Interrelationship between Dilauryl Succinate Feeding and Nutritional Encephalomalacia in Starting Chicks

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Various levels of dilauryl succinate with or without additional *d*- α -tocopheryl acetate and of diethyl succinate were fed to chicks for 4 weeks to examine the interrelationship between the esters and nutritional encephalomalacia.

Chicks fed dilauryl succinate at the level higher than 3% died with lesions in the cerebellum. The lesions were prevented by the supplementation of 25 mg or more of *d*- α -tocopheryl acetate per kg of diet. Median lethal dietary level for males of meat-type and egg-type chicks at 3 weeks of age was 6.3 and 6.0%, respectively. That for females of meat-type at 3 weeks of age was 9.0%, suggesting that males were more sensitive than females. Diethyl succinate did not induce encephalomalacia.

In a series of studies to find new energy sources for domestic animals and poultry among chemical compounds,¹⁻³⁾ meat-type chicks fed an experimental corn-soy type diet containing 5% of an ester, dilauryl succinate, showed typical symptoms of vitamin E deficiency.³⁾ Seventy percent of the 30 chicks on the ester in the previous experiment³⁾ died with the symptoms of encephalomalacia within 44 days on the diet. Administration of 0.5 ml of 4% alcoholic solution of *d*- α -tocopheryl acetate to each of the chicks on the ester at 15 days of age appeared effective to prevent the deficiency symptoms. No deficiency symptoms were observed among the chicks on the ester given 10 I.U. of vitamin E per kg of diet. Since no chicks died on the diets of the similar

composition without the supplementation of the ester,³⁾ it was suggested that feeding the ester, dilauryl succinate, resulted in a great increase of the requirement of vitamin E by the chicks, and therefore, otherwise sufficient dietary level of vitamin E became inadequate to keep chicks healthy.

Accordingly, 4 experiments were carried out to confirm reproducibility of deficiency symptoms of vitamin E by feeding the ester, and to compare median lethal dietary level between males and females and between meat-type and egg-type chicks.

EXPERIMENTAL

Four experiments were carried out. The first one, Expt. 911, was designed to confirm the reproducibility of nutritional encephalomalacia by feeding dilauryl succinate and the preventive effect of *d*- α -tocopheryl acetate to this disease. The second experiment, Expt. 001, was designed to compare the sexual difference of meat-type chicks in the response to dilauryl succinate

1) M. Yoshida, H. Morimoto and R. Oda, *Agr. Biol. Chem.*, **34**, 1301 (1970).

2) M. Yoshida, H. Morimoto and R. Oda, *ibid.*, **34**, 1668 (1970).

3) M. Yoshida, K. Kosaka and H. Hoshii, *Japan. Poultry Sci.*, **8**, 39 (1971).

TABLE I. COMPOSITION OF DIET

Diet	Yellow corn %	Soybean meal, %	Fish meal, %	DL-Me-thionine, %	Vita-mins ^{a1} %	Mine-rals ^{b1} %	Cellu-lose, %	La. suc. ^{c1} %	Et. suc. ^{c1} %
Control	60.0	30.2	5.0	0.2	0.5	4.1	—	—	—
D-4	47.1	32.9	"	"	"	"	6.2	4.0	—
D-10	27.8	36.9	"	"	"	"	15.5	10.0	—
D'-5	53.7	31.5	"	"	"	"	—	—	5.0
D'-20	34.8	35.4	"	"	"	"	—	—	20.0

^{a1} One g of vitamins contains thiamine·HNO₃ 1.38 mg, riboflavin 6.88 mg, niacin 3.06 mg, Ca-pantothenate 3.75 mg, pyridoxine·HCl 1.38 mg, folic acid 0.7 mg, choline chloride 206 mg, vitamin A 2,000 i.u., vitamin D₃ 400 i.u., and vitamin K₃ 53 µg.

^{b1} Minerals are composed of 1.5% of CaCO₃, 2.0% of CaHPO₄, 0.5% of NaCl and 0.1% of trace mineral mixture, which contains 16.6% of Mn, 6.02% of Fe, 0.62% of Cu, 0.06% of Co and 0.02% of Zn.

^{c1} La. suc.: dilauryl succinate; Et. suc.: diethyl succinate.

by estimating median lethal dietary level of the ester. The third experiment, Expt. 003 was designed to estimate median lethal dietary level of dilauryl succinate with egg-type chicks. The last experiment, Expt. 011 was to examine the effect of diethyl succinate to both of meat-type and egg-type chicks.

