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Anti-vitamin E activity of isolated soy protein for the chick*)

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With 6 tables

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Isolated soybean protein has been used as a major protein source in our laboratory in the formulation of semi-purified diets for chicks, rats and rabbits. Recently, we made what we believed to be minor changes in the chick diet and subsequently encountered considerable mortality and typical vitamin E deficiency symptoms apparently caused by an interaction between certain soy protein preparations and refined corn oil.

Experimental

Day-old male chicks of the Vantress breed or from a cross between Columbian females and New Hampshire males were used for these studies in single or duplicate lots of 7 to 10 birds per treatment. The duration of the experiments varied between 25 and 28 days. The basic diets had the following composition in percent: Isolated soy protein, 30.0–35.0; corn starch, 28.0; mineral mix, 4.9[1]); corn oil, 0–10.0; fiber, 3.0; DL-methionine, 0.3–0.7; choline chloride (70% concentrate), 0.3; vitamin mixture, 0.2[2]); glucose to 100.

Except for the last two experiments, vitamin E was provided at a level of 5 mg D-α-tocopheryl succinate/kg basal diet; in the last experiment 5 mg/kg diet DL-α-tocopheryl acetate was added to the basal diet; in the next to the last experiment the basal diet was devoid of vitamin E. The isolated soy protein was produced by Skidmore Enterprises (Cincinnati, Ohio) or, in one instance, by their predecessors, Archer Daniels Midland Corporation. The refined corn oil (A. E. Staley Co., Decatur, Illinois) provided, according to our assay, 1280 mg reducing substances/kg oil (Ferric chloride-Dipyridyl method); if DAM's (1) tocopherol frationation data for corn oil are applicable, this oil should have provided approximately 8 mg α-tocopherol when added to the diet at a level of 5%.

Results and discussion

The standard, semi-purified chick diet in use for many years in our laboratory contained 30% isolated soy-protein and 3% corn oil. This composition was modified, for reasons unrelated to the present studies, to simulate the diet of

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[1]) Composition of Mineral Mix in gm./kg diet: $Ca_3(PO_4)_2$, 8.5; KH_2PO_4, 10.5; NaCl, 8.0; $CaCO_3$, 19.0; $Fe[HOCH_2-(CHOH)_4CO_2]_2$, 0.52; $MgSO_4$, 2.5; $MnSO_4 \cdot H_2O$, 0.2; KI, 0.01; $CuSO_4$, 0.0128; $ZnCO_3$, 0.1; $Na_2MoO_4 \cdot 2H_2O$, 0.01.

[2]) Composition of Vitamin Mix in mg/kg diet: Thiamine HCl, 25; riboflavin, 16; Ca pantothenate, 20; pyridoxine HCl, 6; biotin, 0.6; folic acid, 4; menadione, 5; vitamin B_{12}, 0.02; ascorbic acid, 250; niacin, 150; vitamin A, 10,000 IU; vitamin D_3, 600 IU.

RITCHEY et al. (3) which contained 35% isolated soy protein. With this modified diet, considerable mortality was observed despite the fact that it provided approximately 10 mg/kg α-tocopherol, about half as the pure vitamin, and the other half from the corn oil. At this time the mortality had not been recognized as caused by a vitamin E deficiency.

In the first experiment of this series, the level of corn oil in the diet was increased from 3 to 5% in the belief that the formulation would be improved through the addition of more calories in relation to the relatively high protein content. A comparison was then carried out between 30 and 35% isolated soy protein, with and without the addition of whole egg powder as a source of high quality protein and other nutrients.

The results (Table 1) showed an accentuation of the mortality (in comparison with earlier observations) with either 30 or 35% isolated soy protein, apparently due to the increased corn oil content (from 3 to 5%) and a significant growth improvement and prevention of mortality upon addition of the whole egg powder. Most of the mortality occurred during the fourth week of feeding these diets; symptoms were primarily nervous in appearance and were later identified histologically as encephalomalacia. Complete identification of the disorder as a vitamin E deficiency had not been made, primarily because the 13 mg/kg α-tocopherol provided in the diet should have been adequate to prevent vitamin E deficiency.

