Immune Competence of Chicks from Two Lines Divergently Selected for Antibody Response to Sheep Red Blood Cells as Affected by Supplemental Vitamin E

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ABSTRACT Effects of dietary vitamin E on responses to SRBC antigens and *Escherichia coli* infection were studied in chicks from White Leghorn lines selected for 24 generations for high (HAS) and low (LAS) antibody responses to SRBC. Chicks were fed corn-soybean diets consisting of either high (300 IU per kg feed) or low (10 IU per kg feed) concentrations of vitamin E from the day of hatch through the end of experiment. The LAS chicks were heavier than the HAS chicks at 14 d of age and thereafter; there was no difference in BW between vitamin E concentrations. At 37 d of age, chicks were inoculated via the brachial vein with 0.1 mL of 0.25% SRBC suspension. Antibody titers at 6 and 10 d after inoculation were higher in HAS than in LAS chicks. At 6 and 10 d after inoculation with SRBC, antibody responses were lower in LAS chicks fed the diet containing the higher vitamin E concentration than in those fed the diet containing the lower concentration of vitamin E. At 64 d of age, chicks were injected in the posterior thoracic air sac with 0.1 mL of 10^{-2} or 10^{-4} dilution of *Escherichia coli* and scored for pericardial and air sac lesions. The HAS chicks were more susceptible to *E. coli* infection than LAS chicks as measured by lesion scores and BW changes. Although dietary vitamin E had no effect on lesion scores in either line, BW loss at 24 h after *E. coli* inoculation was significantly reduced in HAS chicks fed the higher concentration of vitamin E. The dosage of *E. coli* had no effect on lesion scores and BW changes. These results suggest that genetic selection might have changed immune competence in relation to responses to dietary vitamin E, and the optimum dietary concentration of vitamin E depends on genotype, among other factors.

(*Key words*: selection, vitamin E, immune response, sheep red blood cells, *Escherichia coli*)

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INTRODUCTION

Enhancement of immune competence of poultry has been sought through development of vaccination, breeding, nutrition, and management programs. In a long-term selection experiment, two lines derived from the same base population were divergently selected for high (HAS) or low (LAS) antibody responses to SRBC (Siegel and Gross, 1980; Martin et al., 1990). These lines differ greatly not only in antibody response to SRBC as growing chicks (Boa-Amponsen et al., 1997), but also in peak and persistence of antibody response to SRBC as adults (Yang et al., 1999). During the selection process, HAS chickens exhibited greater resistance to Marek's disease, Newcastle disease virus, *Mycoplasma gallisepticum*, *Eimeria tenella*, a splenomeglia virus, and feather mites. The HAS chickens also had a greater susceptibility to *Escherichia coli* and *Staphylococcus aureus* infection than did the LAS chickens (Gross et al., 1980; Dunnington et al., 1986, 1992).

Vitamin E is a primary biological antioxidant preferentially retained in cellular membranes that are particularly susceptible to oxidative damage commonly found in normal metabolic processes. More than two decades ago, it was shown that supplementation of vitamin E at higher than traditional dietary concentrations enhanced immune function and disease resistance in poultry (Tengerdy and Nockels, 1973, 1975; Tengerdy and Brown, 1977; Heinzerling et al., 1974; Jackson et al., 1978). Although some recent studies also showed positive effects of vitamin E on immunity of poultry (Haq et al., 1996; Gore and Qureshi, 1997; Erf et al., 1998), Sell

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Abbreviation Key: HAS = high antibody response line; LAS = low antibody response line.

et al. (1997) reported that adding a high concentration (300 IU/kg) of vitamin E did not alleviate the adverse effects of *Escherichia coli* infection in broilers and young turkeys. Whether there is an enhancement of immune competence in poultry fed high concentrations of vitamin E may be dependent on genotype, concentration of vitamin added, and environment. The study reported here was conducted to examine effects of vitamin E on immune competence in the two selected lines with known genetic backgrounds differing in antibody response to SRBC.

