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Brief Communication

VITAMIN E DEFICIENT FAT COMPONENT FOR COMPOSING EXPERIMENTAL DIETS

It is well known that vitamin E is present in the lipids of green leafy plants and in the oils of seeds, and that the latter represent the major source of natural vitamin E for most animal feeds. The fact that vitamin E is fat-soluble results in difficulties when separating it from the lipid material, which in turn is an indispensible step for composing vitamin E deficient diets for animals. Therefore, composing experimental diets involves both practical and economical problems.

Elimination of the vitamin E can be solved in different ways. Molecular distillation, selective absorption, and heating are some examples of these methods which lead to removal or destruction of the vitamin E. However, it is very difficult to achieve in practical circumstances a total absence of vitamin E in the finished product; this would be desirable, for example, in experiments studying in detail the interactions between selenium and vitamin E.

In earlier experimental work we have used molecular distilled cotton seed oil as a fat source in vitamin E deficient diets (e.g., *Bengtsson et al.* 1978, *Hakkarainen et al.* 1978). However, at best the total content of the vitamin E of the cotton seed oil was 62.5 mg/kg (64 % α-tocopherol and 36 % γ-tocopherol) after molecular distillation.

In our efforts to find a fat which would have a still lower vitamin E content, we have used distilled fatty acids from soybean oil (Raision Tehtaat, SF-21200 Raisio, Finland). This fatty acid mixture has been produced from crude soybean oil by hydrolyzation, followed by vacuum distillation. The vitamin E content of the fatty acid mixture was 0.15 mg α-tocopherol/kg. This was used as the sole fat source in a vitamin E deficient diet fed to chicks.

The main fatty acids in cotton seed oil and in distilled soybean fatty acid mixture were palmitic acid, 21.8 and 11.5 %, stearic acid, 2.7 and 4.1 %, oleic acid, 19.0 and 22.5 %, linoleic acid, 52.9 and 53.9 %, and linolenic acid, 0.6 and 6.6 %, respectively.

Fifty-three male White Leghorn chicks of commercial breeding were purchased as experimental animals at the age of 1 day. Four of the chicks were sacrified immediately. The remainder of the chicks were divided into 3 groups. The basal diet was composed of glucose (45.5 %), skim-milk powder (20.0 %), gelatin (10.0 %), casein (8.0 %), cellulose (3.0 %), methionine (0.5 %) and mineral mixture (including sodium selenite 0.1 mg/kg) and vitamin mixture (except for vitamin E). Distilled fatty acids obtained from soybean oil (7.0 %) were used as the sole fat component given Groups 1 and 3. This was replaced by molecular distilled cotton seed oil (7.0 %) in Group 2. Group 3 also received 10 mg of dl-α-tocopheryl acetate/kg diet. The chicks were fed ad libitum. The above mentioned diets allowed a normal growth rate and development of the chicks. There were no differences in body weight gain between the 3 groups during the experimental period.

Two chicks per group were killed at the age of 3, 6 and 9 days. In the vitamin E supplemented control Group 3, two chicks were sacrificed weekly during the rest of the observation period of 42 days. In Groups 1 and 2, chicks older than 9 days were guillotined as soon as they showed clinical signs of illness. Blood samples were taken and the livers were removed without delay and stored at —20°C. Vitamin E analyses in liver and plasma samples were performed by using high pressure liquid chromatography with fluorescence detection.

All the chicks in Groups 1 and 2 at the age of 20 days or older showed clinical signs of nutritional encephalomalacia before they were decapitated. Apparently healthy birds suddenly became atactic and fell on one side. After making attempts to rise, they fell backward. Abnormal twisting of the neck was often observed. Post mortem examination confirmed the clinical diagnosis nutritional encephalomalacia. Hemorrhages, edema, necrosis and gliosis of the cerebellum were the dominating findings. Microscopic examination further revealed hyaline tromboses in the capillaries and vascular alterations, notably in the endothelial cytoplasm. Degenerative neuronal changes occurred particularly in the Purkinje cells and in the large motor nuclei.