Experimental diet. Composition of some of the experimental diets is shown in Table I. The experimental diets in this paper is shown by a sign indicating the dietary level of the succinates. For example, the diet D-4 contained 4% of dilauryl succinate. The diet containing diethyl succinate was expressed by D', so the diet D'-5 contained 5% of diethyl succinate. For each percent of dilauryl succinate, 3.22% of yellow corn was taken out and 0.67% of soybean meal and 1.55% of cellulose were supplemented to keep dietary level of total digestible nutrients and of crude protein constant at 67.7 and 22.3%, respectively. For each percent of diethyl succinate 1.26% of yellow corn was taken out and 0.26% of soybean meal was added to adjust dietary protein level. The simple corn-soy type control diet had been used in this laboratory for many years without any trouble due to nutritional inadequacy.

To indicate the supplementation of *d*-α-tocopheryl acetate, mg of the acetate per kg of diet was added to the sign of the diet. For example, the diet D-10+200 contained 10% of dilauryl succinate together with 200 mg/kg of *d*-α-tocopheryl acetate.

Chicks and feeding. In Expt. 911, 120 3-day-old White Leghorn male chicks were wing-banded and divided into 8 lots of 15 chicks each with uniform

average body weight. The experimental diet, D-10, were fed to 4 lots of them, the diet of the same composition with fortified vitamin E, *i.e.* D-10+200, was fed to the other 2 lots, and the control diet to the rest two.

At 15 days of age, when the first chick fell down with typical symptoms of encephalomalacia, the chicks of the first group on D-10 was divided randomly into 5 sub-groups of 12 chicks each, and fed the experimental diets containing various levels of *d*-α-tocopheryl acetate for different periods as shown in Table II.

Chicks of Sub-group 1 were fed D-10 continuously. Those in Sub-group 2 were fed D-10+25, *i.e.* the diet D-10 fortified by 25 mg/kg of *d*-α-tocopheryl acetate, for 3 days, and thereafter, were switched back to the diet D-10. Chicks in Sub-groups 3 and 4 were given either D-10+50 or D-10+100 for 6 days, then switched back to D-10. The chicks in Sub-group 5 were fed D-10+200, *i.e.* the same diet given to Group 2, until the end of the experiment.

In Expt. 001, 155 male and 164 female one-day-old meat-type cross breeds (White Cornish male×White Plymouth Rock female) were divided into 8 lots for each of the sexes and each of 7 experimental diets was fed to a lot, except D-4, which was fed to duplicated lots. The feeding plan and number of chicks in each lot are shown in Table IV with the data of the experiment.

In Expt. 003, 183 male one-day-old egg-type chicks (White Leghorn) were divided into 8 lots. One of 4 experimental diets were fed to duplicated lots for 4 weeks. The feeding plan and number of chicks in each lot are also shown in Table IV.

TABLE II. FEEDING PLAN AND NUMBER OF CHICKS SHOWING THE LESIONS OF ENCEPHALOMALACIA IN EXPT. 911

No. of chicks	Days of age and experimental diet ^{a)}					Encephalomalacia ^{b)}		No. of chicks survived
	3	15	18	21	31	18 days of age	31 days of age dead survived	
Group 1.								
Sub-g. 1	12	←	← D-10 →			2/6	6/6 —	0
Sub-g. 2	12	←	← D-10 + 25 →	← D-10 →		0/4	7/7 0/1	1
Sub-g. 3	12	←	D-10 →	← D-10 + 50 →	← D-10 →		11/11 0/1	1
Sub-g. 4	12	←		← D-10 + 100 →	← D-10 →		9/9 —	3
Sub-g. 5	12	←		← D-10 + 200 →			2/2 0/4	10
Group 2	30	←		← D-10 + 200 →		0/5	— 0/4	25
Group 3	30	←		← Control →		0/5	— 0/4	25

^{a)} Dietary levels of dilauryl succinate and *d*- α -tocopheryl acetate were shown, for example, the diet, D-10+200, contained 10% of the succinate and 200 mg of the acetate per kg of diet.

^{b)} Number of chicks showing lesions in cerebellum/number of chicks examined.

