PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 279–283 (1974)

Protection of Chicks Against *E. coli* Infection by Dietary Supplementation with Vitamin E[1] (38087)

ROLLIN H. HEINZERLING, CHERYL F. NOCKELS, CAREY L. QUARLES, AND ROBERT P. TENGERDY
(Introduced by Robert E. Moreng)

Department of Microbiology and Department of Animal Sciences, Colorado State University, Fort Collins, Colorado 80521

We have previously reported that dietary supplementation with a 3–6-fold excess of vitamin E significantly increased the humoral immune response of chicks against sheep red blood cells (1), and of mice against sheep red blood cells and tetanus toxoid (2). A noticeable splenomegaly accompanied the elevated antibody titers in mice, suggesting that an increased cell proliferation in lymphopoietic tissues may have contributed to the increased antibody production. The predominant increase in antibody titer was IgG (2). Selenium alone or in combination with vitamin E also enhanced the humoral immune response (3), but synthetic antioxidants did not (2).

An increased antibody level alone may or may not contribute to an increased protection against an infectious agent. The purpose of the study reported here was to demonstrate increased protection of chicks against an *Escherichia coli* infection, afforded by supplemental vitamin E, and a possible correlation of this protection with increased levels of serum antibody. *E. coli* may cause diarrhea and death in young chicks raised on presently used commercial rations. *E. coli* was selected as the test organism because it can be controlled under laboratory conditions, and its antigenic structure and immunogenicity have been studied.

Methods and Materials. The chicks used in these experiments were fed a normal diet supplemented with 150 or 300 mg vitamin E as DL-α-tocopheryl acetate per kg feed. This is 3–6 times the level found in a normal chick starter ration (43.2 mg vitamin E/kg feed). The commercial preparation of Dry Vitamin E acetate 50% SD of Hoffmann La Roche was used as the source of vitamin E. This contained 51.3% of chemically pure DL-α-tocopheryl acetate, the rest was hydrolyzed protein, about 3.0% silicon dioxide, 0.2% sorbic acid, and 0.1% sodium benzoate for compounding and for preservative. Mortality, weight gain, bursa and spleen weights, hemagglutinating antibody titers, and serum globulin fractions were compared as parameters of the protective effect of vitamin E against *E. coli* infection.

The experiment was conducted in two separate trials. Each trial consisted of 1-day-old male broiler chicks randomly assigned to two groups: a control uninfected group and an infected group. Each group was subdivided into three dietary groups of 30–40 chicks each receiving a normal chick starter ration supplemented with 0, 150, and 300 mg vitamin E/kg feed. Each group was further subdivided into three replicates composed of 12–14 chicks.

The chicks were housed in raised, wire-floored, electrically heated battery brooders and received feed and water *ad lib.* All chicks were vaccinated by an aerosol method against Newcastle disease and infectious bronchitis at 13 days of age. At 15 days of age, chicks were challenged with a slow lactose fermenting *E. coli* strain, P.S.U. L-2a (5). Infection was achieved by ad-

[1] This research was partially supported by a Grant from Hoffmann La Roche, Inc., and is published as Scientific Series Number 1905 by the Colorado State University Experiment Station.

ministering orally 1.0 ml and in the post thoracic air sac 0.1 ml of a suspension containing 1×10^9 cells/ml.

Chicks were monitored daily for mortality. Infected chicks usually died 2–4 days following infection, or recovered within 5 days. At the age of 29 days, 4 survivor chicks in trial one and 5 survivor chicks in trial two of each dietary group were bled by heart puncture. The serum protein fractions were separated by standard cellulose acetate electrophoresis and determined by densitometer. Passive hemagglutination titer against a purified *E. coli* lipopolysaccharide LPS antigen was treated with 0.25 *N* NaOH microtiter methods (6). The *E. coli* LPS antigen was prepared by the method of Westphal and Lüderitz (7). This antigen contained most of the somatic antigen specificity and reactivity against *E. coli.* The LPS antigen was treated with 0.25 *N* HCl for 1 hr at 45°C before being fixed to the sheep red blood cell. Spleen and bursa were excised from 6-week-old chicks from each dietary group in trial two and weighed.

