

Performance of Pureline Broiler Breeders Fed Two Levels of Vitamin E

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ABSTRACT Reported in this paper is an experiment designed to evaluate responses of two commercial broiler dam purelines (A and B) continuously fed 10 or 300 IU of vitamin E/kg from 168 to 441 d of age. Prior to Day 168, all pullets were fed diets containing 10 IU of vitamin E/kg. During the early laying period, percentage hen-day ovulations and percentage hen-day normal egg production were similar for both lines and diets. During the latter part of the laying cycle, there were differences between lines for these traits (A > B), as well as for BW and egg weight for which line differences were reversed (A < B). Also during this period, percentage hen-day ovulations and percentage hen-day normal egg production differed between diets (300 > 10 IU/kg). These differences between diets were consistent with the greater number of females that entered lay and higher hen-housed egg production of the 300- than 10-IU/kg group. Although during the laying cycle all females received a fixed

amount of feed, BW gains were greater for Line B than A and for the 300- than the 10-IU/kg level of vitamin E.

Heterophil (H):lymphocyte (L) ratios, percentage livability, and relative asymmetries of shank length and diameter were similar among groups. Pullets from Line B and those fed the higher level of vitamin E exhibited more fear than their counterparts. Head shaking did not differ between vitamin E levels; however, there was a line-by-time of day interaction for this behavior. The 30-fold difference in dietary vitamin E was reflected by a 15-fold difference in plasma vitamin E levels in both lines. For vitamin E level in the yolk, however, there was a line-by-diet interaction. The interaction resulted from no difference between lines at the 10 IU/kg level and differences of 10- and 6+-fold in Lines B and A at the 300 IU/kg level, respectively. Overall, responses to continuous feeding of vitamin E at these levels were influenced by genetic stock, age, duration of feeding, and measurement criteria.

(*Key words:* broiler pureline, vitamin E, egg production, relative asymmetry, body weight)

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INTRODUCTION

Broiler breeding programs have a pyramid structure with the foundation being highly selected elite sire and dam purelines (Ewart, 1993; Pollock, 1999). Intense selection in closed pureline populations may be viewed as a genetic stressor (Moller and Swaddle, 1997), resulting in greater relative asymmetries of bilateral traits in purelines than in F₁ crosses between them (Yang et al., 1997).

Dietary supplements to enhance and maintain performance are routinely used in poultry production. One dietary supplement receiving considerable emphasis for its role in alleviating effects of stressors and enhancing immunocompetence in broilers, turkeys, and layers is vitamin E (e.g., Cherian and Sim, 1997; Gore and Qureshi, 1997; Sell et al., 1997; Bollengier-Lee et al., 1998; Erf et al.,

1998; Gebert et al., 1998; Hossain et al., 1998; Surai et al., 1999; Siegel et al., 2000; Yang et al., 2000). The focus of these studies has varied, as has the level and duration of feeding diets supplemented with vitamin E, genetic stocks, and measurement criteria. In this paper results are presented for growth-, reproductive-, and stress-related traits measured over time in two broiler breeder dam purelines fed two levels of vitamin E.

MATERIALS AND METHODS

Lines, Husbandry, and Diets

Eggs from two commercial broiler dam purelines (A and B) of a comparable age were obtained from a primary breeder and were incubated in the same machine at Virginia Tech. At hatch (March 17) chicks were wing-banded,

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Abbreviation Key: A, B = commercial broiler purelines; H = heterophil; L = lymphocyte.

vaccinated against Marek's Disease, and placed in light-proof pens with wood shavings for bedding. Lighting was continuous to 7 d of age and from 0500 to 2000 h to Day 37 when chicks were moved to grower pens and exposed to natural day length. At 126 d of age, pullets were individually housed in three-tier battery cages in a light-proof room where the photoperiod was 14 h of light:10 h of darkness.

Feed was provided ad libitum to Day 14. From then to Day 168, the amount of feed fed was adjusted weekly to attain BW recommended by the primary breeder. Thereafter, each pullet was provided a daily allowance of 130 g of feed. Diets containing 20% CP and 2,685 kcal ME/kg were fed from 0 to 56 d of age; diets containing 14% CP and 2,827 kcal ME/kg were fed from 56 to 168 d of age. Both diets contained 10 IU/kg of vitamin E. Commencing at 168 d of age, 80 pullets from each line were randomized into two groups of 40 each and were fed a diet containing 16% CP and 2,752 kcal ME/kg supplemented with 10 or 300 IU/kg of vitamin E. Lower and higher denote the 10 and 300 IU/kg levels of vitamin E, respectively. All diets were in the form of mash, and vitamin E supplementation was as dl- α -tocopherol acetate.