In Expt. 011, 40 female one-day-old meat-type cross breeds as used in Expt. 001 were divided into 5 lots of 8 chicks each, and 100 male one-day-old egg-type chicks as used in Expt. 003 were divided into 5 lots of 20 chicks each. One of 5 experimental diets were fed to each of the female and male lots for 4 weeks. The feeding plan and number of chicks in each lot are shown in Table VI.

All of the chicks were reared for 4 weeks in an electrically-heated wire-floor battery brooder. Feed and water were given *ad libitum* throughout the experimental period.

Post-mortem examination. In Expt. 911, 6 chicks and 2 dead chicks from Sub-group 1, 4 chicks from Sub-group 2, 5 chicks each from Groups 2 and 3, were sent to National Institute of Animal Health at 18 days of age for histopathological examination by the veterinarians. All of the other chicks which died during the experimental period of Expt. 911 were examined for lesions in the cerebellum in this laboratory. At the end of the experiment, all of the chicks survived in Sub-groups 2 and 3, and 4 chicks each from Sub-group 5 and Groups 2 and 3 were sacrificed and examined.

In Expt. 001, among 117 chicks which died during the experimental period, 113 chicks was examined for lesions in the cerebellum and cerebrum in this laboratory. The rest, *i.e.* 4 chicks were not examined, since they died on holiday. At the end of the experiment,

2 or 3 chicks from each lots and one female surviving on D-12 were sacrificed for the examination.

The first and the following 4 chicks died on dilauryl succinate in Expt. 003 were examined for lesions in the cerebellum and cerebrum. All of the chicks died on di-ethyl succinate in Expt. 011 were examined also.

Determination of peroxide in the diet. Peroxide value in the experimental diets used in Expt. 001 was determined by modified procedure of iodometry proposed by C. H. Lea and adopted by International Chemical Union,⁴⁾ after extracting crude fat in the sample by redistilled peroxide-free ethyl ether.

RESULTS

Number of chicks showing the symptoms of encephalomalacia in Expt. 911 is presented in Table II. At 18 days of age, 8 chicks in Sub-group 1, 2 dead and 6 alive, and 4, 5 and 5 chicks in Sub-group 2, Groups 2 and 3, respectively, were examined by the veterinarians in National Institute of Animal Health. Typical lesions in the cerebellum were observed in

4) "Standard Analytical Procedure for Oil and Fat," ed. by Japanese Society of Oil Chemistry, Asakura-shoten, Tokyo, 1966, p. 170.

2 chicks dead and 2 alive in Sub-group 1, while no chicks in Sub-group 2, Groups 2 and 3 showed the symptoms. No other histopathological abnormality was reported by the veterinarians.

Clear lesions in the cerebellum of all of the other chicks died during the experimental period of Expt. 911 were observed on post-mortem examination in this laboratory. On the other hand, no chicks, who survived and were examined at the end of the experiment, showed any of the lesions. These data shown in Table II revealed clearly that dilauryl succinate induced the nutritional encephalomalacia and that *d*- α -tocopheryl acetate at the dietary level of 200 mg/kg was effective to prevent the disease completely.

Two chicks in Sub-group 5, who suffered severely at 15 days of age, died within 3 days after administration of *d*- α -tocopheryl acetate, but none of the other chicks in this subgroup died thereafter. Even the feeding of *d*- α -tocopheryl acetate at the level of 25 mg/kg was apparently effective, since no chicks examined at the third day on *d*- α -tocopheryl acetate showed any abnormality, as shown in Table II.

In order to understand the preventive effect of *d*- α -tocopheryl acetate, a curve of pooled mortality of Sub-groups 3 and 4 is shown in

FIG. 1. Mortality of 24 Chicks by Encephalomalacia.

Fig. 1. The chicks in these sub-groups were given either 50 or 100 mg/kg of *d*- α -tocopheryl acetate at 15 days of age, and after 6 days, i.e. at 21 days of age, they were returned to the diet D-10 without additional *d*- α -tocopheryl acetate. Two chicks died at 15 days of age, one at 16 and one at 17 days of age, respectively, but no chicks died within the following 6 days. After 3 days of withdrawal of additional *d*- α -tocopheryl acetate, mortality due to encephalomalacia increased rapidly and only 4 chicks out of 24 survived at the end of the experiment.

