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Influence of the level of dietary linoleic acid on the amount of d-α-tocopherol acetate required for protection against encephalomalacia

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With 1 figure and 3 tables

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In earlier studies (1) we have found that diets containing 30% lard, corresponding to a dietary level of linoleic acid between 1.5 and 1.6%, are suitable for production of encephalomalacia in young chicks under the conditions usually employed in our laboratory.

Under these conditions, an incidence of encephalomalacia of 50% of the chicks could be obtained with a dietary level of 0.37 mg d-α-tocopherol acetate per 100 g diet.

In the present experiments we have examined the question whether the requirement for d-α-tocopherol acetate increases when the diet contains higher amounts of linoleic acid than used in the above-mentioned study.

To this end we have recorded the incidence of encephalomalacia in chicks reared on diets with different levels of linoleic acid and different levels of d-α-tocopherol acetate.

Experimental

The different levels of dietary linoleic acid were provided in the form of lard and lard supplemented with corn oil or sunflower seed oil freed from tocopherols by adsorption on filtrol.

The adsorption procedure was carried out in the following way: Corn oil and sunflower seed oil were dissolved in petroleum ether, b. p. 50–60 °C, 20 vol. oil in 100 vol. of the solution. The solutions were passed through approximately 7 cm layers of activated filtrol (from Filtrol Corporation, Vernon, Calif., U.S.A.) on glass fritted filters under light suction. The filtrol was activated by being heated at 100 °C for 48 hours. Approximately 500 g filtrol was used for 2 liter solution. The filtrate was tested for reducing material with EMMERIE-ENGEL reagent, and when the test was positive, filtered again through activated filtrol until the test was negative. The filtrol treated solution was concentrated by distilling off the main part of the petroleum ether, whereafter the rest of the solvent was removed in vacuo.

The fatty acid pattern of the filtrol treated oils was determined by gas-liquid chromatography on a Podbielniak Chromacon apparatus with a diethyleneglycol-succinate phase, coated on acid-washed Chromosorb W (15% by weight, 80–100 mesh).

The experimental diets were modifications of diet no. 4 in our previous paper (1). The compositions, except the additions of d-α-tocopherol acetate, are indicated in table 1.

The additions of d-α-tocopherol acetate are indicated in table 3.

Weighed amounts of d-α-tocopherol acetate were dissolved in ethyl ether and mixed with casein, whereafter the ether was evaporated at room temperature. Portions of the casein supplemented with tocopherol acetate in this way were mixed with unsupplemented casein in amounts adjusted to secure the desired levels of tocopherol acetate and mixed

with the other non-lipid constituents of the diet. Lard and filtrol treated oils were then added in the proportions indicated. Amounts of diet sufficient for one week were prepared at a time and stored in closed containers at minus 20 °C.

Table 1. Composition of the experimental diets

Diet no.	I	II	III	IV	V
	g	g	g	g	g
Casein, Vitamin Test[1])	30	30	30	30	30
Gelatin	3	3	3	3	3
Salt mixture[2])	5.17	5,17	5,17	5.17	5.17
Vitamin B mixture[2])	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.2	0.2	0.2	0.2	0.2
Corn starch	31.53	31.53	31.53	31.53	31.53
Lard[3])	30	27	24	27	22
Filtrol-treated corn oil		3	6		
Filtrol-treated sunflower seed oil				3	8
	100.00	100.00	100.00	100.00	100.00
Vitamin K substitute[4])	1 mg	1 mg	1 mg	1 mg	1 mg
Selenium dioxide	0.14 μg	0.14 μg	0.14 μg	0.14 μg	0.14 μg

[1]) From Genatosan Ltd., Loughborough, England. [2]) DAM and SØNDERGAARD (2). [3]) From Andelssvineslagteriet, Hillerød, Denmark. [4]) Synkavit, Roche (di-calcium salt of 2-methyl-1,4-naphthohydroquinonediphosphoric acid ester).

Vitamins A and D_3 were given in the form of 0.1 ml of an aqueous solution (3) twice a week, corresponding to 250 i.u. vitamin A and 20 i.u. vitamin D_3 per day.

