It has been pointed out by Bunnell *et al.* (1956) and Markson *et al.* (1957) that the rate of liver storage of vitamin E decreases with age and presumably with live weight, from birth to 6–8 weeks of age. The mean fresh-liver weights of birds on the maize-starch diets was 16·6 g, compared with 20·0 g on the wheat-starch diets; further, from the limited amount of information available on excretion levels it would appear that utilization of tocopherols is more efficient on a maize-starch diet.

The increase in liver fatty acids with increasing α-tocopherol storage might be due to either or both of two causes:

(*a*) fatty material is taken into the liver as a carrier for tocopherol;

(*b*) α-tocopherol is stored in the liver in esterified form, probably as tocopheryl acetate.

Table 5. *Distribution of α-tocopherol in blood and liver of chicks on the different diets*

Diet	D-α-tocopheryl acetate equivalent in diet* (mg/100 g)	Total food intake (g)	Total intake of α-tocopherol (mg)	Total tocopherols† in serum (mg)	α-Tocopherol in liver (mg)	Recovered α-tocopherol: Serum† (%)	Recovered α-tocopherol: Liver (%)	Percentage not accounted for
W 65	0·28	1587	4·5	0·04	0·01	0·89	0·22	98·9
	2·28	1435	32·8	0·60	0·08	1·83	0·24	97·9
	4·28	1514	64·6	1·15	0·13	1·78	0·21	98·0
	8·28	1569	130·0	2·32	0·44	1·78	0·33	97·9
M 65	0·28	1183	3·3	0·03	0·02	0·90	0·60	98·5
	2·28	1080	24·7	0·42	0·09	1·70	0·36	97·9
	4·28	1236	52·8	0·96	0·19	1·82	0·36	97·8
	8·28	1128	93·5	1·49	0·48	1·60	0·51	97·9
W 55	0·28	1580	4·4	0·08	0·01	1·80	0·23	98·0
	2·28	1585	36·2	0·53	0·08	1·46	0·22	98·3
	4·28	1430	61·1	0·75	0·11	1·23	0·18	98·6
	8·28	1415	117·5	1·16	0·18	0·98	0·16	98·9
M 55	0·28	1058	3·0	0·06	0·01	2·0	0·33	97·7
	2·28	1111	25·4	0·59	0·10	2·30	0·39	97·3
	4·28	1117	47·6	0·81	0·16	1·70	0·34	98·0
	8·28	1087	90·0	1·82	0·24	2·02	0·27	97·7

* See p. 270.

† Taken as α-tocopherol.

Tocopherols are known to be stored in most organs and tissues of the body, without a main deposition site (Harris & Mason, 1955), but blood and liver contents of tocopherol have been used as indications of vitamin E status. From the values recorded above, some figures for the quantitative distribution and recovery of tocopherols have been calculated (Table 5). For the purposes of these calculations total blood volume has been taken as equivalent to 9% of live weight (Sturkie, 1954). It can be seen that, irrespective of source of starch or level of tocopherol intake, only about 2% of the ingested vitamin E was found in blood and liver. Calculations based on the figures given by Johnson, Carrik & Hauge (1948) show that the efficiency of liver

storage of vitamin A is more than ten times that of vitamin E. There was no evidence of better utilization of dietary tocopherol at any particular dietary level or any indication of relative depletion of liver storage on the basal diets low in vitamin E.

Chemical, histological and biochemical evidence supports the view that, under the conditions of stress imposed by these experiments, the requirement of young chicks for maintaining a reasonable rate of weight increase from birth to 6 weeks of age is less than 0·3 mg α-tocopherol/100 g diet. The biochemical findings suggest that in general a linear relationship exists between vitamin E intake and body storage, and this observation may prove useful in planning further experimental work.

SUMMARY

1. Groups of ten or fifteen day-old chicks were fed for 6 weeks on a series of purified basal diets to which DL-α-tocopheryl acetate was added at various levels. The basal diets were low in vitamin E and differed only in that they contained either 65 or 55% of wheat or maize starch. Live weight and food consumption were recorded. The concentrations of tocopherols in the serums and livers were determined at 6 weeks of age.

2. Addition of vitamin E did not result in significantly faster live-weight gains. Better weight gains occurred on both the wheat-starch diets than on the maize-starch diets, but food utilization was generally better on maize starch.

3. A low incidence of exudative diathesis occurred on all diets. It was a type not prevented by vitamin E or selenium. No other signs attributable to vitamin E deficiency were found.

4. A linear relationship was found to exist between the logarithms of the values for serum tocopherol and dietary vitamin E levels.

5. A higher level of α-tocopherol storage in liver was found on the maize-starch than on the wheat-starch diets. Increasing the starch content of the diet modified the storage pattern of α-tocopherol.

6. It is concluded that the efficiency of liver storage of vitamin E is low, and not influenced by changes in the dietary level.

7. Under the conditions of the experiments reported here the vitamin E requirement of young chicks was less than 0·3 mg/100 g diet.

I am grateful to Dr J. L. Williams for helpful suggestions and to Mr M. W. Barwick for the histological examination of brain specimens.

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Printed in Great Britain