TABLE III. GROWTH RATE, FEED INTAKE AND FEED EFFICIENCY OF CHICKS ON DILAURYL SUCCINATE FOR 4 WEEKS

Diet ^{a)}	Sex	Body wt. gain	Feed intake, g	Feed efficiency, g/kg
Expt. 911 ^{b)}				
control	male	305	593	515
D-10+200	male	169	390	433
Expt. 001 ^{c)}				
D-1	male	503	818	567
"	female	494	735	620
D-2	male	516	750	606
"	female	484	730	606

^{a)} See footnote 1 in Table II.

^{b)} White Leghorns

^{c)} Cross breeds of White Cornish male $\times$ white Plymouth Rock female.

Growth rate, feed intake and feed efficiency (gain/feed) of the chicks of Groups 2 and 3 are summarized in Table III. The performance of chicks on dilauryl succinate with 200 mg/kg of *d*- α -tocopheryl acetate was inferior to those of the control chicks, the data suggesting rather low possibility to use this ester as an energy source of poultry feed, besides the problem of nutritional encephalomalacia.

Mortality of the chicks on dilauryl succinate is presented in Table IV. The data with meat-type chicks revealed that male chicks were much more sensitive to dilauryl succinate in the diet than female chicks were. Variance

TABLE IV. MORTALITY FROM ENCEPHALOMALACIA INDUCED BY DILAURYL SUCCINATE IN EXPT. 001 AND 003

Expt. No., type of chicks and diet	Dietary peroxide mM/kg	Mortality ^{a)}				Encephalomalacia ^{b)}	
		3 weeks of age		4 weeks of age		4 weeks of age	
		male	female	male	female	male	female
Expt. 001, Meat-type:							
D-1	0	0/10	0/17	0/10	0/10	0/2	0/2
D-2		0/24	0/18	0/24	0/18	0/3	0/3
D-3		1/15	0/24	4/15	0/24	0/3	0/2
D-4		5/46	3/48	12/46	6/48	0/4	0/5
D-5	0.38	5/15	2/13	8/15	4/13	0/2	0/2
D-8	0.81	16/23	13/22	23/23	17/22	—	1/3
D-12	1.15	21/22	14/22	22/22	21/22	—	0/1
Expt. 003, Egg-type:							
D-5		16/38		28/38			
D-6		14/40		32/40			
D-7		26/39		32/39			
D-8		29/38		36/38			

^{a)} Number of chicks died/total chicks tested.^{b)} Number of chicks showing encephalomalacia/number of chicks examined.

TABLE V. MEDIAN LETHAL DIETARY LEVEL OF DILAURYL SUCCINATE

Age, type and sex of chicks	Average %	95% limits %
3 weeks Meat-type M	6.3	5.6~ 7.1
" " F	9.0	7.4~10.4
" Egg-type M	6.0	5.2~ 6.6
4 weeks Meat-type F	6.2	5.5~ 6.9

analyses of the mortality after inverse sine transformation revealed that the difference in mortality between males and females at 3 and 4 weeks of age were both significant at 5% level.

From the percentage of mortality of meat-type males at 3 weeks age, working probit was estimated as described by C.I. Bliss,⁵⁾ and the following Equation 1 was induced,

$$y = 0.537 + 5.582 \log x \quad (1)$$

where, y is working probit and x is dietary

level of dilauryl succinate. The linearity of the equation was confirmed by χ^2 -test. From the Equation 1, median lethal dietary level and its 95% fiducial limits were estimated and are shown in Table V. By the same procedure, median lethal dietary level and 95% fiducial limits for meat-type females and for egg-type males were estimated and are shown in Table V.

Mortality, incidence of encephalomalacia, body weight gain and feed intake of egg-type males and meat-type females fed graded levels of diethyl succinate are presented in Table VI. Only one egg-type chicks died at 20 days of age, whose body weight was only 38 g. No typical symptom in cerebellum was observed. It was concluded that the chick died by emaciation on the diet containing as high as 20% of diethyl succinate after the cold shock due to the accidental cut of electric current for 5 hr at that day. Except this chick, no chicks died during the experimental period and no chicks showed any change in cerebellum after the post-mortem examination. It

5) "The Statistics of Bioassay," ed. by C. I. Bliss, Academic Press Inc., New York, N.Y., 1952, p. 536.