MATERIALS AND METHODS

Stock and Diet

Two lines of White Leghorn chickens, which were derived from the same base population and were divergently selected for high or low antibody response at 5 d after inoculation with 0.1 mL of a 0.25% suspension of SRBC given from 41 to 51 d of age (Siegel and Gross, 1980; Martin et al., 1990), were used in this experiment. Within-line matings of male and female chickens from the 24th generation of continuous selection of these lines were made to produce 100 progeny from each line. At hatch, chicks were wingbanded, given Marek's vaccination, and randomly distributed into two groups. Each group was fed corn-soybean mash diets consisting 20% CP and 2,685 kcal ME/kg feed. Two concentrations of vitamin E in the form of dl-α-tocopherol acetate were fed. These were 10 IU (low) or 300 IU (high)/kg diet. All chicks were reared on litter in floor pens with feed and water provided for ad libitum intake. Lighting was continuous to 21 d of age, after which the photoperiod was from 0600 to 2000 h. Chicks were individually weighed at 14 and 28 d, and their sex was determined at 37 d of age.

SRBC Administration and Antibody Assay

At 37 d of age, each chick was inoculated via the brachial vein with 0.1 mL of 0.25% SRBC suspension. At 6, 10, and 20 d after inoculation, blood samples were obtained from the brachial vein, and total SRBC antibody titers were measured 24 h later by the microtiter hemagglutination procedure of Wegmann and Smithies (1966). Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution producing visible hemagglutination.

E. coli *Challenge*

At 64 d of age, chicks were inoculated in the posterior thoracic air sac with 0.1 mL of either 10^{-2} or 10^{-4} dilution of *E. coli* (serotype 01:K1 incubated for 24 h in tryptose broth). Each dose was administered to a random sample of one-half of the individuals within each vitamin E dosage by line subclass. Body weights were obtained prior to inoculation and 24, 48, and 120 h after inoculation. Mortality was recorded daily. After the last BW measurement, chicks were killed by cervical dislocation and scored for pericardial and air sac lesions. Scores were 1) no lesions, 2) mild air sac lesions, 3) moderate air sac lesions, 4) mild to moderate heart lesions, 5) extensive heart lesions, and 6) dead (Praharaj et al., 1997).

Statistical Analysis

Body weights and antibody titers were subjected to analysis of variance using the General Linear Models procedure (SAS Institute, 1985); line, vitamin E concentration, and sex were the main effects. For data resulting from *E. coli* challenge, changes in BW and lesion scores were analyzed with line, vitamin E concentration, sex, and dosage as main effects. Significance was declared at $P < 0.05$, and, where found, comparisons among multiple means were made by Duncan's Multiple Range Test. Prior to analysis, BW were transformed to common logarithms, and percentage changes in BW were transformed to arc sine square roots.

RESULTS

Growth

The LAS chicks were heavier than the HAS chicks at 14 d of age and thereafter (Table 1). Body weights were similar for vitamin E concentrations, and line by vitamin E interactions were not significant. Males were heavier than females at 28 d of age and thereafter.

Antibody Responses to SRBC Inoculation

Sexes responded similarly to SRBC antigen. The higher antibody titers at 6, 10, and 20 d after inoculation for HAS chicks than for LAS chicks were expected (Table 1). At 6 and 10 d after inoculation with SRBC, differences between vitamin E concentrations were observed in the LAS line but not in the HAS line. There was no effect of vitamin E on antibody titers at 20 d after inoculation.

Response to E. coli *Challenge*

Susceptibility to *E. coli* challenge, as measured by lesion scores and BW changes, was greater in HAS chicks than in LAS chicks. Loss of BW at 24 h after *E. coli* inoculation was less in HAS chicks fed the higher concentration of vitamin E than in HAS chicks fed the lower concentration of vitamin E; no difference was detected between vitamin E concentrations in LAS chicks (Table 2). Mortality during the 5 d after *E. coli* inoculation was 2.0 and 6.8% for HAS chicks fed the higher and lower concentrations of dietary vitamin E, respectively. Percentages for LAS chicks were 2.0 and 4.2 for the higher and lower vitamin E concentrations, respectively.