Body and organ weight gains, serum protein fractions, and hemagglutination titers were statistically analyzed for variance by regression analysis and Tukey's test for multiple comparisons (8).

Results and Discussion. Table I shows that dietary supplementation with 150 and 300 mg vitamin E/kg significantly reduced mortality in *E. coli* infected chicks ($P < 0.01$) at the level of infection which caused 25–30% mortality in normal chicks. Dietary vitamin E supplementation reduced mortality in chicks fed both the 150 mg vitamin E diet/kg ($<10\%$) and in chicks fed the 300 mg vitamin E diet/kg ($<6.0\%$) in both trials. The variation in mortality between the two supplemented diets proved to be within experimental error and thus not significantly different.

In general, infected chicks tended to gain less weight than noninfected chicks, but dietary vitamin E supplementation restored the weight gain of infected chicks to that of the noninfected chicks. The difference in weight gain between infected and noninfected chicks was only significant in chicks fed the unsupplemented diet in trial one but not in trial two. The slightly higher mortality rate of this group (trial two) probably obscured the effect of infection on weight gain, since the weight gain represents only the survivors. Infection also reduced bursa and spleen weights (% body weight). Dietary vitamin E supplementation appeared to counteract the weight loss, although the statistical difference was not significant.

The decrease in chick mortality infected with *E. coli* corresponded to a significant increase ($P < 0.01$) in the titer of hemagglutinating antibody (Table II). Other small variances in the hemagglutination titers were associated with experimental error in that the differences were less than two log titers. It was also observed that 300 mg vitamin E supplementation to the diet/kg did not increase the hemagglutinating antibody titers over those of 150 mg/kg of vitamin E supplementation.

The α_2 globulin fraction of the serum was significantly higher ($P < 0.05$) in all dietary vitamin E supplemented groups, whereas the β globulin fraction was generally lower. The increased α_2 globulin fraction of the dietary vitamin E supplemented chicks suggests that the increased hemagglutination titers were associated with this fraction. The possibility that the α_2 globulin fraction may contain the protective antibody is currently under investigation.

Vitamin E at the levels used in this experiment was not toxic. In another investigation we have found that even 64 g vitamin E/kg diet was not toxic to the chick. The only noticeable effect of this enormous concentration of vitamin E was very waxy feathers and smaller weight gain. A detailed report of these observations is in preparation.

The experimental results indicate that the increased protection of chicks against *E. coli* infection was attributed, at least partially, to an enhanced humoral immune response by supplementing dietary vitamin E. Supplemental dietary vitamin E of 150 or 300 mg/kg provided enhanced protection and immunity to chicks as illustrated in this report.

Summary. Dietary supplementation of 150–300 mg vitamin E (DL-α-tocopheryl acetate)/kg gave increased protection

TABLE I. Effect of Vitamin E on Growth and Survival of *E. coli*-Infected and Noninfected Chicks.[a]

Dietary vitamin E added (mg/kg)	Trial 1					Trial 2			
	E. coli infected	No. of chicks	No. of deaths	Mortality (%)	2–4 Weeks average weight gain (g)	No. of chicks	No. of deaths	Mortality (%)	2–4 Weeks average weight gain (g)
0	+	36	9	25.0 B	284 B ±21	42	13	31.0 B	395 A ±43
	−	36	0	0.0 A	348 A ±34	40	0	0.0 A	411 A ±33
150	+	34	3	9.3 A	313 A ±27	40	2	4.9 A	367 A ±51
	−	36	0	0.0 A	332 A ±28	40	0	0.0 A	396 A ±40
300	+	37	2	5.4 A	330 A ±39	40	0	0.0 A	387 A ±35
	−	36	0	0.0 A	340 A ±32	40	0	0.0 A	408 A ±32

[a] Weight gains represent the average gain of survivors. Means followed by different capital letter indicate significant differences ($P < 0.01$).

