N,N¹-DIPHENYL-P-PHENYLENEDIAMINE IN THE PREVENTION OF VITAMIN E DEFICIENCY IN THE LAMB

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It has been demonstrated that N,N¹-diphenyl-p-phenylenediamine (dppd) is effective in preventing encephalomalacia in the chick (Singsen et al., 1955) and low hatchability of turkey eggs (Jensen et al., 1956) produced by feeding a tocopherol-low diet. Inclusion of this compound in the diet also has been shown to increase the vitamin A and carotenoid levels in the blood plasma and liver (Potter et al., 1956; Monson et al., 1955). Johnson and Goodyear (1955) have reported that dppd is capable of preventing resorption-gestation in female rats maintained continuously on a tocopherol-free diet from weaning age, but that the protective effect disappears during the second or third reproductive cycles. They also have found that dppd fails to regenerate vitamin E-deficient female rats, thereby indicating that the vitamin is specific for some essential function in metabolism. However, Draper et al. (1956) have observed that the quantity of alpha-tocopherol required for regeneration of deficient females is markedly decreased in the presence of dppd in the curative diet.

These results support the popular theory that tocopherols play a specific role in metabolism which cannot be assumed by other compounds, but that part of the dietary requirement for vitamin E represents a requirement for self-preservation or other antioxidation reactions in which the vitamin is replaceable with certain other antioxidants.

The present study was designed to determine whether dppd is capable of preventing vitamin E deficiency in lambs fed a tocopherol-free artificial milk diet. A preliminary report of the experiment has appeared (Draper and Johnson, 1955).

Experimental

The experimental animals were twenty lambs, one to three day old, obtained from the University flock ² and local breeders. They were fed ad libitum a basal diet similar to that described earlier (Draper et al., ¹This project was supported in part by a grant from Armour and Company, by a gift of vitamins from Merck and Co., Inc., and by a supply of N,N¹-diphenyl-p-phenylenediamine from B. F. Goodrich Chemical Company.
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1952) except that the tocopherol-free lard was incorporated at a level of 28% and cerelose was increased to 36% of the dry matter content. The diet contained 16% solids.

The lambs were allotted to five groups of four each. Dppd was added daily in ethanol solution to the diet of three groups at rates calculated to provide 0.001%, 0.025%, and 0.10% of the dry matter intake, respectively. The lowest level was chosen as a result of previous experience with rats which indicated that dppd was toxic when fed at the two higher levels over an extended period of time (Draper et al., 1956). The two remaining groups were given, respectively, the basal diet alone or with a vitamin E supplement (50 mg. a-tocopherol per lb. body weight per week).

Results

Two criteria of vitamin E deficiency were employed, namely, the occurrence of clinical symptoms of "stiff lamb disease" and histological degeneration of skeletal and cardiac muscle. Detailed descriptions of the symptoms and lesion have been given elsewhere (Madsen et al., 1935; Metzger and Hagan, 1927). The lambs were maintained on the experimental diets for a period of 7 weeks or until death or clinical symptoms occurred. The tissue samples were placed in FAA fixative (formalin-acetic acid-alcohol) for 24 hours and were preserved in 70% ethanol. Slices were mounted in paraffin and stained with hematoxylin and eosin.

The performance of the various groups may be summarized by treatments as follows:

Group 1. Basal diet. Two lambs developed muscular dystrophy after 27 and 34 days, respectively, and one died suddenly after 37 days. One animal survived the 7-week experimental period. Upon histological examination, the tissues from all the lambs exhibited local cellular degeneration and nuclear proliferation as described by several other workers for vitamin E-deficient animals.

Group 2. 0.001% dppd. All animals developed "stiff lamb disease", characterized chiefly by muscular weakness of the hind legs, or died suddenly during the fifth week. The histological appearance of the tissues was similar to that of the negative controls. Hence this level of dppd exerted no apparent effect on the course of vitamin E deficiency.

Group 3. 0.025% dppd. Three members of this group survived the experiment without exhibiting any clinical symptoms of vitamin E de-
ficiency. The fourth died suddenly after 41 days, having gained 0.57 lb. per day. The histological examination indicated slight proliferation of nuclei in local areas; otherwise the tissues appeared normal. The single fatality may have been a consequence of the rapid rate of growth which may be presumed to have increased the vitamin E requirement of this animal.

Group 4. 0.10% dppd. All the animals receiving this level of dppd appeared in normal condition, clinically and histologically, at the end of the experiment. Average daily gains for the four lambs were 0.29, 0.30, 0.33 and 0.49 lb. per day.

Group 5. Alpha tocopherol. No evidence of vitamin E deficiency or other manifestations of malnutrition were observed.

The growth rate was not affected by the various treatments imposed, and group averages ranged from 0.22 to 0.35 lb. per day.

Discussion

The foregoing results demonstrate that dppd is capable of preventing muscular dystrophy in lambs receiving a vitamin E-deficient diet, and that, under the conditions imposed, from 0.025% to 0.10% of the dry matter intake is effective. The 0.025% level appears to have been somewhat suboptimal for the complete prevention of muscle degeneration. It should be anticipated that under other experimental conditions, with particular reference to the dietary concentration of unsaturated fatty acids, the effective amount of dppd would be significantly different. The milk of the ewe is substantially higher in fat than the diet used in this study, but the proportion of unsaturated fatty acids is lower, so that the concentration of monoethenoid and polyethenoid acids per unit of dry matter is roughly comparable. To the extent that the concentration of unsaturated fatty acids in the diet contributes to the development of "stiff lamb disease" it may be surmised that 0.1% dppd might approximate the amount effective for lambs receiving vitamin E-low natural milk.

Summary

Newborn lambs were maintained on an artificial milk diet containing tocopherol-free lard as a source of fat. The effectiveness of \( \text{N}_2\text{N}^1\)-diphenyl-p-phenylenediamine (dppd) in preventing the occurrence of clinical and histological signs of muscular dystrophy was determined. Administration of dppd at a level equivalent to 0.10% of the dry matter
content of the diet completely prevented the appearance of vitamin E deficiency. A level of 0.025% was found to be slightly inadequate and 0.001% was ineffective.

Literature Cited


Monson, W. J., E. T. Gade, M. D. Lloyd and A. C. Groschke. 1955. Liver storage of vitamin A in chicks fed graded levels of dehydrated alfalfa meal with and without supplemental DPPD. Poultry Sci. 34:1211. (abst.).