The percentage of HAS chicks with lesion scores lower than 3 was 26 for those fed the higher concentration of vitamin E and 16 for those fed the lower concentration of vitamin E. Percentages were 56 and 60 for the LAS

TABLE 1. Means and SEM for BW and antibody titers to SRBC by line and dietary concentrations of vitamin E

		BW			Antibody titers to SRBC antigen		
Line[1]	Vitamin E	14 d of age	28 d of age	64 d of age	6 d[2]	10 d	20 d
	(IU/kg)	(g)					
HAS	10	68 ± 2[b]	152 ± 4[b]	570 ± 15[b]	7.4 ± 0.3[a]	4.9 ± 0.2[a]	2.4 ± 0.1[a]
	300	71 ± 2[b]	146 ± 3[b]	566 ± 12[b]	6.8 ± 0.3[a]	4.9 ± 0.2[a]	2.5 ± 0.2[a]
LAS	10	79 ± 2[a]	177 ± 4[a]	664 ± 15[a]	2.4 ± 0.3[b]	1.9 ± 0.1[b]	1.1 ± 0.1[b]
	300	80 ± 1[a]	173 ± 4[a]	677 ± 13[a]	1.5 ± 0.1[c]	1.2 ± 0.1[c]	1.1 ± 0.1[b]

[a–c]Means within a column with no common superscripts differed ($P \leq 0.05$).

[1]HAS = line selected for high antibody response to SRBC antigen; LAS = line selected for low antibody response to SRBC antigen.

[2]Days after inoculation with 0.1 mL of a 0.25% SRBC suspension.

line, respectively. Chicks with scores lower than 3 were not affected or only slightly affected by *E. coli*; the LAS chicks had a significant advantage over the HAS chicks in this category. Conversely, percentages of chicks scoring higher than 3 (those with heart damage or even death) were 19 and 25 for the HAS chicks fed higher and lower dietary vitamin E, respectively. Respective percentages were 12 and 8 for the LAS line. More HAS chicks suffered from heart infection and death than LAS chicks; however, the effect of vitamin E concentration was not significant for these comparisons.

Dosage of *E. coli* had no effect on lesion scores or on BW changes after *E. coli* challenge (data not shown). Although there was no sexual dimorphism for lesion scores, BW changes were greater for females than males. Percentage changes in BW at 24, 48, and 120 h after inoculation were –6.5, –3.6, and 1.1 for females and –4.8, –1.6, and 3.5 for males, respectively.

Correlations Among Traits Related to E. coli Challenge

Initial BW prior to *E. coli* inoculation was not correlated with BW changes and lesion scores for the HAS line (Table 3). For the LAS line, initial BW prior to *E. coli* inoculation was not correlated with BW changes at 24 and 48 h after inoculation or with lesion scores. Changes in BW and lesion scores from the *E. coli* inoculation were highly correlated with each other in both lines. Greater BW loss after *E. coli* inoculation was associated with higher lesion scores.

DISCUSSION

Selection for high or low antibody responses to SRBC antigens has changed the immune competence of chickens used in this experiment. Antibody titers were several fold higher in HAS chicks than in LAS chicks, which is consistent with results reported by Boa-Amponsem et al. (1997) and Yang et al. (1999). Correlated responses to selection were also noted for several traits related to immunity. The greater susceptibility of HAS chicks to *E. coli* infection compared with LAS chicks that was observed during the early generations of selection (Gross et al., 1980) still existed in this experiment after 24 generations of divergent selection. The LAS chicks also had heavier BW than did the HAS chicks at 14 d of age and thereafter. In a biological system, advancement in one trait is generally accompanied by a debt or regression in another trait. It seems that selection for antibody response has redirected the original resource allocations in these lines.