Traits

Body weights (10 g) were obtained the day the first egg was laid and at 154 and 441 d of age. Lengths and diameters (0.1 mm) of the left and right shanks (Moller et al., 1999) were measured at 336 d of age, with left-right order randomized to remove systematic biases (David et al., 1999).

Egg production was recorded daily from day of onset of lay to Day 378 and from 410 to 441 d of age. Each egg was classified as normal or defective (double yolk, extra calcified, compressed, broken, soft shell, membrane, yolk, or other). Egg production was then quantified as the percentage normal eggs, percentage hen-day ovulations, and percentage hen-day normal eggs for the two recording periods. In addition, hen-housed egg production to Day 378 was quantified. Normal eggs laid from Days 307 to 311 were weighed (0.1 g) daily, and the mean was considered as the value for each pullet.

Behavior data were obtained for the same 10 pullets from each line-diet subclass three times: as the pullets were entering lay (196 to 208 d of age), near peak production (229 to 238 d of age), and later in the laying period (313 to 318 d of age). The behaviors were "fear" as a reaction to a human and head shaking, a stereotypy that may be indicative of stress (Hughes and Black, 1974). Both "fear" and head shaking were measured according to procedures described by Mauldin and Siegel (1979). For "fear," the observer (the same individual) faced the chicken and slowly moved a pencil from left to right across the front of the cage. Each pullet received a score, based on its responses to the observer and pencil, that was cumulated for eight trials during each testing period. Scoring criteria were (1) peck at pencil, (2) face the front

of the cage, (3) face either side of the cage, (4) face the rear of the cage, and (5) flight.

The procedure for head shaking consisted of observing five pullets for 5 min and recording the cumulative time each pullet shook its head from side to side. Observations for fear data were made from 1300 to 1400 h and for head shaking from 0600 to 0700 h and 1400 to 1500 h.

Blood, obtained from each pullet at 280 d of age, was mixed with EDTA as the anticoagulant. Slides were prepared for determining the number of heterophils and lymphocytes as described by Gross and Siegel (1983). All slides were coded, and 60 cells were classified by the same individual.

Blood obtained from 10 pullets at 420 d of age, from each line-diet subclass, was mixed with EDTA as the anticoagulant. The plasma was analyzed for vitamin E by using a slight modification of the procedures of Catignani and Bieri (1983) and Buttriss and Diplock (1984). Infertile eggs laid between 393 and 396 d of age from five pullets per line-diet subclass were also analyzed for vitamin E using modifications of the same procedure.

Statistical Analysis

Mortality and number of pullets entering lay were analyzed by chi-square. All other traits were analyzed by ANOVA with the GLM procedure (SAS Institute, 1985) in a fixed model

$$Y_{ijk} = \mu + L_i + D_j + (LD)_{ij} + e_{ijk}$$

where $i = 1, 2$ lines; $j = 1, 2$ dietary levels of vitamin E; and $k = 1, 2, \dots, n$ individuals. Time of day and interactions of it with line and level of vitamin E were included in ANOVA for headshakes. Before analysis, BW were transformed to common logarithms, headshakes to square roots, and ratios and percentages to arc sine square roots. Relative asymmetry (RA) of lengths and diameters of the left (L) and right (R) shanks was defined as the ratio of the absolute value of asymmetry (L-R) divided by the size of the bilateral trait:

$$RA = ((L - R) / [(L + R) / 2]) \times 100.$$

RESULTS AND DISCUSSION

Body weights at 154 d of age were similar for lines (B = 2,442 \pm 32 g; A = 2,471 \pm 31 g) and for levels of vitamin E (higher = 2,453 \pm 33 g; lower = 2,460 \pm 31 g). The lack of difference between lines and diets was expected because a single diet had been fed and feed allowances were adjusted to achieve a priori BW. From Day 168 onward, a fixed amount of feed was provided, and by Day 441, there were differences in BW between lines (B = 3,794 \pm 53 g; A = 3,516 \pm 49 g) and dietary levels of vitamin E (higher = 3,732 \pm 59 g; lower = 3,579 \pm 48 g). A lack of line by dietary level of vitamin E interaction for BW at 441 d suggests that the response in BW gain was similar for both lines.