TABLE VI. RESPONSE OF CHICKS ON DIETARY DIETHYL SUCCINATE AT 4 WEEKS OF AGE

Type of chicks, sex and diet	Mortality ^{a)}	Encephalo- malacia ^{a)}	Body weight gain, g	Feed intake, g
Egg-type, male:				
Control	0/20	0/2	258	459
D'-5	0/20	0/2	236	429
D'-10	0/20	0/2	148	285
D'-15	0/20	0/5	67	172
D'-20	1/20 ^{b)}	0/5	26	84
Meat-type, female:				
Control	0/8		510	752
D'-5	0/8		386	554
D'-10	0/8		316	446
D'-15	0/8		218	350
D'-20	0/8		73	141

^{a)} See footnotes a and b in Table IV.

^{b)} One chick died by emaciation, whose dead body weight was 38 g.

was clear that diethyl succinate did not induce encephalomalacia.

Diethyl succinate decreased appetite and therefore growth rate, even at the dietary level of 5%. The findings were quite agreeable with those reported previously.^{2,6)}

DISCUSSION

Data obtained in the first 3 experiments confirms clearly the previous observation³⁾ that dilauryl succinate induces the nutritional encephalomalacia and that *d*- α -tocopheryl acetate is quite effective to prevent the symptoms. Diethyl succinate does not induce such symptoms at all. Since corresponding amount of succinic acid to those of the succinate in these experiments did not result in such a nutritional abnormality,⁶⁾ although dietary succinic acid level higher than 5% depressed the feed intake and retarded the growth of chicks,⁶⁾ the effect of dilauryl succinate on nutritional encephalomalacia of chicks is certainly due to the lauryl alcohol moiety of the ester.

Experimental diets used in Expt. 001 con-

tained peroxide less than 1.2 mM/kg of diet, as shown in Table IV. It is known that dietary peroxide induced vitamin E deficiency of chicks. However, the level of peroxide in the experimental diet of less than 1.2 mM/kg may not be the main cause of nutritional encephalomalacia observed in these experiments, since the control diet which had been used in this laboratory for many years could contain about 2 mM of peroxide per kg diet without any ill influence on the performance of chicks.

Median lethal dose (LD₅₀) is commonly used as a measurement of acute toxicity. Since LD₅₀ is measured from the percentage mortality for a certain days after single administration of various doses of a test material, the procedure can hardly be applied for evaluation of chronic toxicity, such as nutritional encephalomalacia. Therefore, in the experiments reported here, median lethal dietary level of dilauryl succinate was estimated from the percentage mortality of one-day-old chicks fed the diets containing graded levels of the ester for 3 or 4 weeks. Median lethal dietary level can be abbreviated as LDL₅₀. Median lethal dietary level depends certainly upon the length of feeding period, feed intake, age of the

6) M. Yoshida and H. Hoshii, *Agr. Biol. Chem.*, **35**, 201 (1971).

chicks and other biological conditions, so that these conditions should be made clear with measurement of LDL_{50} . However, it is quite useful for practical comparison of toxicity of various materials and sensitivity of chicks to them.

Median lethal dietary levels for meat-type and egg-type male chicks for 3 weeks of feeding period and for meat-type female chicks for 4 weeks were just comparable as shown in Table V. Contrary to the statistics⁷⁾ that meat-type chicks are more sensitive to encephalomalacia on field condition than egg-type chicks are, the sensitivity to encephalomalacia induced by dilauryl succinate can not be distinguished between meat-type and egg-type chicks. There has also been obtained another evidence for general recognition that females are usually more tolerable to poisonous materials than males are. According to the LDL_{50} estimated in Expt. 001, one more week will be necessary for females to show the similar mortality to the males.

7) "Illustrated Chicken Diseases" (2 ed.), ed. by T. Horiuchi, S. Kawamura and R. Seki, Nippon Chikusan Shinkokai, Tokyo, 1969, p. 325.

At the beginning of Expt. 001, LDL_{50} of meat-type chicks at 3 weeks of age was roughly estimated to be 4%, so that the number of chicks given D-4 was made to be the largest and those on D-3 and D-5 was followed to it initially, to make reliability of the estimation of LDL_{50} high. However, fairly large number of the chicks were lost at 3 days of age due to accidents in the brooder. Therefore, actual number of the chicks used on each of the experimental diets was rather skewed as shown in Table IV. However, it is certain that the accident had little influence on the experimental purpose, since no chicks on D-1 and D-2 died after 3 days of age and their growth rate was comparable to that observed in other experiment in this laboratory, as shown in Table III. Several chicks also died in Expt. 003 within 5 days, and actual number of the chicks per each diet was somewhat varied as shown in Table IV.

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