Table 1. Effect of egg powder supplementation on body weight and chick mortality of chicks fed isolated soy-protein

Diet	4-week body weight[2]) (gm.)	Mortality[3])
30% Isolated soy-protein[1])	352 ± 8	10/20
+ 5% Whole egg powder	440 ± 8	2/20
35% Isolated soy-protein	328 ± 20	9/20
+ 5% Whole egg powder	383 ± 14	0/20

While the next experiment was in progress, the disorder was identified as vitamin E deficiency with some degree of certainty and it was decided to check the chemical potency of the D-α-tocopheryl succinate used in the basal diet; it tested within 90% of the stated potency.

In the second experiment, isolated soy protein was compared to casein relative to vitamin E deficiency symptoms and mortality. The results (Table 2) showed better growth, no mortality and no symptoms of vitamin E deficiency for the chicks on the casein diet, while the symptoms and mortality noted in the earlier experiments were again encountered on all levels of the isolated soy protein diets.

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[1]) Assay protein C-1 (Skidmore Enterprises) basal diet contained 5% corn oil and 5 mg/kg D-α-tocopheryl succinate.

[2]) Average ± SE for duplicate groups of 10 male chicks from a cross between Columbian ♀ × New Hampshire ♂.

[3]) Mortality occurred almost exclusively during 4th week.

Table 2. Effect of protein source and level on body weight and mortality from vitamin E deficiency

Dietary protein	Amino acid supplement	4-week body weight[2]) (gm.)	Mortality
Isolated soy-protein, 25%[1])	DL-methionine, 0.20%	314 ± 22	3/20
30%	0.25%	365 ± 11	6/20
35%	0.30%	320 ± 16	7/20
40%	0.35%	314 ± 15	5/20
Crude casein, 25%	DL-methionine, 0.2 % L-arginine HCl, 1.0 % glycine, 1.0 %	424 ± 11	0/20
30%	DL-methionine, 0.25% L-arginine HCl, 1.2 % glycine, 1.2 %	460 ± 15	0/20
35%	DL-methionine, 0.30% L-arginine HCl, 1.4 % glycine 1.4 %	400 ± 15	0/20
40%	DL-methionine, 0.35% L-arginine HCl, 1.6 % glycine, 1.6 %	433 ± 12	0/20

By this time it had become obvious that the mortality and other symptoms were those of classical vitamin E deficiency (encephalomalacia, exudative diathesis) and that isolated soy protein was implicated in its initiation.

Experiment 3 was designed to elucidate further the nature of the disorder by comparing a 30% isolated soy protein diet that produced vitamin E deficiency with various other dietary variations based on the vitamin K-low diet of GRIMINGER (4), which, though it also provided 30% isolated soy protein, did *not* cause any mortality or other vitamin E deficiency symptoms.

The differences between the vitamin K assay diet and the present diet which produced severe vitamin E deficiency included a menadione-free vitamin mix, a small amount of dextrin (5%) and of glycine (.4%) and considerably less corn oil (1 vs. 5%) for the former diet. Table 3 clearly pinpoints the vitamin E deficiency to an interaction between isolated soy protein and the higher of the two levels of corn oil. No mortality or symptoms of vitamin E deficiency were noted on any of the diets that did not provide 5% corn oil. The two diets that did, produced severe mortality and vitamin E deficiency.

In the next experiment, various vitamin E active substances were compared as supplements to a diet providing 30% isolated soy protein (from 2 sources), 5% corn oil and 5 mg/kg D-α-tocopheryl succinate. Since growth had been significantly affected by the vitamin E deficiency in the earlier experiments, it was thought of interest to use a faster growing breed of chicks (Vantress) for these final experiments.

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[1]) Assay protein C-1 (Skidmore Enterprises); basal diet also contained 5% corn oil and 5 mg/kg D-α-tocopheryl succinate.

[2]) Average ± SE for duplicate groups of 10 male chicks from a cross between Columbian ♀ and New Hampshire ♂.