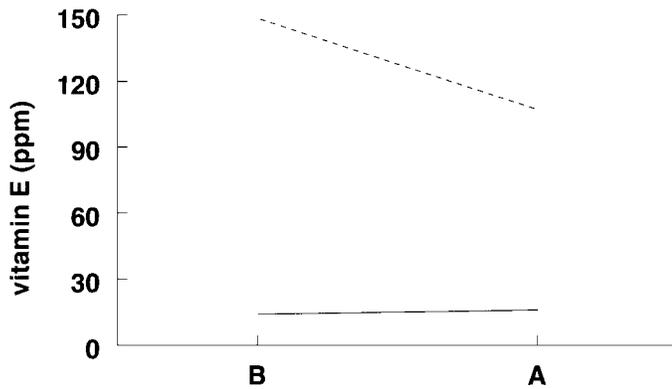


FIGURE 1. Vitamin E content of eggs for pullets from Lines B and A fed 10 (—) or 300 (---) IU/kg of vitamin E.

Continuous feeding of two dietary levels of vitamin E to broiler breeders resulted in differences in plasma and yolk concentrations of this vitamin. At 420 d of age (252 d of treatment), plasma vitamin E was >15-fold greater in hens fed the higher (28.0 ± 3.4 ppm) dietary level of vitamin E than in those fed the lower amount (1.8 ± 0.1 ppm). The response was similar in both lines (B = 15.3 ± 3.5 ppm; A = 14.5 ± 4.2 ppm) with no significant line by dietary level of vitamin E interaction. For vitamin E concentration in yolks of eggs laid between 393 and 396 d of age (225 to 228 d of treatment), however, the interaction of line by dietary level of vitamin E was significant. This interaction (Figure 1) resulted from a difference between lines at the higher, but not the lower, dietary level of vitamin E. In both lines, eggs from hens fed the higher dietary level contained more vitamin E than those fed the lower level, the difference being only of degree. This finding is consistent with results of Meluzzi et al. (2000) that the amount of vitamin E in yolk was related to the amount in the diet.

Line by dietary level of vitamin E interactions were not significant for any of the reproductive traits summarized in Table 1. Although BW at onset of lay was similar for both lines, onset of lay occurred at a younger age in Line A than B. Also, egg weights were greater for Line B than A. No differences were noted between lines to 378 d of age for number of individuals entering lay, percentage normal eggs, percentage hen-day ovulations, and percentage hen-day normal egg production. At older ages (410 to 441 d), however, means for these traits were greater for Line A than B.

Body weight and age at first egg were not influenced by dietary level of vitamin E (Table 1). Egg weight as well as percentage normal eggs, percentage hen-day ovulations, and percentage hen-day normal egg production to 378 d of age were also similar. Although all hens fed the higher vitamin E diet commenced lay, only 92% of those fed the lower level entered lay. This difference contributed to the difference in hen-housed egg number between diets, with greater fecundity becoming evident for percentage hen-day ovulations and percentage hen-day normal egg production from 410 to 441 d of age. Additionally, there was a difference ($P < 0.10$) between dietary levels of vitamin E for hens in lay at this older age (higher = 88%; lower = 77%). This dietary effect was consistent for both lines with the percentage of hens in lay being 82% for Line B and 83% for Line A.

Although livability was similar for both lines and dietary levels of vitamin E (Table 2), general fitness based on criteria used in this experiment appeared to be superior for Line A over B and for the higher than lower dietary level of vitamin E. This effect of age and duration of feeding on fitness is consistent with results obtained for H:L ratios on Day 280 (112 d of treatment) and relative asymmetries of shank length and diameters measured on Day 336. The H:L ratios were within the range of optimum well-being (Gross and Siegel, 1993), and relative asym-

TABLE 1. Means \pm SEM for reproductive traits for Lines B and A and dietary vitamin E levels of 300 and 10 IU/kg