Table 2. Fatty acid composition of dietary fats. Individual fatty acid methyl esters as per cent of total fatty acid methyl esters[1])

Dietary fat	Lard	Corn oil [3])	Sunflower seed oil [3])
Fatty acid[2])			
14:0	1.2	0	tr
16:0	33.2	10.21	6.81
16:1	4.0	0.76	1.43
18:0	14.4	1.92	4.41
18:1	38.7	28.32	29.13
18:2	6.6	56.42	55,77
18:3		2.37	1.40
20:1	2.0	0	0.51
22:0 (or:1)		tr	0.61

[1]) These analyses were carried out by Mrs. G. HØLMER. [2]) Number of carbon atoms and double bonds. [3]) Filtrol treated

Day-old chicks were kept in brooders with wire screen bottoms and given the vitamin E-free starter ration indicated in our previous paper (1) for 6 days, whereafter they were

Table 3. Results of the feeding experiments

	Experiment no. 1			Experiment no. 2			
Diet no.	I	II	III	I	IV	V	
Calculated level of linoleic acid g 100 g	1.95	3.37	4.79	1.95	3.35	5.69	
d-α-tocopherol-acetate[1]) mg/100 g	Incidence of encephalomalacia: Number of chicks with encephalomalacia in groups of 10 chicks						Average incidence if encephalo-malacia
0	9			8			
0.193	6			8	5	8	6.7
0.269	7	4		5	9	5	6.0
0.373	5	3	4	3	6	3	4.0
0.519	3	3	0	4	4	1	2.5
0.721	0	0	0	2	0	3	0.83
1.00	0	1	0	1	0	0	0.33
1.39	0	0	0	0	0	0	0
1.93		0	0		0	0	0
2.69		0	0				
3.73			0				

[1]) Obtained from Distillation Products Industries, Division of Eastman Kodak Company, Rochester, N. Y., U.S.A.

divided into groups of 10 and given the experimental diets for 38 to 40 days. They were inspected daily; those showing signs of encephalomalacia were killed by decapitation and autopsied. Chicks not having shown signs of encephalomalacia were killed in the same way at the end of the feeding period. Encephalomalacia was diagnosed by the clinical signs and the macroscopic examination of the cerebrum and cerebellum.

Results and discussion

Table 2 shows the results of the gas-liquid chromatographic analyses of the fatty acid pattern of the three fats used in the diets. The fatty acid pattern is expressed as area per cent of the methyl esters. Considering the quantity of nonsaponifiable matter in the fats as negligible, the amount of linoleic acid in the diets can be calculated from the data in table 2, the dietary levels of the fats indicated in table 1, and a correction for the small amount of linoleic acid contained in the corn starch. As shown in our previous paper (1), 31.53 g corn starch furnishes 0.07 g linoleic acid per 100 g diet. The lard used in the present experiment had a somewhat higher content of linoleic acid than that used previously (1).

The calculated levels of linoleic acid in the diets and the incidences of encephalomalacia found at the different levels of d-α-tocopherol acetate are presented in table 3.

If a constant proportion between tocopherol acetate and linoleic acid was required in order to obtain a given value for the incidence of encephalomalacia, then the values for the incidences obtained with diets III and V would have required more than twice the amount of tocopherol acetate necessary for obtaining the same incedences with diet I.

It appears from table 3 that this is not the case. On the contrary, it seems that the variations of the incidence of encephalomalacia found at a given level of d-α-tocopherol acetate are independent of the dietary levels of linoleic acid used.

In other words, it seems justified to calculate average values of the incidences of encephalomalacia found at a given level of tocopherol acetate independently of the level of linoleic acid.

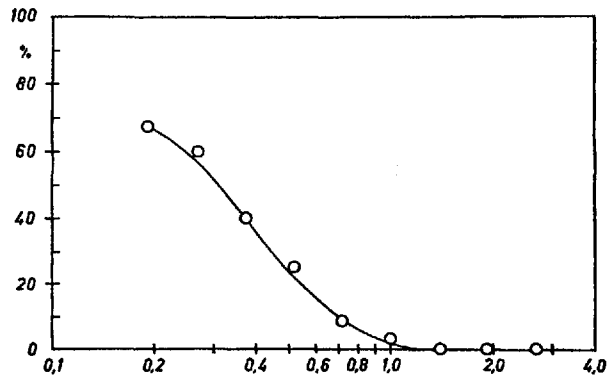

Fig. 1. Average incidence of encephalomalacia as a function of the level of dietary d-α-tocopherol acetate. Logarithmic scale. Vertical axix: incidence of encephalomalacia (per cent). Horizontal axis: dietary level of d-α-tocopherol acetate (mg per 100 g diet). – For details, see text.