TABLE II. Effect of Vitamin E Supplementation of Hemagglutination Titer and Serum Globulin Fractions of *E. coli*-Infected and Noninfected Chicks.[a]

Dietary vitamin E added (mg/kg)	*E. coli* infected	Trial 1 HA titer (log_2)	Trial 1 Serum globulin fractions (%) α_1	α_2	β	γ	Trial 2 HA Titer (log_2)	Trial 2 Serum globulin fractons (%) α_1	α_2	β	γ
0	+	7.75 B	4.9 a	12.2 a	16.0 a	18.9 a	6.66 B	2.8 a	13.5 a	14.5 a	16.9 a
		±0.88	±0.7	±1.3	±1.0	±1.7	±0.65	±0.7	±2.7	±1.0	±1.5
	−		4.0 a	11.0 a	13.7 a	17.7 a		4.0 a	12.1 a	14.4 a	16.8 a
			±0.8	±1.2	±1.5	±1.3		±0.6	±1.3	±1.4	±0.6
150	+	10.75 A	4.0 a	13.3 b	12.8 b	19.0 a	8.83 A	4.8 a	19.3 b	12.3 a	18.9 a
		±0.46	±0.8	±1.0	±1.3	±1.5	±0.71	±1.2	±1.7	±1.7	±1.5
	−		4.9	12.6 a	12.6 b	18.5 a		4.3 a	16.1 b	10.6 b	16.9 a
			±0.7	±1.5	±1.1	±1.0		±0.8	±1.0	±0.8	±0.8
300	+	10.00 A	3.7 a	15.5 b	11.8b	20.7 a	9.66 A	3.4 a	16.1 b	11.9 b	17.7 a
		±0.53	±0.6	±1.1	±1.2	±1.7	±0.88	±0.5	±1.1	±1.5	±1.4
			5.1 a	13.0 b	11.8 b	17.4 a		5.6 a	16.2 b	13.0 a	16.4 a
			±1.2	±0.9	±1.2	±1.3		±1.0	±0.8	±1.7	±0.8

[a] The data are means of four chicks in Trial 1 and five in Trial 2. The means followed by different capital letters indicate significant differences at the ($P < 0.01$) level by the t test means followed by different small-case letters indicate significant differences at the ($P < 0.05$) level by regression analysis. The assays were done from sera obtained from 29-day-old chicks 2 weeks after infection.

against a relatively moderate (25–30% mortality) *E. coli* infection. A correlative 2–3-fold increase in $\log_2$ antibody titer against the *E. coli* indicates that increased chick survival was in part immunologic.

We thank Hoffmann La Roche, Inc., for supplying the vitamin E and for the vitamin E assays of the diets.

1. Tengerdy, R. P., Heinzerling, R. H., and Nockels, C. F., Inf. and Immunol. **5**, 987 (1972).

2. Tengerdy, R. P., Heinzerling, R. M., Brown, G. L., and Mathias, M. M., Int. Arch. Allergy Appl. Immunol. **44**, 221 (1973).

3. Spallholz, J. E., Martin, J. C., Gerlach, M. L., and Heinzerling, R. H., Proc. Soc. Exp. Biol. Med. **143**, 685 (1973).

4. Dorr, P., and Nockels, C. F., Poultry Sci. **50**, 1375 (1971).

5. Glantz, P. J., Avian Diseases **9**, 2 (1965).

6. Herbert, W. V., *in* "Handbook of Experimental Immunology" (D. M. Weir, ed.), pp. 720–744, Blackwell Sci. Publ., Oxford, England (1967).

7. Westphal, O., and Lüderitz, O., Angew. Chem. **66**, 407 (1954).

8. Snedecor, G. W., and Cochran, W. G. "Statistical Methods," 6th ed., Iowa State University Press, Ames, Iowa (1967).

Received Nov. 12, 1973. P.S.E.B.M., 1974, Vol. 146.