Trait	Line		Vitamin E	
	B	A	300	10
Age at 1st egg (d)	203 \pm 2	* 197 \pm 3	198 \pm 3	203 \pm 3
BW at 1st egg (g)	3,450 \pm 37	3,438 \pm 41	3,464 \pm 34	3,422 \pm 44
% Entering lay	96	96	100	* 92
Egg weight ¹ (g)	63.9 \pm 0.5	* 62.1 \pm 0.5	63.1 \pm 0.5	62.9 \pm 0.5
Egg production to 378 d of age ²				
% Normal	95.3 \pm 1.0	97.0 \pm 0.4	96.6 \pm 0.7	95.7 \pm 0.7
% HD ovulations	46.0 \pm 1.6	46.3 \pm 1.8	47.0 \pm 1.7	45.2 \pm 1.7
% HD normals	45.3 \pm 1.6	44.6 \pm 1.7	45.0 \pm 1.6	43.1 \pm 1.6
HH egg number	77.3 \pm 3.3	80.1 \pm 4.2	84.0 \pm 3.7	* 73.4 \pm 3.8
Egg production from 410 to 441 d of age ²				
% Normal	95.5 \pm 1.1	* 98.9 \pm 0.7	97.5 \pm 0.8	96.8 \pm 1.1
% HD ovulations	27.8 \pm 2.2	* 34.9 \pm 2.7	35.9 \pm 2.4	** 26.9 \pm 2.5
% HD normals	26.4 \pm 2.1	* 34.6 \pm 2.7	34.8 \pm 2.4	** 26.1 \pm 2.5

¹Measured from 307 to 311 d of age.

²HD = hen-day; HH = hen-housed.

* $P \leq 0.05$.

** $P \leq 0.01$.

TABLE 2. Percentage livability from 168 to 441 d of age, and means \pm SEM for heterophil:lymphocyte (H:L) ratios, shank lengths and diameters, and fear scores for lines B and A and dietary vitamin E levels of 300 and 10 IU/kg

Trait	Line		Vitamin E		
	B	A	300	10	
% Livability	99	96	96		
H:L at 280 d of age	0.42 \pm 0.03	0.45 \pm 0.03	0.45 \pm 0.03	0.42 \pm 0.03	
Shank (mm) at 336 d of age					
Length:					
left	111.7 \pm 0.4	111.6 \pm 0.4	111.2 \pm 0.4	112.2 \pm 0.4	
right	112.1 \pm 0.4	112.1 \pm 0.4	111.6 \pm 0.4	112.6 \pm 0.4	
RA ¹	0.37 \pm 0.12	0.42 \pm 0.10	0.40 \pm 0.10	0.39 \pm 0.12	
Diameter:					
left	18.6 \pm 0.1	18.6 \pm 0.1	18.6 \pm 0.1	18.6 \pm 0.1	
right	18.5 \pm 0.1	18.7 \pm 0.1	18.6 \pm 0.1	18.6 \pm 0.1	
RA ¹	0.16 \pm 0.46	0.17 \pm 0.38	0.25 \pm 0.38	0.25 \pm 0.48	
Fear scores by periods (d of age)					
196 to 208	13.5 \pm 0.4	**	8.4 \pm 0.4	11.4 \pm 0.4	10.6 \pm 0.6
229 to 238	12.5 \pm 0.5	**	8.4 \pm 0.4	11.2 \pm 0.6	* 9.7 \pm 0.5
313 to 318	13.9 \pm 0.3	**	11.0 \pm 0.8	13.8 \pm 0.6	** 11.1 \pm 0.7

¹RA = relative asymmetry.

**P* \leq 0.05.

***P* \leq 0.01.

metries were similar for both lines and diets. Line B females were more fearful than Line A pullets at all ages (Table 2). Although there were no differences between dietary levels of vitamin E near the age of onset of lay, thereafter values were greater for the higher than lower dietary level of vitamin E. Dietary level of vitamin E had no effect on frequency of headshakes; however, the line by time of day interaction was significant within each age period. As shown in Figure 2, there was a difference between lines at 0600 h but not at 1400 h. The former was approximately 2 h prior to feeding, and the latter was

approximately 2 h after the daily food supply was consumed.