In fig. 1, these average values are plotted against the levels of tocopherol acetate, the latter in logarithmic scale. It is seen the average values of the incidences are lying on a smooth curve, and that a 50% incidence of encephalomalacia corresponds approximately 0.32 mg d-α-tocopherol acetate per 100 g diet. The curve is similar to that found in our previous study (1) for the incidences of encephalomalacia obtained with varying levels of tocopherol acetate, but with one level of linoleic acid (1.57%). Then a 50% incidence of encephalomalacia was obtained with 0.37 mg d-α-tocopherol acetate per 100 g diet, a result which is in fairly good agreement with that of the present study. (It is of minor importance for the conclusions drawn from these studies that somewhat higher values for the level of tocopherol acetate corresponding to a 50% incidence of encephalomalacia might have been found if the feeding period had been prolonged considerably.)

Encephalomalacia does not occur in the absence of dietary fatty acids of the linoleic acid series (4). Therefore, in experiments in which the encephalomalacia producing agent is linoleic acid, the incidence of the disease will increase as the content of linoleic acid in the diet increases from zero up to a certain level. In this range, the amount of tocopherol acetate necessary for prevention of encephalomalacia will increase with the level of linoleic acid, since the requirement must be correlated with the ability of the unsupplemented diet to produce the disease. But beyond this range, the requirement for tocopherol acetate apparently remains constant.

The level of linoleic acid beyond which the requirement for tocopherol acetate remains constant is probably not much different from that used in our

previous study (1), viz. 1.57%, since in that study lower levels of linoleic acid lessened the occurrence of encephalomalacia among chicks on the unsupplemented diet.

The reason why the requirement for tocopherol acetate remains constant beyond a certain level of linoleic acid might be that there is a limit for the conversion of linoleic acid to arachidonic acid. In earlier studies (4), we found that encephalomalacia developed faster with 1.5% ethyl arachidonate than with 1.5% ethyl linoleate and with 30% lard. If the present experiments had been carried out with arachidonic acid instead of linleic acid it is likely that the requirements for tocopherol acetate would have been higher, and, if a limit for the requirement had been reached, it would probably be determined by the amount of arachidonic acid (or its metabolites) that can be incorporated into lipids in the brain.

It is not known whether results similar to those of the present study can be applied to other manifestations of vitamin E deficiency than encephalomalacia.

Summary

The incidence of encephalomalacia in chicks reared on diets with different levels of linoleic acid and d-α-tocopherol acetate were recorded.

The experimental feeding began when the chicks were 6–7 days old and lasted 38 to 40 days.

Two experiments were carried out, each with 24 groups of 10 chicks.

In the first experiment, the different levels of linoleic acid were provided in the form of lard and lard supplemented with corn oil freed from tocopherol by adsorption on filtrol; in the second experiment, the linoleic acid levels were provided in the form of lard and lard supplemented with sunflower seed oil freed from tocopherol in the same way. The level of total fat, 30%, was the same in all diets.

The dietary levels of linoleic acid were: in the first experiment, 1.95, 3.37, and 4.79 per cent; in the second experiment, 1.95, 3.35, and 5.69 per cent.

Eleven different levels of d-α-tocopherol acetate ranging from 0 to 3.73 mg per 100 g diet were used.

Within the range of linoleic acid levels used, the incidences of encephalomalacia were determined by the levels of d-α-tocopherol acetate and independent of the levels of linoleic acid.

Mean values of the incidences of encephalomalacia found at a given level of d-α-tocopherol acetate were found to lie on a smooth curve when plotted against the logarith of the tocopherol level.