These chicks were more sensitive to the dietary stress than the Columbian-New Hamshire chicks (Table 4) as illustrated by the appearance of vitamin E deficiency symptoms (both encephalomalacia and exudative diathesis) even on the diet which provided only 1% corn oil, which had not produced the disorder with the slower growing breed of chicks (see Table 3).

Table 3. Comparison of two diets containing similar amounts of isolated soy-protein on body weight gain, mortality and exudative diathesis of chicks

Dietary variables[1])			4-week body weight[2]) (gm.)	Mortality	Exudative diathesis[3])
Vitamin mix[4])	Corn oil	Other			
A + menadione	1%	Glycine, 0.4%	261 ± 7	0/18	0
B	1%	Glycine, 0.4%	258 ± 6	0/18	0
A + menadione	1%	Glycine, 0.4% +dextrin, 5 %	280 ± 6	0/18	0
A + menadione	5%	Glycine, 0.4%	251 ± 19	7/18	7/18
B	5%	Dextrin, 5 %	255 ± 28	11/18	2/18

Table 4. Effect of corn oil, antioxidant, selenium and α-tocopherol on growth and vitamin E deficiency symptoms of chicks on an isolated soy-protein diet

Source of soy-protein[5])	Diet Supplements Corn oil	Vitamin E active compound	25-day body weight[6]) (gm.)	Vitamin E deficiency symptoms[7])
A	1%	5 mg/kg D-α-tocopheryl succinate	360 ± 26	3/14
A	5%	5 mg/kg D-α-tocopheryl succinate	403 ± 21	4/14
A	5%	50 mg/kg DL-α-tocopheryl succinate[9])	438 ± 17	0/14
A	5%	0.02% ethoxyquin[8,9])	445 ± 24	0/14
A	5%	1 mg/kg selenium dioxide[9])	383 ± 29	6/14
B	5%	5 mg/kg D-α-tocopheryl succinate	365 ± 23	9/14
B	5%	50 mg/kg DL-α-tocopheryl acetate[9])	463 ± 19	0/14

[1]) Diets contained 30% isolated soy-protein (Skidmore Enterprises) and 5 mg/kg D-α-tocopheryl succinate.

[2]) Average ± SE for duplicate groups of 9 males from a cross between Columbian ♀ and New Hampshire ♂.

[3]) Number of chicks with exudative diathesis are in addition to those that died, some of which also showed vitamin E deficiency symptoms.

[4]) Mix A was the same as Mix B except that it was devoid of menadione.

[5]) 30% isolated soy-protein; A – Skidmore Enterprises, B – Archer Daniels Midland Co.

[6]) Average ± SE for duplicate groups of 7 male Vantress chicks.

[7]) Includes birds that died; symptoms were exudative diathesis and histologically verified encephalomalacia.

[8]) Santoquin (Monsanto Chemical Company, St. Louis, Mo.).

[9]) In addition to 5 mg/kg D-α-tocopheryl succinate.

The experiment also suggested that one of the isolated soy protein products (Archer Daniels Midland) was possibly a more potent vitamin E antagonist than the other (Skidmore Enterprises) with a greater tendency for growth inhibition and other symptoms of vitamin E deficiency (Table 4). The addition of 50 mg/kg DL-α-tocopheryl acetate or of 0.02% ethoxyquin completely prevented growth inhibition and deficiency symptoms. Selenium addition, as selenium dioxide, was ineffective in this experiment in overcoming either symptom of vitamin E antagonism.

The next experiment was undertaken to study the interaction of protein and fat source in the presence of two levels of vitamin E (DL-α-tocopheryl acetate). The basic diet was free of vitamin E. Table 5 shows that the vitamin E antagonism manifested itself with the isolated soy protein-5% corn oil diet even in the presence of 10 mg/kg DL-α-tocopheryl acetate (in comparison with the 5 mg/kg D-α-tocopheryl succinate used in the earlier experiments). No vitamin E deficiency was noted with this tocopherol level on either the casein-gelatin diets, or on the isolated soy protein-glycerol diets.