General Comments

The results of the present study suggest that effects of dietary levels of vitamin E on broiler breeders are more pronounced near the end of the laying cycle than at the beginning. The difference in BW at 441 d between lines was 8% (B > A), and that for vitamin E was 4% (higher > lower). These differences suggest differences between

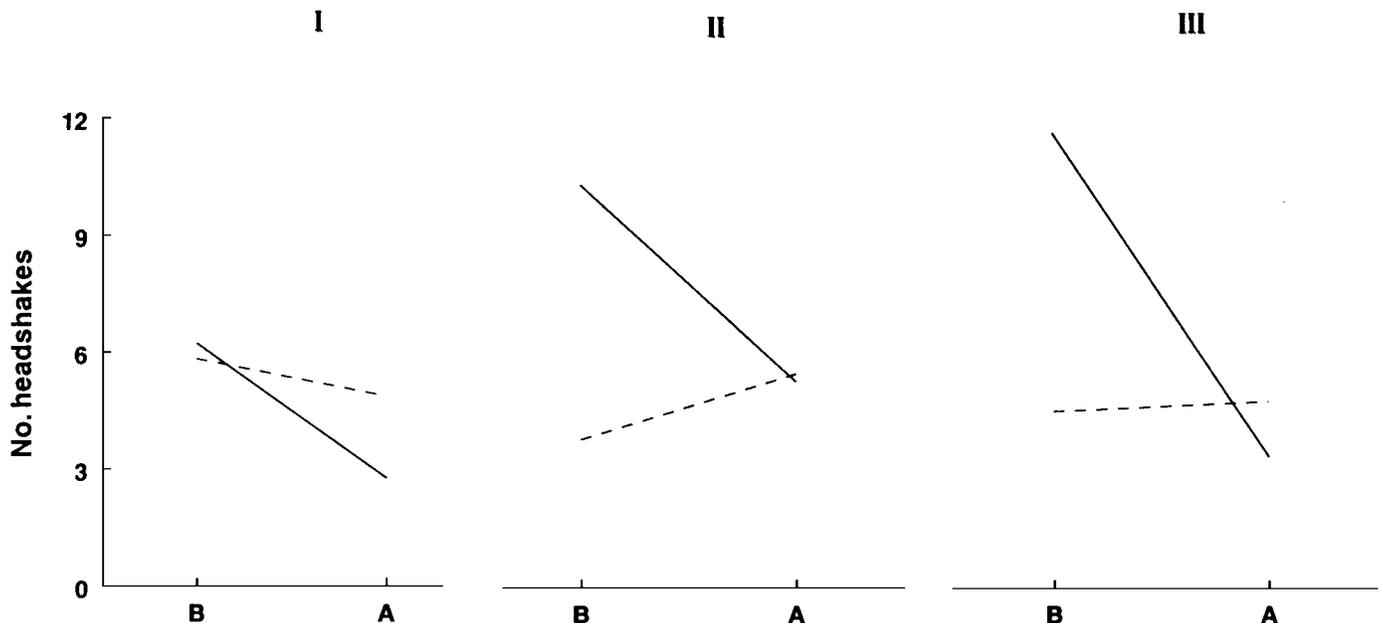


FIGURE 2. Mean frequency of headshakes during four 5-min observations at 0600 h (—) and again at 1400 h (---) for Lines B and A, between ages 196 to 208 d (I), 229 to 238 d (II), and 313 to 318 d (III). At all ages, there was a significant difference between lines at 0600 but not 1400 h.

lines in allocation of resources because egg production was greater for the higher than lower dietary level of vitamin E and for Line A than B. Thus, at the higher level of vitamin E more resources were allocated to BW and reproduction than at the lower level. The results suggest that supplementing diets with vitamin E may provide a strategy for reducing or delaying effects associated with aging. The dietary level of vitamin E by line interaction for vitamin E in the egg but not in blood plasma is consistent with status in the blood and elimination of excess E via the egg yolk. That is, the line with lower egg production (B) had a higher concentration of vitamin E in its egg yolks. These observations suggest that results obtained with the feeding of different dietary levels of vitamin E are influenced by genetic stock, duration and level of feeding dietary vitamin E, age, and measurement criteria. Moreover, they may have an influence on progeny when fed to breeders.

