Israel Journal of Entomology Vol VII, 1972

VITAMIN E, TOCOPHEROL QUINONE AND SELENIUM IN THE DIET OF THE HOUSE CRICKET, *ACHETA DOMESTICUS* (L.)

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ABSTRACT

The effects of vitamin E in the house cricket, *Acheta domesticus* (L.) have been further investigated, with emphasis on the threshold for the effects and the possible significance of tocopherol quinone and selenium in replacing vitamin E. Pigmentation and reproduction are affected at the same concentrations of vitamin E, and tocopherol quinone and selenium do not replace vitamin E. A possible involvement of vitamin E with copper is discussed.

INTRODUCTION

The pioneer workers in insect nutrition were alive to the possibility that vitamin E was required in the diet. Fraenkel and Blewett (1946) showed that the effect of dietary vitamin E in various Lepidoptera was that of an antioxidant for required polyunsaturated fatty acids. This was the only known role of vitamin E in insect nutrition until it was indicated by Chumakova (1962) that vitamin E was essential for the reproduction of a beetle *Cryptolaemus montrouzieri* Mulsant. Meikle and McFarlane (1965) showed conclusively that vitamin E was required for spermatogenesis in *Acheta domesticus(*L.), and House (1966) showed that vitamin E was required for female reproduction in *Agria affinis* (Fallen). Vitamin E is not, however, an essential nutrient for many other insect species.

In further work with vitamin E in the house cricket, McFarlane (1972a) has found that vitamin E in the diet at physiological levels resulted in a loss of cuticular pigmentation in 30 — 50% of adult males. The 'albino' effect was not found in adult females or in the larval stages. Sclerotization appeared not to be affected, and it was suggested there was a specific effect of vitamin E on a phenolase system. It was also shown that vitamin E could inhibit a phenolase system in the egg (McFarlane, 1972b).

The view that vitamin E acts solely as a physiological antioxidant is still strongly held (Tappel 1965), but most workers are of the opinion that this is just one of its functions, although other precise actions remain to be discovered (Boguth, 1969). The complexity of the vitamin E picture may be illustrated by the relationship of vitamin E to selenium, which can replace vitamin E for some of the functions of the vitamin, and yet has functions distinct from those of vitamin E (Schwarz, 1965).

The purpose of the work presented in this article was primarily to investigate the metabolic interrelationships of vitamin E, tocopherol quinone and selenium in the house cricket.

MATERIALS AND METHODS

The basic diet used was that of Meikle and McFarlane (1965). It consisted of vitamin-free casein (40g), D(+) dextrose (20g), 'Alphacel' non-nutritive cellulose (30g), salt mixture U.S.P. XIV (2g), cholesterol (lg) and the B – vitamin mixture of Ritchot and McFarlane (1962).

Sixty newly hatched larvae were placed on each test diet, ten per jar, in each experiment. The temperature of rearing was 29±1°C. and the R.H. 50%. Adults were collected daily and weighed, then transferred to 1-gallon candy jars where they were accumulated. Eggs were collected twice weekly and incubated on moist filter paper in an air-tight jar. After three days, the eggs were examined for for evidence of development.

The nutritional chemicals were obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. These included DL-α-tocopherol (vitamin E), α-tocopherol quinone, DL-α-tocopherol acetate, and sodium selenite.

Means and standard deviations are given where appropriate.

RESULTS

The results of a typical experiment are given in Table I. Vitamin E at all three levels improved the growth of females, but this was only significant at the lowest level of vitamin E in this experiment. Such an improvement is always found though it is not always significant with the number of test insects routinely used. There is no improvement in the growth of males with vitamin E, and this is always the case. Meikle and McFarlane (1965) found that vitamin E at 86 μg/g of diet significantly improved female weight.

There was a marked improvement in survival on diets with 17.2μg/g vitamin E or more and this is usually, but again not always, found in similar experiments. Survival on the basic diet varies from 50 – 80%.

Tocopherol quinone, which in other respects has little or no vitamin E activity, as will be seen, did not improve the growth or survival of larvae.

Pigmentation

Males with no visual evidence of melanization or with just a slight darkening were considered to be 'albino' forms. These are quite distinct from normally pigmented insects, though doubtful intermediates do occur. Doubtful cases were considered to be normal for the presentation of results.

Table II gives the combined results of three (3) experiments in which the level of vitamin E was varied and the effect on pigmentation determined.

TABLE I.

Average weight of the adults (⩽24 hr. old), average duration of the larval stage and percentage survival to the adult stage on various artificial diets.