Zusammenfassung

Gruppen von Küken wurden mit Nahrungen mit verschiedenen Höhen von Linolsäure und d-α-Tocopherolacetat verfüttert. Die Häufigkeit des Auftretens von Encephalomalazie in den verschiedenen Gruppen wurde ermittelt.

Die Experimentalfütterung wurde angefangen, wenn die Küken 6 bis 7 Tage alt waren und dauerte 38–40 Tage.

Zwei Versuchsreihen, jede mit 24 Gruppen von 10 Küken wurden ausgeführt.

Die Beibringung der verschiedenen Höhen von Linolsäure erfolgte in der ersten Versuchsreihe mit Hilfe von Schweineschmalz und Schweineschmalz versetzt mit Maisöl, das vorher durch Adsorption auf Filtrol von Tocopherol befreit worden war; in der zweiten Versuchsreihe wurden zu dem selben Zweck Schweineschmalz und Schweineschmalz versetzt mit in ähnlicher Weise von Tocopherol befreitem Sonnenblumöl, verwendet. Der Gehalt an Gesamt-Fett war in allen Nahrungen 30%.

Die verschiedenen Höhen von Linolsäure waren in der ersten Versuchsreihe 1,95, 3,37 und 4,79%; in der zweiten Versuchsreihe 1,95, 3,35 und 5,69%.

Elf verschiedene Höhen von d-α-Tocopherolacetat im Bereich von (einschließlich) 0 mg bis 3,73 mg per 100 g Nahrung wurden verwendet.

Innerhalb des Bereichs der verwendeten Höhen von Linolsäure waren die Häufigkeiten von Encephalomalazie in den verschiedenen Gruppen durch die Höhen des d-α-Tocopherolacetats bestimmt und abhängig von den Höhen von Linolsäure.

Die Mittelwerte der bei einer gegebenen Höhe von Tocopherol gefundenen Häufigkeit von Encephalomalacie liegen auf einer gleichmäßig verlaufenden Kurve, wenn dieselben gegen den Logarithmus der Tocopherolhöhe graphisch aufgeführt werden.

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Thermo-analytische Untersuchungen von Sterinen

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Mit 9 Abbildungen und 10 Tabellen

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Über die aromatischen Crackprodukte von Sterinen wurde von L. SCHMID und seinen Mitarbeiter veröffentlicht (1, 2, 3). Die Verfasser bestätigten, daß die untersuchten Stoffe (Cholesterin, Phytosterin und Ergosterin) verschiedene, aromatische Produkte lieferten, wenn diese einem Crackprozeß unterworfen wurden. Unter diesen waren Phenantrenabkömmlinge, Zersetzungsprodukte des Cholansystems, sogar auch Methylcyclophenantren.

Inzwischen haben wir mehrere thermo-analytische Untersuchungen mit dem Gerät „Derivatograph" durchgeführt (4, 5, 6), deren Ergebnisse uns veranlaßten, die Zersetzung von Steroiden auch thermo-analytisch zu untersuchen. Damit konnten wir viele analytische und andere Fragen lösen.

Über die Thermo-analyse, bzw. das Derivatograph findet man in der Fachliteratur viele Publikationen (7, 8), hier möchten wir über unseres Gerät nur eine kurze Zusammenfassung geben.

Das Gerät ist eigentlich eine thermo-analytische Waage, welche zur gleichen Zeit drei Funktionen erfüllt:

1. Durch das Gerät wird die Gewichtsveränderung des zur Untersuchung eingewogenen Stoffes unter der Hitzeeinwirkung kontinuierlich registriert, Kurve TG.

2. Ebenso, wird die Temperaturveränderung (Kurve T) während des Aufheizen des elektrischen Gerätsofen, durch welchem man die Hitzeeinwirkung auf den zu untersuchenden Stoff zustande bringt, registriert. Aber nicht nur die Temperaturmessung des Ofenraumes wird durchgeführt, sondern auch dieselbe im Inneren des untersuchenden Stoffes und auch eines im Raum des Ofens anwesenden inerten Stoffes, Al_2O_3; die Temperatur des ersten läuft abhängend von dem exo-, bzw. endothermen Weg der Reaktionen vorwärts oder bleibt