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REFERENCES

- Bollengier-Lee, S., M. A. Mitchell, D. B. Utomo, and P. E. V. Williams, 1998. Influence of high vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. *Br. Poult. Sci.* 39:106–112.
- Buttriss, J. L., and A. T. Diplock, 1984. High-performance liquid chromatography methods for vitamin E in tissues. *Methods in Enzymology* 105:131–138.
- Catignani, G. L., and J. G. Bieri, 1983. Simultaneous determination of retinol and alpha-tocopherol in serum and plasma by liquid chromatography. *Clin. Chem.* 29:708–712.
- Cherian, G., and J. G. Sim, 1997. Egg yolk polyunsaturated fatty acids and vitamin E content alters the tocopherol status of hatched chicks. *Poultry Sci.* 76:1753–1759.
- David, P., A. Hingle, K. Fowler, and A. Pomiankowski, 1999. Measurement bias and fluctuating asymmetry estimates. *Anim. Behav.* 57:251–253.
- Erf, G. F., W. G. Bottje, T. K. Bersi, M. D. Headrick, and C. F. Fritts, 1998. Effects of dietary vitamin E on the immune system of broilers: Altered proportions of CD4 T cell in the thymus and spleen. *Poultry Sci.* 77:529–537.
- Ewart, J., 1993. Evolution of genetic selection techniques and their application in the next decade. *Br. Poult. Sci.* 34:3–10.
- Gebert, S., R. Messikommer, H. P. Pfirter, G. Bee, and C. Wenk, 1998. Dietary fats and vitamin E in diets for laying hens: Effects on laying performance, storage stability and fatty acid composition of eggs. *Arch. Geflügelkd.* 62:214–222.
- Gore, A. B., and M. A. Qureshi, 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. *Poultry Sci.* 76:984–991.
- Gross, W. B., and H. S. Siegel, 1983. Evaluation of the heterophil:lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* 27:972–979.
- Gross, W. B., and P. B. Siegel, 1993. General principles of stress and welfare. Pages 21–34 *in: Livestock Handling and Transport*. T. Grandin, ed. CAB International Wallingford, Oxon, UK.
- Hossain, S. M., S. L. Barreto, A. G. Bertechini, A. M. Rios, and C. G. Silva, 1998. Influence of dietary vitamin E level on egg production of broiler breeders, and on the growth and immune response of progeny in comparison with the progeny from eggs injected with vitamin E. *Anim. Feed Sci. Technol.* 78:307–317.
- Hughes, B. O., and A. J. Black, 1974. The effects of environmental factors on activity, selected behaviour patterns and “fear” of fowl in cages and pens. *Br. Poult. Sci.* 15:375–380.
- Mauldin, J. M., and P. B. Siegel, 1979. “Fear”, headshaking, and production in five populations of cage chickens. *Br. Poult. Sci.* 20:39–44.
- Meluzzi, A., F. Sirri, G. Manfreda, N. Tallarico, and A. Franchini, 2000. Effects of vitamin E on the quality of table eggs enriched with n-3 long chain fatty acids. *Poultry Sci.* 79:539–545.
- Moller, A. P., G. S. Sanotra, and K. S. Vestergaard, 1999. Developmental instability and light regime in chickens. *Appl. Anim. Behav. Sci.* 62:57–71.
- Moller, A. P., and J. P. Swaddle, 1997. *Asymmetry, Developmental Stability, and Evolution*. Oxford Univ. Press, New York, NY.
- Pollock, D., 1999. Geneticist’s perspective from within a broiler primary breeder company. *Poultry Sci.* 78:414–418.
- SAS Institute, 1985. *SAS7 User’s Guide: Statistics*. Version 5 Edition. SAS Institute Inc., Cary, NC.
- Sell, J. L., M. F. Soto-Salanova, P. Palo, and M. Jeffrey, 1997. Influence of supplementing corn-soybean meal diets with vitamin E on performance and selected physiological traits of male turkeys. *Poultry Sci.* 76:1405–1417.
- Siegel, P. B., C. T. Larsen, D. A. Emmerson, P. A. Geraert, and M. Picard, 2000. Feeding regimen, dietary vitamin E, and genotype influence on immunological and production traits of broilers. *J. Appl. Poult. Res.* 9:269–278.
- Surai, P. F., R. C. Noble, and B. K. Speake, 1999. Relationship between vitamin E content and susceptibility to lipid peroxidation in tissues of the newly hatched chick. *Br. Poult. Sci.* 40:406–410.
- Yang, A., E. A. Dunnington, and P. B. Siegel, 1997. Developmental stability in stocks of White Leghorn chickens. *Poultry Sci.* 76:1632–1636.
- Yang, N., C. T. Larsen, E. A. Dunnington, P. A. Geraert, M. Picard, and P. B. Siegel, 2000. Immune competence of chicks from two lines divergently selected for antibody response as affected by supplemental vitamin E. *Poultry Sci.* 79:799–803.