Table 5. Effect of protein and fat source on vitamin E deficiency of chicks

Diet variables[1])					
Protein source	Fat	DL-α-tocopheryl acetate mg/kg	25-day body weights[2]) (gm)	Vitamin E deficiency symptoms[3])	Plasma tocopherol[4]) mg/100 ml
Isolated soy-protein, 30%[5])	Glycerol, 5%	10	422 ± 14	0	0.21
		50	438 ± 19	0	0.64
	Corn oil, 5%	10	410 ± 24	4/10	0.18
		50	471 ± 24	0	1.36
Crude casein, 20% + gelatin, 10%	Glycerol, 5%	10	384 ± 27	0	0.25
		50	383 ± 22	0	1.72
	Corn oil, 5%	10	375 ± 26	0	0.20
		50	334 ± 31	0	1.30

Since SCOTT and STOEWSAND (5) and CALVERT et al. (6) had shown that as little as 100 mcg selenium per kg diet was adequate for the prevention of exudative diathesis, yet selenium had been ineffective in experiment 4 (Table 4), we carried out a final experiment with selenium again the dietary variable. In this experiment a new supply of isolated soy protein had to be used and the results (Table 6) point up the difficulties encountered with these soy protein isolates. With this soy preparation symptoms of vitamin E deficiency were observed even on the vitamin E control diet. On the other hand, the selenium supplements significantly improved body weight gains in comparison to the control or the vitamin E supplemented group.

[1]) Added at expense of glucose.
[2]) Average ± SE for single groups of 10 male Vantress chicks.
[3]) Exudative diathesis.
[4]) Average of two determinations made on pooled plasma from 5 chicks per sample.
[5]) Skidmore Enterprises.

Table 6. Effect of α-tocopherol and selenium on growth of chicks fed an isolated soy-protein diet supplemented with 10% corn oil

Diet Supplement[1])	23-Day Body Weight[2]) (gm)
None	382 ± 17
50 mg/kg DL-α-tocopheryl acetate	363 ± 22
1 mg/kg Na_2SeO_3	427 ± 15
1 mg/kg H_2SeO_3	421 ± 15

Plasma tocopherol levels do not offer an adequate explanation for the hypothesis that isolated soy protein in the presence of corn oil destroys dietary and/or tissue tocopherol. One one hand the group of chicks fed isolated soy protein-5% corn oil, supplemented with 10 mg/kg DL-α-tocopheryl acetate had the lowest plasma tocopherol level of any of the treatment groups, and was lower than the corresponding groups fed either isolated soy protein-5% glycerol or casein with either glycerol or corn oil. On the other hand, the group receiving isolated soy protein-5% glycerol supplemented with 50 mg/kg DL-α-tocopheryl acetate had a lower plasma tocopherol level than those chicks fed the corresponding isolated soy protein-corn oil diet. Nevertheless, there is a suggestion of an interaction between tocopherol and corn oil even for the casein groups, as between those supplemented with glycerol versus those given corn oil; this interaction appears to be exaggerated by isolated soy protein.

General comments

These studies show a vitamin E antagonism traceable to an interaction between corn oil and isolated soybean protein. DAM and coworkers (1, 7) have shown that 12.5 mg/kg tocopherol was adequate to prevent encephalomalacia in diets similar to those used here except that casein-gelatin rather than isolated soy was the protein source. In the present study, diets supplemented with 10 mg/kg DL-α-tocopheryl acetate and supplying approximately 8 mg/kg tocopherol from corn oil did not prevent vitamin E deficiency symptoms. DAM et al. (1) have suggested rapid destruction of the tocopherol present in vegetable oils but it is not known whether this is a factor also for the relatively stable tocopheryl acetate or succinate used in the present experiments.

The confusing response to selenium suggests its possible involvement in the isolated soy protein-corn oil interaction. In one experiment in which the isolated soy protein produced marked severity of exudative diathesis, selenium dioxide was ineffective in ameliorating the disorder. In the final experiment with a new supply of isolated soy protein, no deficiency symptoms were noted but growth was significantly improved with selenite and selenous acid. We do not believe that the source of selenium played a role in the different responses obtained. We have previously observed (8) excellent prevention of exudative

[1]) Diet contained a new batch of isolated soy-protein (Skidmore Enterprises) not used in any of the previous trials; it further contained 10% corn oil and 5 mg/kg DL-α-tocopheryl acetate.