Treatment	Adult males obtained			Adult females obtained			
	No.	Mean wt. (mg)	Mean duration larval stage (days)	No.	Mean wt. (mg)	Mean duration larval stage (days)	Survival (%)
Basic diet	12	278.2±30.4	63.8±4.9	19	346.7±66.5	58.3±6.5	52
+8.6 μg vit E/g	15	276.7±51.5	65.0±7.1	18	405.5±57.1*	60.4±6.3	55
+17.2 μg vit. E/g	20	281.4±39.0	66.8±6.9	26	372.4±50.9	59.0±7.2	77
+86 μg vit. E/g	17	286.5±50.4	67.3±6.8	25	384.9 ±62.3	58.0±7.2	70
+17.9 μg Tocopherol Quinone /g	20	271.0±47.9	64.4±5.7	16	335.4 ±47.0	55.6±8.3	60
+89.3 μg/Tocopherol Quinone /g	11	289.5 ±47.4	65.4 ±6.6	19	352.4 ± 52.9	59.3 ±6.1	50

*Significantly different from basic diet at P=0.01

TABLE II. The percentage of 'albino' forms on various vitamin E diets. Combined data from three (3) trials.

	Total No. Males	No. 'Albino' Males	% 'Albino' Males
Basic diet	52	2	4
+8.6 μg vit. E/g	47	5	11
+17.2 μg vit. E/g	53	13	25
+86 μg vit. E/g	56	22	39

'Albino' forms were not produced on the basic diet in the original experiments of this series (McFarlane, 1972a), but they have since occurred occasionally on the basic diet. The incidence of 'albino' forms increases rapidly at 17.2μg of vitamin E/g of diet, but a fivefold increase to 86 μg/g does not give a large increase in the percentage of 'albino' forms. The level for maximum activity lies between 17.2 and 86μg/g, and probably closer to the lower level. Levels higher than 86 μg/g were not used, as these would surely be outside the physiological range.

Reproduction

Eggs were collected from the insects referred to in the section on pigmentation, and the results presented (Table III) have been combined from three experiments.

TABLE III. The number of eggs laid on various vitamin E diets and percentage of eggs developing. Combined data from three (3) trials.

	No. Eggs Laid	No. Eggs Developing	% Developing
Basic diet	607	2	0.3
+8.6 μg vit. E/g	177	9	5.0
+17.2 μg vit. E/g	2437	1603	65.8
+86 μg vit. E/g	3113	2270	72.9

Virgin females lay fewer eggs and at a slower rate than inseminated females (Meikle and McFarlane 1965). Generally speaking, the percentage of eggs developing from females on the basic diet is very low, as shown in Table III. However, from time to time, the basic diet gives egg production as good as that on a vitamin E diet. In one instance of this, three males were removed from the diet and mated separately with

virgin females. None of these produced viable eggs. It would, of course, require only one fertile male to give viable eggs, and in fact Meikle and McFarlane (1965) did find some viable sperm in males fed the basic diet, although they did not get survival of eggs from adults on such a diet.

The threshold for vitamin E activity on reproduction seems to be around 17.2 μg/g, and this is probably close to the level for maximal effect. Thus two functions of vitamin E, the reduction of pigmentation and the reproductive effect, require similar levels of vitamin E in the diet.

Tocopherol quinone and selenium

Tocopherol quinone was investigated for its vitamin E activity, and particularly because quinones can inhibit phenolase activity (Webb, 1966). Tocopherol quinone added at two levels equimolar with the two highest vitamin E levels did not improve growth of females or survival (Table I), did not appreciably improve egg production or survival (Table IV), and gave no 'albino' forms.

TABLE IV. The number of eggs laid on various diets and percentage of the eggs developing.

	No. Eggs Laid	No. Eggs Developing	% Developing
Basic	74	0	0
+17.9 μg Tocopherol Quinone/g	25	2	8.0
+89.3 μg Tocopherol Quinone/g	560	21	3.8
Basic	34	0	0
+18.9 μg Tocopherol Acetate/g	858	668	77.9
Basic	68	2	2.9
+0.2 ppm selenium	33	0	0
+1.0 ppm selenium	9	0	0

Selenium in concentrations of 0.2 and 1.0 ppm, added as sodium selenite, was completely negative in vitamin E effects (Table IV), and was perhaps slightly toxic at the higher level.

DL-a-tocopherol acetate, as expected, gave similar results to vitamin E at the 17.2μg/g, or equimolar, level (Table IV). It also improved female weight significantly (P=0.01), increased survival, and gave 24% 'albino' forms.

