# Effect of Long Chain Keto Acids on Encephalomalacia in Chicks.* (25768)

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The presence of unsaturated fatty acids and absence of Vit. E have served as dietary prerequisites to development of a high incidence of encephalomalacia in chicks(1-4). However, as synthetic antioxidants have been shown to decrease the rate of incidence(5-7), exposure of usaturated dietary fats to autoxidation may also represent a factor in the rate of incidence. In the present study 3 possible oxidation products of long chain unsaturated fatty acids were therefore fed to chicks kept on a Vit. E deficient diet and the effect of oxidation products on rate of incidence determined.

*Methods.* The long chain oxidation products or keto acids (Table I) were prepared from castor oil(8). The 12-oxo-octadecanoic acid was prepared from 12-oxo-*cis*-9-octadecenoic acid by hydrogenation under 50 lb pressure in the presence of palladium-aluminum oxide catalyst. The constants of all the synthesized keto acids agreed with those previously reported(8). Five groups of week-old male chicks (New Hampshire Columbia cross) were fed *ad lib* a Vit. E-deficient diet† and 0.25% of the keto acid. When clinical signs of encephalomalacia(9) such as ataxia, head retraction, and spasms followed by complete prostration were observed in the chick, it was removed from the cage for further histological and chemical determinations. Onset of encephalomalacia in the chick was confirmed by observing gross lesions, accompanied by hemorrhage and edema of the cerebellum. In another study with 12 groups of 6 birds each, levels of both keto (12-oxo-*cis*-9-octadecenoic) acid and corn oil were varied at the expense of glucose on a weight basis.

*Results.* Addition of long chain keto acids to a typical Vit. E-deficient diet (Table I) induced symptoms of encephalomalacia after 7 days of feeding as compared with 15 to 28 days previously reported(1-4). When the experiment was terminated at the end of 11 days on this diet, all chicks on diet 2 showed positive symptoms of encephalomalacia. 12-Oxo-*cis*-9-octadecenoic acid or its methyl ester caused the highest rate of incidence. The 12-oxo-*trans*-10-octadecenoic acid and 12-oxo-octadecanoic acid were not as potent as 12-oxo-*cis*-9-octadecenoic acid. Furthermore, rate of incidence depended on level of dietary fat as well as level of keto acid. A higher rate of incidence was noted at a 10 than at a 5 or 2.5% level of corn oil (Table II). A level of keto acid as low as 0.05% increased rate of incidence.

*Discussion.* The presence of oxidation products of long chain unsaturated fatty acids in a diet which contained unsaturated fat was shown to accelerate development of encephalomalacia in chicks. The presence of a dietary source of Vit. E, namely corn oil, which contained a reported level of 100 mg % of mixed tocopherols(10), did not counteract the effect of keto acids. The influence of keto acids on metabolism of Vit. E *in vivo* will be discussed in a subsequent paper.

*Summary.* The presence of oxidation products of long chain unsaturated fatty or "keto" acids, accelerated development of encephalomalacia in chicks kept on a diet containing corn oil. 12-Oxo-*cis*-9-octadecenoic acid was

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† Each 100 g of basal ration contained 68.18 g glucose, 35.3 g isolated soybean protein, 0.75 g DL-methionine, 0.3 g glycine, 0.2 g choline chloride, 2.166 g calcium carbonate, 1.05 g potassium phosphate (monobasic), 0.940 g calcium phosphate (dibasic), 0.80 g sodium chloride, 0.25 g magnesium sulfate, 0.03 g ferrous sulfate, 0.02 g manganous sulfate, 0.01 g zinc carbonate, 0.002 g cupric sulfate, 0.001 g potassium iodide, 0.001 g sodium molybdate, 10 mg thiamine hydrochloride, 10 mg niacin, 1.6 mg riboflavin, 2.0 mg calcium pantothenate, 0.002 mg vit. $B_{12}$, 0.6 mg pyridoxine hydrochloride, 0.006 mg biotin, 0.4 mg folic acid, 0.2 mg p-aminobenzoic acid, 0.5 mg menadione, 1000 I.U. vit. A acetate and 60 I.U. vit. $D_3$. The keto acid was mixed with the basal ration at the beginning of the experimental period and the diets kept in metal cans at room temperature.

TABLE I. Effect of Keto Acids on Severity of Encephalomalacia.

| Diet | Supplements to basal diet* | Feed intake, 7 days (g) | Wt gain, 7 days (g) | Positive symptoms |
|---|---|---|---|---|
| 1 | 10% corn oil | 99 | 100 | 2/12† |
| 2 | Diet 1 + 0.25% 12-oxo-*cis*-9-octadecenoic acid | 98 | 98 | 12/12 |
| 3 | Diet 1 + 0.25% 12-oxo-octadecanoic acid | 100 | 105 | 5/12 |
| 4 | Diet 1 + 0.25% 12-oxo-*trans*-10-octadecenoic acid | 85 | 96 | 5/12 |
| 5 | Diet 1 + 0.25% methyl ester of 12-oxo-*cis*-9-octadecenoic acid | 99 | 96 | 10/11 |

* Supplements to basal diet were made at expense of glucose on weight basis.
† Results expressed as fraction with No. of chicks showing advanced stages of encephalomalacia in numerator and total No. of chicks in denominator.

TABLE II. Effect of 12-Oxo-*cis*-9-octadecenoic Acid and Corn Oil Levels on Severity of Encephalomalacia.

| Treatment No. | Test diet: Supplements to basal, % keto acid | % corn oil | Feed intake, 7 days (g) | Wt gain, 7 days (g) | Positive symptoms |
|---|---|---|---|---|---|
| 1 | 0 | 2.5 | 129 | 95 | 0/6 |
| 2 | 0 | 5.0 | 127 | 94 | 1/6 |
| 3 | 0 | 10.0 | 126 | 107 | " |
| 4 | .05 | 2.5 | 123 | 97 | 0/6 |
| 5 | .05 | 5.0 | 124 | 100 | 3/6 |
| 6 | .05 | 10.0 | 117 | 94 | " |
| 7 | .1 | 2.5 | 127 | 98 | 1/6 |
| 8 | .1 | 5.0 | 128 | 100 | 2/6 |
| 9 | .1 | 10.0 | 116 | 100 | 4/6 |
| 10 | .25 | 2.5 | 124 | 92 | 1/6 |
| 11 | .25 | 5.0 | 117 | 93 | 2/6 |
| 12 | .25 | 10.0 | 118 | 92 | 6/6 |

most potent keto acid tested to date and was most effective in presence of dietary source of unsaturated fatty acid such as corn oil. With basal diet containing 10% corn oil, clinical symptoms of encephalomalacia could be induced at end of 7 days by supplementing the diet with 0.25% of 12-oxo-*cis*-9-octadecenoic acid.

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