The liver α-tocopherol content showed a clear response to the vitamin E levels of the diets during the 6 week experiment (Fig. 1). The diet containing 7 % distilled fatty acids from soybean oil without vitamin E supplement (Group 1) resulted in the lowest α-tocopherol concentration in the liver, which decreased

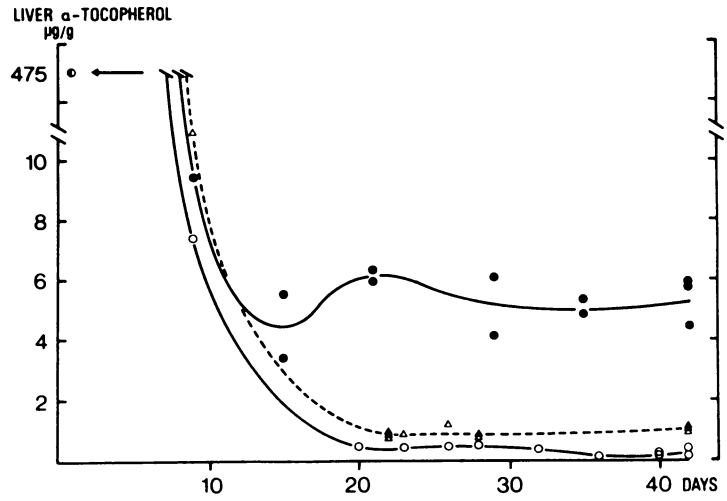

Figure 1. Liver α-tocopherol content in chicks fed vitamin E deficient basal diets with 2 different fat components. Group 1: distilled fatty acids from soybean oil (○——○). Group 2: molecular distilled cotton seed oil (△- - -△). Group 3: distilled fatty acids from soybean oil with 10 mg of dl-α-tocopheryl acetate/kg diet (●——●). For further details, see the text.

from ca. 475 μg/g liver at the start to a level of about 0.20 μg/g during the final week of the experimental period. However, the fastest rate of decrease took place during the first 3 weeks and before the onset of clinical symptoms. The diet containing 7 % cotton seed oil, also without vitamin E supplement (Group 2), resulted in a similar decrease but liver α-tocopherol concentration leveled off on a clearly higher level (ca. 1 μg/g) compared to the soybean oil Group 1. Supplementation of the soybean oil diet with 10 mg dl-α-tocopheryl acetate/kg (Group 3) limited the depletion of liver α-tocopherol to a final level of about 5 μg/g liver. Changes in plasma α-tocopherol showed the same pattern as liver α-tocopherol. The final levels of plasma α-tocopherol in Groups 1, 2 and 3 were 0.05, 0.15, and 0.9 mg per g of total lipid in plasma, respectively. No clinical symptoms or post mortem signs referring to encephalomalacia or to other diseases were found in Group 3. They remained healthy.

Nutritional encephalomalacia is a well established disorder of the central nervous system which appears in young chicks predominantly at the age of 3 to 8 weeks when they are fed a vitamin E deficient diet containing polyunsaturated fatty acids;

the linoleic acid content of the diet evidently accelerates the induction of the disease (*Lannek & Lindberg* 1975, *Scott* 1978).

It is apparent that the experimental diet containing 7 % distilled fatty acids from soybean oil as the sole fat component produced a rapid response of deficiency and disease. The distilled fatty acids from soybean oil are practically devoid of vitamin E (< 0.2 mg/kg oil) and proved to be of great value in composing vitamin E deficient experimental diets.

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REFERENCES

Bengtsson, G., J. Hakkarainen, L. Jönsson, N. Lannek & P. Lindberg: Requirement for selenium (as selenite) and vitamin E (as α-tocopherol) in weaned pigs: I. The effect of varying α-tocopherol levels in a selenium deficient diet on the development of the VESD syndrome. J. Anim. Sci. 1978, *46*, 143—152.

Hakkarainen, J., P. Lindberg, G. Bengtsson, L. Jönsson & N. Lannek: Requirement for selenium (as selenite) and vitamin E (as α-tocopherol) in weaned pigs: III. The effect on the development of the VESD syndrome of varying selenium levels in a low-tocopherol diet. J. Anim. Sci. 1978, *46*, 1001—1008.

Lannek, N. & P. Lindberg: Vitamin E and selenium deficiences (VESD) of domestic animals. Advanc. vet. Sci. comp. Med. 1975, *19*, 127—164.

Scott, M. L.: Vitamin E. *In:* The Fat-Soluble Vitamins. Ed.: H. F. De Luca, Plenum Press, New York 1978, p. 133—210.

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