Results in this experiment showed that feeding diets containing 300 IU supplemental vitamin E/kg alleviated some of the adverse effects of *E. coli* infection in chicks. At the higher concentration of vitamin E, loss of BW at 24 h after *E. coli* challenge was reduced in HAS chicks, and the difference in lesion scores and BW loss at 24 h after infection between lines was not significant. Improvements caused by supplemental vitamin E, however, were not as dramatic as those observed by Heinzerling et al. (1974), Tengerdy and Nockels (1975), and Tengerdy and Brown (1977), who reported that adverse

TABLE 2. Means and SEM for lesion scores and percentage change in BW by line and dietary concentrations of vitamin E after *Escherichia coli* challenge

		Percentage change in BW after inoculation[2]			
Line[1]	Vitamin E	24 h	48 h	120 h	Lesion scores
HAS	10	–8.5 ± 0.7[c]	–4.4 ± 1.1[b]	1.3 ± 0.9[a]	3.5 ± 0.2[a]
	300	–6.4 ± 0.8[b]	–4.1 ± 1.0[b]	0.9 ± 1.3[a]	3.1 ± 0.2[ab]
LAS	10	–3.5 ± 0.7[a]	–1.1 ± 0.7[a]	3.7 ± 0.9[a]	2.5 ± 0.2[c]
	300	–4.4 ± 0.9[ab]	–1.2 ± 0.6[a]	3.0 ± 0.6[a]	2.7 ± 0.2[bc]

[a–c]Means within a column with no common superscripts differed ($P \leq 0.05$).

[1]HAS = line selected for high antibody response to SRBC antigen; LAS = line selected for low antibody response to SRBC antigen.

[2](BW after inoculation – BW before inoculation)/BW before inoculation × 100.

TABLE 3. Correlations among BW, BW changes, and lesion scores after *Escherichia coli* challenge by line[1,2]

	BW64	BW69	BWCHG1	BWCHG2	BWCHG5	Lesion scores
BW64		0.90**	0.05	–0.05	–0.10	–0.10
BW69	0.95**		0.27**	0.29**	0.34**	–0.18
BWCHG1	–0.01	0.17		0.57**	0.53**	–0.55**
BWCHG2	0.09	0.33**	0.50**		0.81**	–0.70**
BWCHG5	0.21*	0.51**	0.51**	0.73**		–0.64**
Lesion scores	–0.03	–0.18	–0.34**	–0.39**	–0.51**	

[1]BW64 and BW69 = BW at 64 and 69 d of age, respectively; BWCHG1, BWCHG2, and BWCHG5 represent percentage changes in BW at 24, 48, and 120 h after *E. coli* challenge, respectively.

[2]Results of the high antibody response line are presented above the diagonal; those for the low antibody response line are presented below the diagonal.

*$P \leq 0.05$.

**$P \leq 0.01$.

effects of *E. coli* infection on growth and livability decreased significantly when feeding diets that contained 150 or 300 IU supplemental vitamin E/kg. Sell et al. (1997), finding that 300 IU/kg addition of vitamin E in diets did not alleviate the adverse effects of *E. coli* infection in young turkeys, suggested that differences in age, dosages, and strains of *E. coli* could have contributed to the different findings among experiments.

Friedman et al. (1998) reported that higher antibody production in response to *E. coli* and Newcastle disease virus was observed in chicks that received 0 and 10 mg added vitamin E/kg feed than in chicks that received 30 and 150 mg added vitamin E/kg feed; similar results were obtained in turkeys. In contrast, Haq et al. (1996) reported that chicks from hens receiving additional dietary vitamin E had higher antibody titers to Newcastle disease virus, and Gore and Qureshi (1997) found that the humoral and cellular immunity of turkeys and chicks was enhanced by embryonic exposure to vitamin E. Results of the present study suggest that genetic selection could initiate direct and correlated changes of immune competence in relation to the effects of vitamin E. Results from the present study suggest that genetic stocks may vary in immune responses to supplemental dietary vitamin E. For example, high yielding broiler stocks may need higher concentrations of vitamin E to optimize performance and disease resistance. Therefore, the optimum requirement of vitamin E may be stock-specific.

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