[2]) Average ± SE for duplicate groups of 6 and 7 male Vantress chicks, respectively; no vitamin E deficiency symptoms were observed in any chicks.

diathesis with the same selenium dioxide preparation used in the present studies.

Finally, it is interesting to note that isolated soy protein has recently been shown to possess considerable rachitogenic activity for the chick necessitating higher levels of dietary vitamin D (9).

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Summary

Day-old male chicks of two breeds were fed diets providing from 1–5% corn oil and 13 or more mg/kg α-tocopherol. When the source of dietary protein was isolated soy protein, growth depression, exudative diathesis, and encephalomalacia accompanied by mortality occurred. These vitamin E deficiency symptoms were prevented by higher levels of α-tocopherol (50 mg/kg) or by an antioxidant (ethoxyquin, 0.02% of diet) but were not relieved by selenium in one experiment. In another experiment, with a different supply of isolated soy protein, selenium significantly improved growth while no deficiency symptoms were noted with this soy protein preparation. With casein as the dietary protein, no vitamin E deficiency symptoms developed with 13 mg/kg α-tocopherol. The results point to a vitamin E antagonism that appears to be related to the processing of isolated soy protein and its interaction with corn oil.

Zusammenfassung

Eintagshähnchen zweier verschiedener Rassen erhielten Rationen mit 1–5% Maisöl und 13 oder mehr mg α-Tocopherol per kg Futter. Wenn das Eiweiß der Rationen aus isoliertem Soja-Protein bestand, war das Wachstum der Hähnchen vermindert, die Mortalität nahm zu und Exudative Diathese und Encephalomalacia wurden beobachtet. Diese Symptome des Vitamin E Mangels wurden durch zusätzliches Tocopherol (50 mg/kg) oder durch ein Antioxidans (Ethoxyquin, 0,02% der Ration) verhindert; in einem Experiment wurden die Symptome aber nicht durch Selenium behoben. In einem anderen Experiment, in welchem ein neues Präparat von isoliertem Soja-Protein verwendet wurde, förderte Selenium das Wachstum erheblich. Wenn das Soja-Protein durch Casein ersetzt wurde, entstanden keine Symptome des Vitamin E Mangels mit der Grundration die 13 mg/kg α-Tocopherol enthielt. Die Ergebnisse weisen auf einen Vitamin E Antagonismus hin, der durch eine Interaktion zwischen dem isolierten Soja-Protein und Maisöl hervorgerufen wird.

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The lipid composition of brain and serum of young rats in relation to age and diet

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As a consequence of the possible relationship between low intake of essential fatty acids and diseases such as multiple sclerosis (1, 2) and atherosclerosis (3), several reports have appeared on metabolism of the polyunsaturated fatty acids (4, 5, 6, 7). However, little is known about the effect of the intake of so-called non-essential fatty acids such as stearic and oleic acid, apart from their function as competitive inhibitors for the conversion of linoleic and linolenic acid to the longer chain fatty acids of the *w*6- and *w*3- series (8, 9).

In our laboratory, we have previously studied the fatty acid composition of the human foetal brain and found that the content of palmitic acid was higher and oleic acid lower as a percentage of the total fatty acids than the corresponding values for the adult brain (10). As a result of this observation we decided to study the change in fatty acid composition with age in the brain of rats receiving a fat-free diet compared to rats receiving the same diet supplemented with stearic, oleic and linoleic acid respectively. In addition, two groups of rats were raised on fat-free diets supplemented with cholesterol and lauric acid. The latter group was included because lauric acid is a main lipid constituent of many commercial baby food products (11).

Materials and methods

1. Diet.

Six groups, each consisting of 3 pregnant Wistar rats, were given the following diets 1–2 weeks before delivery:

A: Basal diet
B: Basal diet containing 5% stearic acid (w/w)
C: Basal diet containing 5% oleic acid (w/w)
D: Basal diet containing 5% linoleic acid (w/w)
E: Basal diet containing 5% lauric acid (w/w)
F: Basal diet containing 5% cholesterol (w/w)