Non-reversibility of 'albino' condition

Four 'albino' males obtained from vitamin E – containing diets were placed on the basic diet (no vitamin E) for the remainder of adult life (the same number of controls was also run). They did not darken further.

Similarly, three 'albino' males were placed on rabbit pellets, the diet which is used to culture the insects, and which has never produced 'albino' crickets, for the remainder of life. They also did not darken further. It has already been shown that vitamin E added to rabbit pellets at a level of 86 μg/g will not produce 'albino' forms (McFarlane 1972a).

In vitro experiments with cuticle

Fraenkel and Rudall (1947) used the technique of incubating cuticles in solutions of phenols with the view to understanding hardening and darkening.

Similar experiments were conducted with pieces of abdominal tergal cuticle from both 'albino' and normal adult male insects. Solutions used were all 1%, or if the phenol was relatively insoluble, a saturated solution was used. The cuticles were incubated in one ml, and to the appropriate tubes one drop of 1% thiourea was added as a Cu-enzyme inhibitor. The tubes were incubated at 33°C. and examined at hourly intervals for seven (7) hours and finally after twenty-four (24) hours.

No monophenolase activity, using tyrosine and phenol as substrates, could be detected in 'albino' cuticles or in normal cuticles, although in the latter the normal pigmentation of course interfered. Polyphenolase activity could be readily demonstrated in both types of cuticle with DL-dihydroxy phenylalanine (dopa), where a black color resulted (which could be inhibited), and catechol, where a red brown color resulted (which also could be inhibited).

While these experiments do not rule out the presence of monophenolase activity in 'albino' cuticles, they do establish that polyphenolase activity is present. It had previously been concluded that 'albino' cuticles show sclerotization (McFarlane, 1972a).

Second generation on an artificial diet

The possibility was tested that depletion of some unknown nutrient by rearing through a second generation on an artificial diet might effect the production of 'albino' forms. Accordingly, 'albino' males were mated to females from the same diet and offspring were collected. One hundred of these were reared on the basic diet with no vitamin E, and one hundred were reared on a diet with 86 μg/g vitamin E. No 'albinos' were produced on the diet with no vitamin E, whereas 33% of the males on the diet with vitamin E were 'albinos'. There was, then, no change from the results with the first generation on the artificial diet.

Copper

In view of the fact that low copper can produce defects in pigmentation in mammals (Underwood, 1971), and that a Cu-enzyme was possibly involved in the 'albino' effect, the level of copper in both the artificial diet and rabbit pellets was examined. The amount of copper in the artificial (basic) diet was 0.67 ppm. The amount of copper in rabbit pellets is at least 10.8 ppm., as this amount is added to the feed (Dr. N. Hussar, Ogilvie Flour Mills, Montreal, personal communication). Rabbit pellets thus contain at least 16x as much copper as the artificial diet.

DISCUSSION AND CONCLUSIONS

The addition of vitamin E in adequate amounts to an artificial diet for the house cricket produces four effects: 1) an elevated female weight, 2) an increase in survival, 3) a great increase in the percentage of 'albino' forms, and 4) it enables the insects to reproduce. The latter two effects invariably accompany the addition of vitamin E.

Vitamin E exerts no appreciable effect on pigmentation or reproduction below 17.2 μg/g of diet, and the maximal effect is probably produced at a level that is not much higher. It is interesting that the two seemingly different functions should have similar requirements. Whether the basic action of vitamin E is similar in both instances remains to be seen. It is also surprising that the effects should require such a high level of vitamin E when compared with other animals, although it is still a physiological level. It is possible that the reproductive factor in rabbit pellets (and in nature) is not vitamin E, but at any rate it is not selenium.

In the case of the effect on pigmentation, it has been shown that vitamin E can inhibit a phenolase (in this case probably a polyphenolase) system in the egg of the house cricket (McFarlane, 1972b), although the precise mechanism of inhibition remains to be worked out. None of the results with the house cricket, however, contradict the view of vitamin E as an antioxidant.

The possibility of an involvement between vitamin E and copper is intriguing, and readily amenable to testing. It seems unlikely, however, that the 'albino' effect is due to a simple binding of copper ions, because it is not reversible, and because polyphenolase activity is unaffected. Binding at the time of enzyme formation, however, could possibly prevent the enzyme being formed. It will be important to establish whether monophenolase activity is in fact present, and, if it is, to see whether phenolic materials are incorporated into the adult cuticle after the normal stage of cuticular hardening and darkening has passed.

ACKNOWLEDGEMENTS

The technical assistance of Mrs. Jean Dubreuil is gratefully acknowledged.

This work was supported by a grant from the National Research Council of Canada.

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